Final Report

Evaluation of the Certus, Inc. and Lone Mountain Processing, Inc. Natural Resource Damage Assessment and Restoration Cases to Restore Mussels in the Clinch and Powell Rivers in Virginia and Tennessee

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Contents

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 1

Production and Release of Propagated Mussels in the Clinch and Powell Rivers for the Certus, Inc. and Lone Mountain Processing, Inc. NRDAR Cases

Abstract

Two Natural Resource Damage Assessment and Restoration (NRDAR) cases in the upper Tennessee River basin are among the first and largest cases in the United States involving injury to freshwater mussels due to release of hazardous substances. The Certus, Inc. spill occurred in 1998 in the upper Clinch River in Virginia, killing an estimated 18,000 mussels, including individuals of three endangered species. The Lone Mountain Processing, Inc. spill occurred in 1996 in the Powell River in Virginia, affecting mussels over a 65-mile section of river. Settlement money from these two cases was used to propagate and release mussels at population restoration (i.e., release of organisms within indigenous range) sites in the upper Clinch River, VA and in the Powell River, TN and VA. Mussel production and release data are here summarized from 2003–2019 for the Virginia Department of Wildlife Resources' Aquatic Wildlife Conservation Center (AWCC) and Virginia Tech's Freshwater Mollusk Conservation Center (FMCC). A total of 8,456,191 juvenile mussels of 34 species were produced by AWCC and FMCC, with a total of 861,845 mussels of 26 species released at sites in Virginia and Tennessee over this time period. Of the released mussels, a total of 152,182 were 20–40 mm long and 1–3 years old. Of these larger and older mussels, 127,574 were released for the Certus, Inc. NRDAR case and 24,608 were released as part of restoration efforts for the Lone Mountain Processing, Inc. NRDAR case. Until 2008, most mussels released were typically a few weeks old and <1 mm long. However, by 2011, both facilities were consistently growing mussels to larger sizes before release. This allowed mussels to settle into substrate more quickly and improved survivability of released mussels at restoration sites.

Introduction

Two Natural Resource Damage Assessment and Restoration (NRDAR) cases in the upper Tennessee River basin in Virginia (VA) are among the first and largest cases in the United States involving injury to freshwater mussels due to release of hazardous substances. The Certus, Inc. chemical spill released 1,350 gallons of Octocure-554 revised, a rubber accelerant, into a tributary of the Clinch River when a tanker truck overturned on U.S. Route 460 in Tazewell County, VA on August 27, 1998. The river was turned a snowy white color downstream of the spill for six miles (Figure 1.1) and took at least 12 hours to clear. The spill affected all organisms in the Clinch River within an approximately seven mile impact zone from Cedar Bluff, VA downstream to Richlands, VA (Figure 1.2). An extensive proportion of the fish population, as well as most aquatic macroinvertebrates were killed, including local populations of three mussel species listed as federally endangered (Golden Riffleshell, Purple Bean, and Rough Rabbitsfoot). This spill also eliminated one of the last two known reproducing populations of the Golden Riffleshell, making the spill one of the worst kills of species listed as federally endangered since the inception of the Endangered Species Act (U.S. Fish and Wildlife Service 2004).

The U.S. Fish and Wildlife Service (USFWS), acting on behalf of the U.S. Department of the Interior (DOI), evaluated injuries to natural resources as a result of the spill. The Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA) gives authority to federal and state trustees to "assess damages for injury to, destruction of, or loss of natural resources." In this context, an injury is "a measurable adverse change in the chemical or physical quality or viability of a natural resource", while damages are the "amount of money sought by the natural resource trustee as compensation for injury ... of natural resources". The CERCLA Natural Resource Damage Assessment and Restoration (NRDAR) regulations (43 CFR Part 11) provide a general process for evaluating and, if appropriate, restoring injuries to natural resources and services where a hazardous substance has been released. A total of 6,207 dead mussels were collected from the surface of the substrate immediately following the spill, including 250 individuals of the three federally listed endangered species. At any given time, however, only a fraction of mussels are expected to be on the substrate surface and available for capture or collection (Schwalb and Pusch 2007). To include buried mussels in the injury quantification, the total number of dead mussels was multiplied by 3. This extrapolation resulted in an estimated injury of 18,621 mussels, including 750 individuals of three endangered species (Table 1.1). More sophisticated methods of injury quantification, such as Resource Equivalency Analyses, had not been developed for NRDAR incidents involving mussel species at the time of the Certus, Inc. spill. Thus, 18,621 mussels were used as the baseline condition and ultimate restoration target for the Certus, Inc. NRDAR case.

As part of a settlement between Certus, Inc. and the DOI, on behalf of the United States of America, Certus, Inc. paid $3,800,000 in natural resource damages to compensate for the injuries from the spill. An amount of $92,567.16 went toward unreimbursed natural resource damage assessment costs incurred by the DOI (Jones 2003). The remaining $3,707,432.84 was placed in an interest-bearing account to be used by the Trustees for restoration, rehabilitation, replacement, or acquisition of equivalent natural resources injured or potentially injured by the spill and for the planning, implementation oversight, and monitoring of restoration projects related to this release.

The Trustee Council in this case consisted of the Commonwealth of Virginia and the U.S. DOI, and was formed to administer settlement funds to restore the natural resources injured by the spill (Commonwealth of Virginia and U.S. Department of the Interior 2003). The council's decision makers were the Deputy Director for Operations of the Department of Environmental Quality (State Trustee) and the Regional Director of Region 5 of the USFWS (Federal Trustee).

The principle goal for the Certus, Inc. NRDAR case was to restore the mussel assemblage and its supporting habitats to approximate baseline conditions (U.S. Fish and Wildlife Service 2004). Under the preferred restoration alternative, the bulk of settlement funds (~$2.8 million) went towards supporting propagation of all impacted mussel species at sites within the spill area as well as selected sites in the Upper Clinch River outside of the spill area. Several sites in the Upper Clinch River in Russell County, VA, from Nash Ford downstream to Cleveland Islands were stocked with mussels to reduce the risk of only stocking mussels at a single, relatively short, urban stream reach. These sites outside of the impact zone also were chosen because *Epioblasma capsaeformis* and *E. brevidens* were used as surrogates species for the critically endangered *E. aureola* to develop propagation, culture, and monitoring techniques for *E. aureola*, which was difficult to successfully propagate and monitor in 2004 and even years later. However, *E. brevidens* and *E. capsaeformis* did not historically occur in the impact zone and had to be stocked downstream, necessitating the use of additional sites where they had occurred historically or currently.

The Lone Mountain Processing, Inc. (LMPI) spill was the result of the failure of a coal slurry impoundment at a coal processing plant in Lee County, VA, on October 24, 1996. Coal slurry entered a system of unused underground mineworks and ultimately exited to the surface at Gin Creek (U.S. Fish and Wildlife Service 2003). From the impoundment, 6,000,000 gallons of coal slurry were released into a series of tributaries of the Powell River. The resulting "blackwater", a mixture of water, coal fines, and clay, impacted a large section of the Powell River, and coal particle sediment ultimately was deposited as far downstream as Norris Reservoir, TN, 65 miles downstream from the release site. Fifteen species of federally listed endangered mussels (3 were listed after the spill) as well as critical habitat of two fish species listed as federally threatened were impacted. The Virginia Department of Environmental Quality (VDEQ) also estimated that at least 11,240 fish of various species were directly killed (U.S. Fish and Wildlife Service 2003). These fishes included species that serve as hosts to endangered mussels.

As part of a settlement between LMPI, Inc. and the DOI, on behalf of the United States of America, LMPI, Inc. paid $2,376,500 in damages. After paying for reimbursement of past assessment costs, certain administrative expenses, and reimbursement of litigation costs, the remaining $2,040,000 was placed in an interest-bearing account to be used by the Trustees for restoration, rehabilitation, replacement, or acquisition of equivalent natural resources injured or potentially injured by the spill and for the planning, implementation oversight, and monitoring of restoration projects related to this release. The Trustees in this case include the U.S. DOI (Federal Trustee) and the USFWS's Region 5 Regional Director was given decision making authority and the Commonwealth of Virginia (State Trustee).

While both cases involved injuries to mussels, there were distinct differences between them. The Certus spill killed almost every mussel within a relatively short, seven-river mile length of stream (i.e., acute impact). Although a discrete event, the LMPI spill may have exposed mussels to chronic levels of contaminants (e.g., PAHs and trace metals), potentially causing chronic sublethal effects to mussels over a much larger area (i.e., chronic impact), including 65 river-miles of the main stem of the Powell River as well as several smaller tributaries. Coal slurry remained in the river for months after the spill event, and was periodically resuspended during high discharge events, likely chronically affecting mussels over a longer time period.

Due to the large amount of mussel propagation needed for both NRDAR cases, two facilities were used. The Freshwater Mollusk Conservation Center (FMCC) at Virginia Polytechnic Institute and State University (Virginia Tech) and the Virginia Department of Wildlife Resources' Aquatic Wildlife Conservation Center (AWCC) were responsible for propagating freshwater mussels at restoration sites in the Clinch and Powell Rivers over a 15-year period. Money from the legal settlements of both spills was principally used to fund propagation activities at these facilities.

The goals of this chapter were to: 1) summarize the number of mussels produced by AWCC and FMCC associated with the Certus, Inc. and LMPI NRDAR cases, 2) summarize the number of mussels released at restoration sites in the Clinch and Powell Rivers as part of the restoration effort for these two NRDAR cases, and 3) broadly assess survival of propagated mussels at AWCC and FMCC during the grow-out period at each hatchery. Summarization of this data is critical for determining whether restoration goals for the two NRDAR cases were met and for developing guidelines for injury and damage assessment of mussels in future cases (e.g., by developing a resource equivalency analysis for assessing injury, determining the cost of producing a given number of mussels in a hatchery, etc.).

Methods

Study areas

Two release sites, Payne Property (RM 322.1) and Sycamore Lane (RM 320), are located in the Clinch River, Tazewell County, VA, in the immediate impact zone of the Certus, Inc. NRDAR spill (Table 1.2). These two restoration sites were chosen because they generally had the best habitat in the seven-mile impact zone. Mussels also were released at four additional sites in the Clinch River, Bennett Property (RM 277.5), Artrip (RM 274.5), Whited Property (RM 272.7), and the left and right descending channels at Cleveland Islands (RM 270), ~40 miles downstream in Russell County, VA, outside of the immediate impact zone (Figure 1.3). These sites were selected to decrease the risk of released mussels being impacted by a single future event at the release sites in the impact zone in Tazewell County. These four sites were selected based on good physical habitat, presence of native mussel fauna, and the presence of fish hosts. For the LMPI NRDAR case, six sites in the Powell River were selected as release sites due to the presence of diverse preexisting mussel assemblages and suitable habitat, including 833 Bridge (RM 120.2) and Fletcher Ford (RM 117.3) in Lee County, VA, and Buchannan Ford (RM 99.2), Upper Brooks Bridge (RM 95.3), Lower Brooks Bridge (RM 94.7), and Oakley Property (RM 89.7) in Claiborne County, TN (Figure 1.3). Together, these sites represent the principle restoration sites used for population restoration for these two NRDAR cases. We use the term "population restoration" to refer to the translocation of either propagated mussels from the lab or wild mussels from other sites to locations within the indigenous range of the mussel species. Population restoration sites included both reinforcement sites (release of mussel species into existing population of conspecifics) and reintroduction sites (release of mussel species in areas from which it is extirpated) (IUCN/SSC 2013). All population restoration sites were typically 100 to 300 meters long and are comprised of high quality mussel habitat. All sites other than the Payne Property and Sycamore Lane (i.e., in the impact zone of the Certus, Inc. Spill) were considered reinforcement sites.

Summarizing mussel production and release data

Data records for total numbers of newly transformed juvenile mussels produced and total number of mussels released in the Clinch and Powell Rivers from 2003–2019 by AWCC and FMCC were summarized, checked for accuracy, and collated. Data included all juvenile mussels produced at these facilities, while the number of mussels released included only those released as part of the Certus, Inc. and LMPI NRDAR cases (releases from other projects are summarized in Appendices A and B). Until 2009, mussels generally were released at very young ages, within days or weeks of excysting from host fish (i.e, dropping off fish host to settle onto river substrate). Starting in 2010, all propagated mussels were allowed to grow to older ages and larger sizes in both facilities to ensure higher survival when released at restoration sites. Therefore, we designated all mussels released at population restoration sites into two categories: those released at <6 months old (typically 2–4 weeks old and <1 mm long) and those released at >6 months old (typically 1–2 years old and 20–40 mm long). Mussels released by AWCC in the Powell River from 2003–2014 were designated to replace mussels lost from the LMPI, Inc. NRDAR case, while mussels released by AWCC from the headwaters of the Clinch River near Tazewell, VA (RM 350.5), downstream to St. Paul, VA (RM 255.7), were designated to replace mussels lost from the Certus, Inc. NRDAR case. Mussels released by FMCC in the Powell River from 2003 to 2014 were designated to the LMPI NRDAR case, while mussels released in the Powell River after 2014 were designated to the Nature Conservancy/Tennessee Valley Authority and Tennessee Wildlife Resources Agency partnerships. Mussels released by FMCC from the headwaters of the Clinch River near Tazewell, VA (RM 350.5), to Cleveland Islands near Cleveland in Russell County, VA (RM 270), were designated to the Certus, Inc. NRDAR case. All scientific names of mussels follow Williams et al. (2017).

These data were summarized by facility (AWCC or FMCC), project (Certus, Inc. or LMPI), and by each individual population restoration and monitoring site in the Clinch and Powell rivers. These data are used in Chapter 2 to estimate the expected number of surviving mussels at monitoring sites using a Leslie matrix model developed in collaboration with U.S. Department of the Interior Economist Kristin Skrabis and mussel survival data published in Jones, Neves, and Hallerman (2012). Estimates of expected number of surviving mussels at release sites were compared to monitoring data collected from 2015—2017 to estimate the actual number of surviving mussels at each site. Further, mussel release data were used in an economic analysis to estimate the cost of producing mussels at each facility.

Survival of propagated mussels at AWCC and FMCC

From 2010 to 2019, we estimated survival of hatchery-reared mussels to stocking size (e.g., 20-40 mm long) by assessing the number of mussels surviving from production as age-0 excysted juveniles to their eventual release at typically 1-2 years by dividing the number of mussels >6 months old released in each year by the number of mussels produced in the previous year. We chose 2010 to begin analysis because all releases from this year onwards were >6 months old, with the exception of: one fish infected with *Epioblasma aureola* that was released in Indian Creek in 2010 (with an estimated 2000 individuals of *E. aureola*), 217 *Villosa vanuxemensis* released in the South Fork Holston in 2010 for a different project, 21 3-month old *E. aureola* placed in silos in Indian Creek in 2016, and three *Lampsilis ovata* released in the Little Tennessee River in 2019 for another project. Starting the analysis in 2010 facilitated comparison to production, and we estimated survival separately for each facility.

Results

Juvenile mussel production

Total numbers of juvenile mussels produced by both AWCC and FMCC from 2004 to 2018 for the LMPI and Certus, Inc. NRDAR cases varied from 134,130 to 1,077,786 juveniles per year with a total of

8,456,191 juveniles and 34 species produced during this period (Table 1.3). *Lampsilis fasciola* was the species with the largest number of individuals produced with 1,798,722 mussels, while *Theliderma intermedia* had the fewest individuals produced, with only one mussel produced. Of the 8,456,191 mussels produced in total, 6,211,202 were produced at AWCC (Table 1.4) and 2,244,989 were produced at FMCC (Table 1.5). *Lampsilis fasciola* was the species with the most individuals produced at AWCC, where 32 species were produced overall, and *Epioblasma capsaeformis* had the most individuals produced at FMCC, where 22 species were produced overall.

Total mussels released

A total of 861,845 mussels representing 26 species – ranging from 3 *Plethobasus cyphus* to 181,995 *L. fasciola* – were released by AWCC and FMCC from 2003 to 2019 to replace mussels lost from the LMPI and Certus, Inc. NRDAR cases (Table 1.6). This number includes 17,802 mussels released jointly by AWCC and FMCC, of which 1,502 were translocations, while the remaining were juvenile mussels that excysted from infected host fishes that were released *in situ* at sites in the Upper Clinch River and Indian Creek at Cedar Bluff, VA. Beginning in 2010, almost all mussels were released at larger sizes by each facility to ensure higher survival and retention at monitoring sites. Of the 861,845 total mussels released, 152,182 were of larger size (20–40 mm long) and generally 1–3 years old (Table 1.7 and Figure 1.4). This number includes 1,502 translocated individuals, primarily of *Actinonaias pectorosa*, *Elliptio dilatata*, *Medionidus conradicus*, and *Ptychobranchus subtentus* collected downstream in the Clinch River in Russell County, VA, and that were released jointly by AWCC and FMCC. Twenty-three species of larger mussels were released, with *E. capsaeformis* and *E. brevidens* being the two species with the greatest numbers of individuals released.

Of the 152,182 mussels released at >6 months old, 127,574 mussels representing 24 species were released in the Clinch River, VA, for the Certus, Inc. NRDAR case (Table 1.8). *Epioblasma brevidens* and *E. capsaeformis* had the greatest number of mussels released, with 36,618 and 25,300 mussels, respectively, while only three *P. cyphyus* were released. For the LMPI NRDAR case, a total of 24,608 mussels representing 11 species were released (Table 1.9). *Epioblasma capsaeformis* had the most released mussels with 11,398 mussels, while only 3 *Actinonaias pectorosa* were released.

Mussels released by AWCC

Of the 861,845 total mussels released of all ages, 632,002 individuals representing 25 species were released by AWCC to replace mussels lost from the LMPI and Certus, Inc. NRDAR cases (Table 1.10). Releases ranged from 3 *P. cyphyus* to 179,832 *Actinonaias pectorosa*. Of these, 73,425 individuals representing 24 species were >6 months old (Table 1.11). *Epioblasma capsaeformis* and *E. brevidens* were the two species with the greatest number of individuals released that were >6 months old.

Of the 73,425 mussels >6 months old released by AWCC, 62,472 individuals representing 24 species were released for the Certus, Inc. NRDAR restoration project (Table 1.12). *Epioblasma brevidens* and *E. capsaeformis* were the species with the greatest numbers of mussels released, with 20,765 and 9,533 individuals, respectively. For the LMPI NRDAR restoration project, 10,953 mussels representing 9 species were released (Table 1.13). *Villosa iris* was the species with the greatest number of mussels released, at 2,977 individuals.

Mussels released by FMCC

Of the 861,845 total mussels released of all ages, 212,041 individuals representing 15 species were released by FMCC to replace mussels lost from the LMPI and Certus, Inc. NRDAR cases (Table 1.14). Releases ranged from 58 *Pleuronaia barnesiana* to 75,495 *Epioblasma capsaeformis*. Of these, 77,255 individuals representing 13 species were >6 months old (Table 1.15). *Epioblasma capsaeformis* was the species with the greatest number of mussels >6 months old released, at 25,235 individuals.

Of the 77,255 mussels >6 months old released by FMCC, 63,600 individuals representing 12 species were released to replace mussels lost from the Certus, Inc. NRDAR restoration project (Table 1.16). *Epioblasma brevidens* had the most mussels released, with 15,853 mussels released. For the LMPI NRDAR restoration project, 13,655 mussels representing 7 species were released (Table 1.17). *Epioblasma capsaeformis* was the species with the greatest number of mussels released, at 9,468 individuals.

Number of mussels released at restoration sites

At the 13 population restoration and monitoring sites in the Clinch and Powell rivers, 128,531 mussels >6 months old were released, including 106,865 individuals at sites in the Clinch River and 21,666 individuals at sites in the Powell River (Table 1.18). Of the three monitoring sites located in the Clinch River impact zone of the Certus, Inc. spill, 15,314 mussels representing 11 species were released at the Payne Property. The majority of these mussels were *Lampsilis fasciola*, *V. iris*, and *V. vanuxemensis*. At Sycamore Lane, the second site in the impact zone, 21,417 mussels representing 11 species were released. The greatest number of mussels released was of *V. iris*, followed by *L. fasciola*, *Lampsilis ovata*, *Medionidus conradicus*, *Ptychobranchus fasciolaris*, and *P. subtentus*. At the Perry Property, the most upstream site in the impact zone, 370 mussels representing 3 species were released. Of the sites located downstream of the impact zone in the Clinch River in Russell County, VA, 28,538 mussels representing 20 species were released at the Bennett Property, the majority of which were *Epioblasma capsaeformis* and *E. brevidens*. At Artrip, 11,066 mussels representing 11 species were released, with the majority being *E. capsaeformis* and *E. brevidens*. Only 1,297 mussels representing 3 species were released at the Whited Property, most of which were *E. capsaeformis*. At Cleveland Islands in the right descending channel (RDC), 7,344 mussels were released, most of which were *E. capsaeformis*, and 12,241 mussels were released in the left descending channel (LDC), most of which were *E. capsaeformis* and *E. brevidens*. The LDC at Cleveland Islands was not monitored as part of this project, but was monitored in 2011 and 2012 by Carey et al. (2015).

For the LMPI NRDAR case, 4,211 mussels representing 5 species were released at Upper Brooks Bridge and 4,583 mussels representing 4 species were released at Lower Brooks Bridge. Most of these were *E. capsaeformis* and *E. brevidens*. Only 1,205 were released at the Oakley Property, almost all of which were *E. capsaeformis*.

As part of the LMPI NRDAR case, mussels also were released at the Route 833 Bridge, Fletcher Ford, and Buchannan Ford in the Powell River. These sites were not monitored as part of this study, but have been monitored in the past (Eckert et al. 2007). At the 833 Bridge site, 1,706 mussels were released, most of which were *Villosa iris*, 7,964 mussels were released at Fletcher Ford, most of which were *E. brevidens* and *E. capsaeformis*, and 1,997 mussels were released at Buchannan Ford, most of which were *E. capsaeformis*. The number of mussels released do not include additional mussel releases from 2015–2017 funded by VDWR's State Wildlife Grant program (SWG) (see Appendix A).

Production and survival of propagated mussels from 2010 to 2019

Production of mussels at AWCC was highest in 2010 (662,930), and from 2013 to 2018 remained between 100,000 and just over 200,000 (Table 1.4; Figure 1.5).

Release of mussels >6 months old to replace mussels lost from the Certus, Inc. and LMPI cases by AWCC was highest in 2011 (12,547), decreased to 2,406 in 2016, and then increased to 6,670 in 2018 (Table 1.11). Release of mussels >6 months old for all projects by AWCC was highest in 2013 (21,672), decreased to 6,256 in 2016, and then increased to 12,883 in 2017 and 11,975 in 2019 (Figure 1.5).

Production of mussels at FMCC in 2011 was 214,585, decreased to 19,825 in 2015, and increased to a high of 273,966 in 2017 (Table 1.5). Release of mussels >6 months old for the Certus, Inc. and LMPI cases by FMCC was highest in 2012 (16,400), decreased to 981 in 2016, and then increased to 12,528 by 2019 (Table 1.15). Release of mussels >6 months old for all projects by FMCC was highest in 2012 (19,569) decreased to 1,533 in 2016, and then increased to 13,231 by 2019 (Figure 1.5). Of the two facilities, AWCC had the higher number of releases from other projects, with 65,318 mussels released versus 10,690 released by FMCC (see Appendices A and B).

The most produced species at AWCC was *Lampsilis fasciola* (588,147), followed by *Epioblasma brevidens* (482,472) and *L. abrupta* (427,172) (Table 1.19). *Epioblasma brevidens* was the species with the most mussels released for the Certus, Inc. and LMPI cases, followed by *E. capsaeformis* and *L. fasciola*. The species with the highest survival to >6 months old for the Certus, Inc. and LMPI cases was *Pleuronaia dolabelloides* (21.9%), followed by *E. aureola* (15.5%) (Table 1.19). Survival of mussels to >6 months old increased when including mussel release data from all projects, i.e., for mussels that were not released as part of the Certus, Inc. and LMPI cases (e.g., *Lampsilis abrupta* and *Fusconaia cuneolus*). The most produced species at FMCC was *E. capsaeformis* (312,638), followed by *Villosa iris* (272,946), *Lampsilis fasciola* (187,695) and *E. brevidens,* (250,176) which also had high production (Table 1.20). *Epioblasma capsaeformis* had the highest number of mussels >6 months old released (25,179) as well as the third-highest survival (8.1%) for the Certus, Inc. and LMPI NRDAR cases. *Medionidus conradicus* had the highest survival to release >6 months old (10.0%), while all other species' survival was less than 10%. When including data from all projects, survival to release >6 months old did not increase as much for AWCC (Figure 1.5).

Discussion

The Certus, Inc. and Lone Mountain, Inc. cases were the first NRDAR cases involving injuries to freshwater mussels in the United States. Consequently, these cases provided a unique opportunity to conduct mussel restoration at a larger scale than ever practiced before. Before these NRDAR cases, there were no full-time, professionally staffed hatcheries to propagate mussels, and the state of propagation technology was underdeveloped.

The settlement money from these cases allowed the hiring of full-time professional-level personnel at both AWCC and FMCC. This investment of resources supported consistent improvement in culture technology of freshwater mussels. For example, numerous host fishes were identified for mussel species whose hosts were previously unknown, allowing for larger-scale production of juveniles. There also was a transition away from propagation and release of very young juveniles (<6 months old). Before 2008, most mussels released for these projects were typically 2–4 weeks old and <1 mm long. However, these mussels had very low survival after release. Early successes of growing mussels to larger sizes and older ages had occurred from 2003 through 2008, but by 2009, both AWCC and FMCC began to release mussels that had grown large enough to have higher survival rates in the wild. By 2010, both facilities were almost exclusively releasing only individuals typically 20–40 mm long and 1–3 years old. These larger individuals were able to settle more quickly into substrate, increasing their survival rate (Jones, Mair, and Neves 2005). This necessitated the development of techniques to culture and maintain mussels in the hatchery over the course of 1–3 years.

While production varied greatly among facilities and years, it was always much higher than the number of mussels being released. Survival of mussels at these hatcheries to larger sizes suitable for release never exceeded 20% in any year and the total average was less than 5%. This highlights the challenges of propagating freshwater mussels for the purposes of restoration; the target number of mussels produced must be much higher than the target number of mussels to be released for a given restoration project. These data provide valuable estimates for these targets for future restoration projects.

In addition to the development of new culture techniques, the nature of these projects promoted collaboration among a number of stakeholders throughout southwest Virginia and northeast Tennessee. The Mussel Recovery Group (MRG) was formed in 2004 to include federal, state, and non-governmental partners that encouraged the sharing of information to most efficiently use the resources of AWCC and FMCC. The development of new culture techniques and technology, as well as ongoing partnerships developed during these projects demonstrate the efficacy of using mussel propagation for restoring mussel populations impacted by chemical spills in the future.

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Tables

Table 1.1. Mussel age and kill estimates from the Certus, Inc. chemical spill that occurred in the Clinch River at Cedar Bluff, Tazewell County, VA, on August 27, 1998.[1]

Species	Min. Age	Max. Age	Mean Age	**Number** Collected	USFWS Kill Estimate
Actinonaias pectorosa	6	32	15.5	135	405
Epioblasma aureola	2	11	4.9	178	534
Lampsilis fasciola	8	33	18.5	962	2,886
Lampsilis ovata	5	38	14.2	62	186
Lasmigona costata	4	33	16.5	84	252
Medionidus conradicus	2	14	6.2	219	657
Pleuronaia barnesiana/ Pleurobema oviforme	4	51	18.8	610	1,830
Ptychobranchus fasciolaris	7	85	31.0	579	1,737
Ptychobranchus subtentus	9	55	21.9	35	105
Theliderma strigillata	11	63	44.5	20	60
Venustaconcha trabalis	4	29	11.3	52	156
Villosa iris	2	20	7.2	3,247	9,741
Villosa vanuxemensis	6	22	11.4	24	72
Total				6,207	18,621

[1]U.S. Fish and Wildlife Service 2004. Final restoration plan and environmental assessment for the Certus chemical spill natural resource damage assessment. Technical Report. Gloucester, VA: US Fish and Wildlife Service: 45 pp.

Table 1.2. Location information for thirteen restoration and monitoring sites for the LMPI and Certus, Inc. NRDAR mussel restoration cases in the Clinch and Powell Rivers, Tennessee and Virginia. RDC = right descending channel and LDC = left descending channel.

Site	River	River Mile	Latitude/Longitude
Payne Property, VA	Clinch	322.1	37.081642°, -81.778816°
Sycamore Lane, VA	Clinch	320	37.095162°, -81.785898°
Bennett Property, VA	Clinch	277.5	36.959511°, -82.097550°
Artrip, VA	Clinch	274.5	36.961647°, -82.119429°
Whited Property, VA	Clinch	272.7	36.948771°, -82.139325°
Cleveland Islands - RDC, VA	Clinch	270	36.938084°, -82.164613°
Cleveland Islands - LDC, VA	Clinch	270	36.937047°, -82.166494°
State Route 833 Bridge, VA	Powell	120.2	36.620940°, -83.284570°
Fletcher Ford, VA	Powell	117.3	36.604622°, -83.295228°
Buchannan Ford, TN	Powell	99.2	36.558269°, -83.423269°
Upper Brooks Bridge, TN	Powell	95.3	36.534982°, -83.442999°
Lower Brooks Bridge, TN	Powell	94.7	36.536824°, -83.451406°
Oakley Property, TN	Powell	89.7	36.535212°, -83.467035°

Table 1.3. Total juvenile mussels produced by AWCC and FMCC from 2004 to 2018 for the Certus, Inc. and LMPI NRDAR cases in the Clinch and Powell rivers in Virginia and Tennessee.

Species (34)	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
Actinonaias ligamentina	24,867	0	54,260	0	41,684	0	0	0	0	0	0	0	0	0	0	120,811
Actinonaias pectorosa	3,092	65,921	48,789	189,602	218,472	134,950	88,958	0	0	0	0	0	0	0	0	749,784
Alasmidonta viridis	0	0	0	0	0	0	0	0	0	0	0	0	0	850	4,773	5,623
Cyprogenia stegaria	0	0	0	0	6,415	7,305	2,208	0	5,755	0	0	0	201	28	173	22,085
Dromus dromas	1,053	0	5,856	6,436	21,092	25,765	28,302	0	34,476	1,688	39	1,777	431	240	0	127,155
Epioblasma aureola	4,864	2,293	10,888	51	0	0	0	0	0	1,159	3,119	296	0	0	0	22,670
Epioblasma brevidens	12,136	52,314	127,072	47,773	50,255	40,916	58,346	95,422	120,822	79,632	75,268	23,470	71,749	133,697	74,242	1,063,114
Epioblasma capsaeformis	65,542	81,476	135,439	136,132	106,679	75,452	92,160	142,666	81,212	60,219	15,576	16,923	55,535	30,749	51,365	1,147,125
Epioblasma triquetra	0	9,965	256	1,734	3,519	9,050	14,782	1,220	1,080	1,608	0	1,543	2,516	10,646	5,519	63,438
Eurynia dilatata	0	0	147	0	0	35,657	0	0	7,069	0	0	0	0	0	0	42,873
Fusconaia cor	0	0	128	39	103	0	0	80	2,135	67	0	0	0	0	0	2,552
Fusconaia cuneolus	0	0	0	0	0	0	0	0	150	0	0	0	0	363	185	698
Hemistena lata	0	3	0	53	20	0	0	0	145	0	0	0	0	1	2	224
Lampsilis abrupta	0	0	0	0	0	0	186,045	120,811	78,635	0	41,681	0	0	0	0	427,172
Lampsilis fasciola	16,631	69,298	103,614	290,885	277,901	264,551	164,834	242,718	151,803	7,549	14,121	38,615	52,421	70,542	33,239	1,798,722
Lampsilis ovata	15,542	72,409	90,558	198,501	99,128	122,656	45,093	35,461	31,404	0	12,298	22,631	6,288	9,496	61,653	823,118
Lasmigona costata	0	0	0	4,648	4,980	4,646	1,908	827	0	63,655	0	0	0	0	0	80,664
Lasmigona holstonia	0	0	0	0	0	53,025	16,268	51,655	0	6,097	52,266	0	0	0	11,654	190,965
Lemiox rimosus	114	124	0	96	139	2,853	5,946	97	12,802	2,846	68	1,682	6,163	37,684	14,775	85,389
Ligumia recta	0	17,791	32,184	132	44,052	0	295	0	21,138	43,464	897	0	0	0	9,469	169,422
Medionidus conradicus	0	456	0	0	71	407	6,031	591	9,123	300	10,472	0	16,838	9,662	0	53,951
Plethobasus cyphyus	0	0	0	0	0	0	0	516	0	0	0	0	0	0	7	523
Pleurobema oviforme	0	0	0	46	0	0	0	0	0	0	0	0	0	0	0	46
Pleuronaia barnesiana	0	0	0	0	0	0	0	0	0	0	0	0	1,171	0	0	1,171
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	457	0	0	0	0	0	0	457
Potamilus alatus	0	0	0	215	0	0	0	7,634	0	0	0	0	0	0	0	7,849
Ptychobranchus fasciolaris	0	0	1,218	5,040	0	0	173	14,681	11,109	22,950	5,532	0	8,716	0	0	69,419
Ptychobranchus subtentus	0	3,658	6,207	0	756	41,849	3,002	848	1,681	20,559	0	0	25,001	21,554	0	125,115
Strophitus undulatus	0	0	0	0	0	0	916	0	0	0	0	0	0	0	4,701	5,617
Theliderma cylindrica	0	187	0	0	0	0	0	310	60	0	0	0	0	211	7	775
Theliderma intermedia	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Venustaconcha trabalis	3,603	6,405	10,706	4,207	14,459	4,148	2,916	6,474	2,809	14,209	10,181	8,765	5,795	11,681	45,896	152,254
Villosa iris	32,590	106,091	113,140	74,041	142,259	58,852	39,393	26,400	50,883	39,179	5,189	11,685	15,697	105,613	28,426	849,438
Villosa vanuxemensis	0	6,767	20,074	47,731	45,802	8,122	25,449	37,189	9,724	6,004	423	6,743	5,979	19,576	6,388	245,971
Total	180,035	495,158	760,536	1,007,362	1,077,786	890,204	783,025	785,600	634,472	371,185	247,130	134,130	274,501	462,593	352,474	8,456,191

Table 1.4. Total juvenile mussels produced by AWCC from 2004 to 2018 for the Certus, Inc. and LMPI NRDAR cases in the Clinch and Powell rivers in Virginia and Tennessee.

Species (32)	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
Actinonaias ligamentina	24,867	0	52,889	0	41,684	0	0	0	0	0	0	0	0	0	0	119,440
Actinonaias pectorosa	3,092	65,921	48,789	187,519	218,472	131,850	88,958	0	0	0	0	0	0	0	0	744,601
Alasmidonta viridis	0	0	0	0	0	0	0	0	0	0	0	0	0	850	4,773	5,623
Cyprogenia stegaria	0	0	0	0	607	6,017	475	0	5,755	0	0	0	201	28	8	13,091
Dromus dromas	0	0	3,567	1,429	5,565	25,765	23,420	0	28,753	448	0	1,777	431	240	0	91,395
Epioblasma aureola	17	0	0	0	0	0	0	0	0	1,159	3,119	296	0	0	0	4,591
Epioblasma brevidens	1,018	4,092	35,242	24,120	21,935	33,666	25,884	63,872	99,480	59,979	75,268	22,458	58,878	76,653	0	602,545
Epioblasma capsaeformis	8,154	2,420	58,746	28,951	54,399	58,823	36,885	45,282	30,990	33,204	9,023	11,338	41,085	22,659	3,301	445,260
Epioblasma triquetra	0	9,965	256	310	3,519	8,542	14,782	0	0	0	0	0	1,040	1,038	2,904	42,356
Eurynia dilatata	0	0	147	0	0	35,657	0	0	7,069	0	0	0	0	0	0	42,873
Fusconaia cor	0	0	128	39	103	0	0	80	2,135	67	0	0	0	0	0	2,552
Fusconaia cuneolus	0	0	0	0	0	0	0	0	150	0	0	0	0	363	185	698
Hemistena lata	0	0	0	0	20	0	0	0	145	0	0	0	0	1	2	168
Lampsilis abrupta	0	0	0	0	0	0	186,045	120,811	78,635	0	41,681	0	0	0	0	427,172
Lampsilis fasciola	7,616	62,286	103,614	272,174	277,901	261,474	164,834	208,715	111,765	2,598	12,811	38,615	25,221	0	23,588	1,573,212
Lampsilis ovata	15,542	55,964	90,558	171,891	90,634	122,656	45,093	35,461	29,761	0	12,298	22,631	6,288	9,496	30,670	738,943
Lasmigona costata	0	0	0	4,648	4,980	4,646	1,908	827	0	63,655	0	0	0	0	0	80,664
Lasmigona holstonia	0	0	0	0	0	53,025	16,268	51,655	0	6,097	52,266	0	0	0	11,654	190,965
Lemiox rimosus	0	0	0	0	139	2,853	5,817	0	12,134	1,008	0	1,682	1,390	37,684	14,039	76,746
Ligumia recta	0	17,791	32,184	0	43,400	0	0	0	21,138	43,464	897	0	0	0	9,469	168,343
Medionidus conradicus	0	456	0	0	0	0	5,867	482	6,370	0	4,759	0	7,429	145	0	25,508
Plethobasus cyphyus	0	0	0	0	0	0	0	516	0	0	0	0	0	0	7	523
Pleurobema oviforme	0	0	0	46	0	0	0	0	0	0	0	0	0	0	0	46
Pleuronaia barnesiana	0	0	0	0	0	0	0	0	0	0	0	0	1,171	0	0	1,171
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	457	0	0	0	0	0	0	457
Ptychobranchus fasciolaris	0	0	392	5,040	0	0	173	3,326	0	49	8	0	0	0	0	8,988
Ptychobranchus subtentus	0	1,784	0	0	756	41,849	3,002	0	0	0	0	0	3,113	10,773	0	61,277
Strophitus undulatus	0	0	0	0	0	0	916	0	0	0	0	0	0	0	4,701	5,617
Theliderma cylindrica	0	0	0	0	0	0	0	310	60	0	0	0	0	211	7	588
Venustaconcha trabalis	1,272	2,961	7,965	3,764	13,858	4,148	2,285	6,474	2,809	8,914	8,009	8,765	5,795	8,910	45,896	131,825
Villosa iris	32,590	58,777	71,752	30,741	100,722	56,839	16,918	3,925	28,676	0	0	0	0	0	0	400,940
Villosa vanuxemensis	0	3,488	14,337	42,936	45,802	8,122	23,400	29,279	0	0	0	6,743	5,979	19,576	3,362	203,024
Total	94,168	285,905	520,566	773,608	924,496	855,932	662,930	571,015	466,282	220,642	220,139	114,305	158,021	188,627	154,566	6,211,202

Table 1.5. Total juvenile mussels produced by FMCC from 2004 to 2018 for the Certus, Inc. and LMPI NRDAR cases in the Clinch and Powell rivers in Virginia and Tennessee.

Species (22)	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
Actinonaias ligamentina	0	0	1,371	0	0	0	0	0	0	0	0	0	0	0	0	1,371
Actinonaias pectorosa	0	0	0	2,083	0	3,100	0	0	0	0	0	0	0	0	0	5,183
Cyprogenia stegaria	0	0	0	0	5,808	1,288	1,733	0	0	0	0	0	0	0	165	8,994
Dromus dromas	1,053	0	2,289	5,007	15,527	0	4,882	0	5,723	1,240	39	0	0	0	0	35,760
Epioblasma aureola	4,847	2,293	10,888	51	0	0	0	0	0	0	0	0	0	0	0	18,079
Epioblasma brevidens	11,118	48,222	91,830	23,653	28,320	7,250	32,462	31,550	21,342	19,653	0	1,012	12,871	57,044	74,242	460,569
Epioblasma capsaeformis	57,388	79,056	76,693	107,181	52,280	16,629	55,275	97,384	50,222	27,015	6,553	5,585	14,450	8,090	48,064	701,865
Epioblasma triquetra	0	0	0	1,424	0	508	0	1,220	1,080	1,608	0	1,543	1,476	9,608	2,615	21,082
Hemistena lata	0	3	0	53	0	0	0	0	0	0	0	0	0	0	0	56
Lampsilis fasciola	9,015	7,012	0	18,711	0	3,077	0	34,003	40,038	4,951	1,310	0	27,200	70,542	9,651	225,510
Lampsilis ovata	0	16,445	0	26,610	8,494	0	0	0	1,643	0	0	0	0	0	30,983	84,175
Lemiox rimosus	114	124	0	96	0	0	129	97	668	1,838	68	0	4,773	0	736	8,643
Ligumia recta	0	0	0	132	652	0	295	0	0	0	0	0	0	0	0	1,079
Medionidus conradicus	0	0	0	0	71	407	164	109	2,753	300	5,713	0	9,409	9,517	0	28,443
Potamilus alatus	0	0	0	215	0	0	0	7,634	0	0	0	0	0	0	0	7,849
Ptychobranchus fasciolaris	0	0	826	0	0	0	0	11,355	11,109	22,901	5,524	0	8,716	0	0	60,431
Ptychobranchus subtentus	0	1,874	6,207	0	0	0	0	848	1,681	20,559	0	0	21,888	10,781	0	63,838
Theliderma cylindrica	0	187	0	0	0	0	0	0	0	0	0	0	0	0	0	187
Theliderma intermedia	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Venustaconcha trabalis	2,331	3,444	2,741	443	601	0	631	0	0	5,295	2,172	0	0	2,771	0	20,429
Villosa iris	0	47,314	41,388	43,300	41,537	2,013	22,475	22,475	22,207	39,179	5,189	11,685	15,697	105,613	28,426	448,498
Villosa vanuxemensis	0	3,279	5,737	4,795	0	0	2,049	7,910	9,724	6,004	423	0	0	0	3,026	42,947
Total	85,867	209,253	239,970	233,754	153,290	34,272	120,095	214,585	168,190	150,543	26,991	19,825	116,480	273,966	197,908	2,244,989

Table 1.6. Total mussels of all ages released by AWCC and FMCC from 2003 to 2019 for the Certus, Inc. and LMPI NRDAR cases in the Clinch and Powell rivers in Virginia and Tennessee.

Species (26)	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Actinonaias ligamentina	0	22,300	0	15,623	0	6,257	0	0	0	0	0	0	0	0	0	0	0	44,180
Actinonaias pectorosa	0	2,613	39,467	12,230	92,051	32,990	272	450	248	9	1	0	0	0	0	0	0	180,331
Cyprogenia stegaria	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	38
Dromus dromas	0	101	0	6	0	0	0	0	0	0	27	0	0	4	4	0	0	142
Epioblasma aureola	0	5,570	3,054	3,000	8,000	300	4,500	2,000	0	0	0	0	0	21	710	0	0	27,155
Epioblasma brevidens	0	2,372	2,386	36,596	0	46	154	1,461	1,139	2,100	4,519	5,425	1,584	1,224	5,538	11,545	6,750	82,839
Epioblasma capsaeformis	0	11,637	2,463	36,835	3,648	1,962	274	2,786	2,836	11,370	8,342	3,952	503	859	740	566	4,463	93,236
Epioblasma triquetra	0	0	0	0	0	0	0	40	339	257	7	0	0	201	68	1,348	161	2,421
Eurynia dilatata	0	0	0	53	0	110	200	224	0	0	0	337	348	0	0	0	0	1,272
Fusconaia cor	0	0	0	10	0	0	0	0	0	0	0	131	0	4	0	0	0	145
Lampsilis fasciola	80	70	39,806	21,430	68,182	36,839	526	929	3,679	4,120	2,981	143	209	22	1,704	599	676	181,995
Lampsilis ovata	0	6,496	21,041	5,789	61,671	26,610	1,603	1,788	474	200	263	200	0	0	174	421	1,669	128,399
Lasmigona costata	0	0	0	0	542	0	0	69	0	3	0	10	0	0	0	0	0	624
Lasmigona holstonia	0	0	0	0	0	0	0	93	878	61	0	0	0	0	0	0	21	1,053
Lemiox rimosus	0	67	0	0	0	0	0	0	19	0	126	0	0	39	25	0	148	424
Ligumia recta	0	0	0	150	173	150	50	46	0	0	0	311	188	421	0	0	0	1,489
Medionidus conradicus	0	0	445	0	0	251	250	0	75	464	151	1,562	50	237	100	2,078	8	5,671
Plethobasus cyphyus	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3
Pleuronaia barnesiana	0	0	58	0	0	0	0	0	0	0	0	0	0	0	0	0	99	157
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	100
Ptychobranchus fasciolaris	0	0	0	5	1,542	0	0	0	0	1,117	558	45	766	1	208	1	0	4,243
Ptychobranchus subtentus	0	0	1,497	72	0	59	133	223	0	64	250	785	586	0	0	105	185	3,959
Venustaconcha trabalis	0	2,289	1,982	0	2,056	1,445	1,500	10	110	139	0	0	0	70	445	893	258	11,197
Villosa iris	765	11,664	21,788	10,728	1,203	3,632	150	1,227	5,372	1,681	0	701	554	163	268	289	2,279	62,464
Villosa vanuxemensis	0	0	3,279	0	9,508	4,212	45	1,441	3,303	2,370	314	0	187	101	824	1,164	1,560	28,308
Total	845	65,179	137,266	142,527	248,576	114,863	9,657	12,787	18,472	23,955	17,539	13,702	4,975	3,408	10,808	19,009	18,277	861,845

Table 1.7. Total mussels >6 months old released by AWCC and FMCC from 2003 to 2019 for the Certus, Inc. and LMPI NRDAR cases in the Clinch and Powell rivers in Virginia and Tennessee.

Species (24)	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Actinonaias pectorosa	0	0	0	0	2	250	272	450	248	9	1	0	0	0	0	0	0	1,232
Cyprogenia stegaria	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	38
Dromus dromas	0	0	0	0	0	0	0	0	0	0	27	0	0	4	4	0	0	35
Epioblasma aureola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	710	0	0	710
Epioblasma brevidens	0	0	0	0	0	5	154	1,461	1,139	2,100	4,519	5,425	1,584	1,224	5,538	11,545	6,750	41,444
Epioblasma capsaeformis	0	0	0	0	0	7	274	2,786	2,836	11,370	8,342	3,952	503	859	740	566	4,463	36,698
Epioblasma triquetra	0	0	0	0	0	0	0	40	339	257	7	0	0	201	68	1,348	161	2,421
Eurynia dilatata	0	0	0	0	0	110	200	224	0	0	0	337	348	0	0	0	0	1,219
Fusconaia cor	0	0	0	0	0	0	0	0	0	0	0	131	0	4	0	0	0	135
Lampsilis fasciola	80	70	50	10	184	133	526	929	3,679	4,120	2,981	143	209	22	1,704	599	676	16,115
Lampsilis ovata	0	0	0	345	213	902	1,603	1,788	474	200	263	200	0	0	174	421	1,669	8,252
Lasmigona costata	0	0	0	0	0	0	0	69	0	3	0	10	0	0	0	0	0	82
Lasmigona holstonia	0	0	0	0	0	0	0	93	878	61	0	0	0	0	0	0	21	1,053
Lemiox rimosus	0	0	0	0	0	0	0	0	19	0	126	0	0	39	25	0	148	357
Ligumia recta	0	0	0	150	173	150	50	46	0	0	0	311	188	421	0	0	0	1,489
Medionidus conradicus	0	0	0	0	0	251	250	0	75	464	151	1,562	50	237	100	2,078	8	5,226
Plethobasus cyphyus	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3
Pleuronaia barnesiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99	99
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	100
Ptychobranchus fasciolaris	0	0	0	0	0	0	0	0	0	1,117	558	45	766	1	208	1	0	2,696
Ptychobranchus subtentus	0	0	0	0	0	59	133	223	0	64	250	785	586	0	0	105	185	2,390
Venustaconcha trabalis	0	66	0	0	4	0	0	10	110	139	0	0	0	70	445	893	258	1,995
Villosa iris	0	4	212	125	1,103	50	150	1,227	5,372	1,681	0	701	554	163	268	289	2,279	14,178
Villosa vanuxemensis	0	0	0	0	2,906	0	45	1,441	3,303	2,370	314	0	187	101	824	1,164	1,560	14,215
Total	80	140	262	630	4,585	1,917	3,657	10,787	18,472	23,955	17,539	13,702	4,975	3,387	10,808	19,009	18,277	152,182

Table 1.8. Total mussels >6 months old released by AWCC and FMCC from 2003 to 2019 for the Certus, Inc. NRDAR case in the Clinch River in Virginia.

Species (24)	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Actinonaias pectorosa	0	0	0	0	2	250	269	450	248	9	1	0	0	0	0	0	0	1,229
Cyprogenia stegaria	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	38
Dromus dromas	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	8
Epioblasma aureola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	710	0	0	710
Epioblasma brevidens	0	0	0	0	0	0	0	1,330	490	452	3,355	4,350	1,584	1,224	5,538	11,545	6,750	36,618
Epioblasma capsaeformis	0	0	0	0	0	7	210	2,736	1,289	4,580	5,743	3,604	503	859	740	566	4,463	25,300
Epioblasma triquetra	0	0	0	0	0	0	0	40	339	0	0	0	0	201	68	1,348	161	2,157
Eurynia dilatata	0	0	0	0	0	110	200	224	0	0	0	337	348	0	0	0	0	1,219
Fusconaia cor	0	0	0	0	0	0	0	0	0	0	0	131	0	4	0	0	0	135
Lampsilis fasciola	80	50	50	0	184	20	526	929	3,337	3,620	2,237	143	209	22	1,704	599	676	14,386
Lampsilis ovata	0	0	0	0	63	302	1,200	1,788	458	0	183	200	0	0	174	421	1,669	6,458
Lasmigona costata	0	0	0	0	0	0	0	69	0	3	0	10	0	0	0	0	0	82
Lasmigona holstonia	0	0	0	0	0	0	0	93	878	61	0	0	0	0	0	0	21	1,053
Lemiox rimosus	0	0	0	0	0	0	0	0	19	0	63	0	0	39	25	0	148	294
Ligumia recta	0	0	0	100	123	50	0	46	0	0	0	311	188	421	0	0	0	1,239
Medionidus conradicus	0	0	0	0	0	251	250	0	75	464	151	1,562	50	237	100	2,078	8	5,226
Plethobasus cyphyus	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3
Pleuronaia barnesiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99	99
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	100
Ptychobranchus fasciolaris	0	0	0	0	0	0	0	0	0	1,117	558	45	766	1	208	1	0	2,696
Ptychobranchus subtentus	0	0	0	0	0	59	133	223	0	64	50	785	586	0	0	105	185	2,190
Venustaconcha trabalis	0	66	0	0	4	0	0	10	110	139	0	0	0	70	445	893	258	1,995
Villosa iris	0	4	200	0	1	0	0	0	3,984	1,681	0	701	554	163	268	289	2,279	10,124
Villosa vanuxemensis	0	0	0	0	2,906	0	45	1,441	3,303	2,370	314	0	187	101	824	1,164	1,560	14,215
Total	80	120	250	100	3,283	1,049	2,833	9,379	14,530	14,560	12,655	12,279	4,975	3,387	10,808	19,009	18,277	127,574

Table 1.9. Total mussels >6 months old released by AWCC and FMCC from 2003 to 2014 for the LMPI NRDAR case in the Powell River in Virginia and Tennessee.

Species (11)	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total
Actinonaias pectorosa	0	0	0	0	0	3	0	0	0	0	0	3
Dromus dromas	0	0	0	0	0	0	0	0	0	27	0	27
Epioblasma brevidens	0	0	0	0	5	154	131	649	1,648	1,164	1,075	4,826
Epioblasma capsaeformis	0	0	0	0	0	64	50	1,547	6,790	2,599	348	11,398
Epioblasma triquetra	0	0	0	0	0	0	0	0	257	7	0	264
Lampsilis fasciola	20	0	10	0	113	0	0	342	500	744	0	1,729
Lampsilis ovata	0	0	345	150	600	403	0	16	200	80	0	1,794
Lemiox rimosus	0	0	0	0	0	0	0	0	0	63	0	63
Ligumia recta	0	0	50	50	100	50	0	0	0	0	0	250
Ptychobranchus subtentus	0	0	0	0	0	0	0	0	0	200	0	200
Villosa iris	0	12	125	1,102	50	150	1,227	1,388	0	0	0	4,054
Total	20	12	530	1,302	868	824	1,408	3,942	9,395	4,884	1,423	24,608

Table 1.10. Total mussels of all ages released by AWCC from 2003 to 2019 for the Certus, Inc. and LMPI NRDAR cases in the Clinch and Powell rivers in Virginia and Tennessee.

Species (25)	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Actinonaias ligamentina	0	22,300	0	15,623	0	6,257	0	0	0	0	0	0	0	0	0	0	0	44,180
Actinonaias pectorosa	0	2,613	39,467	12,230	92,051	32,740	23	450	248	9	1	0	0	0	0	0	0	179,832
Cyprogenia stegaria	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	38
Dromus dromas	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	8
Epioblasma aureola	0	2	0	3,000	0	0	0	0	0	0	0	0	0	21	710	0	0	3,733
Epioblasma brevidens	0	0	0	0	0	5	0	1,300	792	992	2,361	5,318	1,584	1,219	3,250	2,745	3,675	23,241
Epioblasma capsaeformis	0	0	0	1,595	3,470	1,220	218	2,736	1,729	1,304	1,793	1,744	503	232	510	566	121	17,741
Epioblasma triquetra	0	0	0	0	0	0	0	40	339	230	1	0	0	0	0	0	10	620
Eurynia dilatata	0	0	0	53	0	0	0	224	0	0	0	337	348	0	0	0	0	962
Fusconaia cor	0	0	0	10	0	0	0	0	0	0	0	131	0	4	0	0	0	145
Lampsilis fasciola	80	70	28,917	21,430	68,182	36,839	526	920	3,676	1,620	2,847	66	202	22	300	348	0	166,045
Lampsilis ovata	0	6,496	14,406	5,689	61,571	26,008	203	894	459	200	263	200	0	0	174	25	14	116,602
Lasmigona costata	0	0	0	0	542	0	0	69	0	3	0	10	0	0	0	0	0	624
Lasmigona holstonia	0	0	0	0	0	0	0	93	878	61	0	0	0	0	0	0	21	1,053
Lemiox rimosus	0	0	0	0	0	0	0	0	19	0	126	0	0	28	25	0	126	324
Ligumia recta	0	0	0	150	173	150	50	46	0	0	0	311	188	421	0	0	0	1,489
Medionidus conradicus	0	0	445	0	0	0	0	0	0	460	101	100	1	234	100	929	0	2,370
Plethobasus cyphyus	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3
Pleuronaia barnesiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99	99
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	100
Ptychobranchus fasciolaris	0	0	0	0	1,542	0	0	0	0	172	265	23	0	0	0	0	0	2,002
Ptychobranchus subtentus	0	0	100	0	0	0	0	223	0	64	0	0	0	0	0	0	185	572
Venustaconcha trabalis	0	187	1,044	0	1,628	1,445	0	5	110	139	0	0	0	70	445	893	258	6,224
Villosa iris	765	11,664	13,356	10,728	26	3,632	150	1,227	1,388	0	0	201	7	50	0	0	0	43,194
Villosa vanuxemensis	0	0	0	0	6,602	4,212	45	1,441	2,909	2,301	2	0	0	81	804	1,164	1,240	20,801
Total	845	43,332	97,735	70,508	235,787	112,508	1,215	9,668	12,547	7,555	7,760	8,541	2,833	2,427	6,322	6,670	5,749	632,002

Table 1.11. Total mussels >6 months old released by AWCC from 2003 to 2019 for the Certus, Inc. and LMPI NRDAR cases in the Clinch and Powell rivers in Virginia and Tennessee.

Species (24)	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Actinonaias pectorosa	0	0	0	0	2	0	23	450	248	9	1	0	0	0	0	0	0	733
Cyprogenia stegaria	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	38
Dromus dromas	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	8
Epioblasma aureola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	710	0	0	710
Epioblasma brevidens	0	0	0	0	0	5	0	1,300	792	992	2,361	5,318	1,584	1,219	3,250	2,745	3,675	23,241
Epioblasma capsaeformis	0	0	0	0	0	7	218	2,736	1,729	1,304	1,793	1,744	503	232	510	566	121	11,463
Epioblasma triquetra	0	0	0	0	0	0	0	40	339	230	1	0	0	0	0	0	10	620
Eurynia dilatata	0	0	0	0	0	0	0	224	0	0	0	337	348	0	0	0	0	909
Fusconaia cor	0	0	0	0	0	0	0	0	0	0	0	131	0	4	0	0	0	135
Lampsilis fasciola	80	70	50	10	184	133	526	920	3,676	1,620	2,847	66	202	22	300	348	0	11,054
Lampsilis ovata	0	0	0	345	213	300	203	894	459	200	263	200	0	0	174	25	14	3,290
Lasmigona costata	0	0	0	0	0	0	0	69	0	3	0	10	0	0	0	0	0	82
Lasmigona holstonia	0	0	0	0	0	0	0	93	878	61	0	0	0	0	0	0	21	1,053
Lemiox rimosus	0	0	0	0	0	0	0	0	19	0	126	0	0	28	25	0	126	324
Ligumia recta	0	0	0	150	173	150	50	46	0	0	0	311	188	421	0	0	0	1,489
Medionidus conradicus	0	0	0	0	0	0	0	0	0	460	101	100	1	234	100	929	0	1,925
Plethobasus cyphyus	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3
Pleuronaia barnesiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99	99
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	100
Ptychobranchus fasciolaris	0	0	0	0	0	0	0	0	0	172	265	23	0	0	0	0	0	460
Ptychobranchus subtentus	0	0	0	0	0	0	0	223	0	64	0	0	0	0	0	0	185	472
Venustaconcha trabalis	0	66	0	0	4	0	0	5	110	139	0	0	0	70	445	893	258	1,990
Villosa iris	0	4	12	125	26	50	150	1,227	1,388	0	0	201	7	50	0	0	0	3,240
Villosa vanuxemensis	0	0	0	0	0	0	45	1,441	2,909	2,301	2	0	0	81	804	1,164	1,240	9,987
Total	80	140	62	630	602	645	1,215	9,668	12,547	7,555	7,760	8,541	2,833	2,406	6,322	6,670	5,749	73,425

Table 1.12. Total mussels >6 months old released by AWCC from 2003 to 2019 for the Certus, Inc. NRDAR case in the Clinch River in Virginia.

Species (24)	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Actinonaias pectorosa	0	0	0	0	2	0	20	450	248	9	1	0	0	0	0	0	0	730
Cyprogenia stegaria	0	0	0	0	0	0	0	0	0	0	0	0	0	38	0	0	0	38
Dromus dromas	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	0	0	8
Epioblasma aureola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	710	0	0	710
Epioblasma brevidens	0	0	0	0	0	0	0	1,300	490	398	1,861	4,243	1,584	1,219	3,250	2,745	3,675	20,765
Epioblasma capsaeformis	0	0	0	0	0	7	186	2,736	1,229	754	1,293	1,396	503	232	510	566	121	9,533
Epioblasma triquetra	0	0	0	0	0	0	0	40	339	0	0	0	0	0	0	0	10	389
Fusconaia cor	0	0	0	0	0	0	0	0	0	0	0	131	0	4	0	0	0	135
Lampsilis fasciola	80	50	50	0	184	20	526	920	3,334	1,120	2,103	66	202	22	300	348	0	9,325
Lampsilis ovata	0	0	0	0	63	0	0	894	443	0	183	200	0	0	174	25	14	1,996
Lasmigona costata	0	0	0	0	0	0	0	69	0	3	0	10	0	0	0	0	0	82
Lasmigona holstonia	0	0	0	0	0	0	0	93	878	61	0	0	0	0	0	0	21	1,053
Lemiox rimosus	0	0	0	0	0	0	0	0	19	0	63	0	0	28	25	0	126	261
Ligumia recta	0	0	0	100	123	50	0	46	0	0	0	311	188	421	0	0	0	1,239
Medionidus conradicus	0	0	0	0	0	0	0	0	0	460	101	100	1	234	100	929	0	1,925
Plethobasus cyphyus	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3
Pleuronaia barnesiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99	99
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	100
Ptychobranchus fasciolaris	0	0	0	0	0	0	0	0	0	172	265	23	0	0	0	0	0	460
Ptychobranchus subtentus	0	0	0	0	0	0	0	223	0	64	0	0	0	0	0	0	185	472
Venustaconcha trabalis	0	66	0	0	4	0	0	5	110	139	0	0	0	70	445	893	258	1,990
Villosa iris	0	4	0	0	1	0	0	0	0	0	0	201	7	50	0	0	0	263
Villosa vanuxemensis	0	0	0	0	0	0	45	1,441	2,909	2,301	2	0	0	81	804	1,164	1,240	9,987
Eurynia dilatata	0	0	0	0	0	0	0	224	0	0	0	337	348	0	0	0	0	909
Total	80	120	50	100	377	77	777	8,441	9,999	5,481	5,872	7,118	2,833	2,406	6,322	6,670	5,749	62,472

Table 1.13. Total mussels >6 months old released by AWCC from 2004 to 2014 for the LMPI NRDAR case in the Powell River in Virginia and Tennessee.

Species (9)	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total
Actinonaias pectorosa	0	0	0	0	0	3	0	0	0	0	0	3
Epioblasma brevidens	0	0	0	0	5	0	0	302	594	500	1,075	2,476
Epioblasma capsaeformis	0	0	0	0	0	32	0	500	550	500	348	1,930
Epioblasma triquetra	0	0	0	0	0	0	0	0	230	1	0	231
Lampsilis fasciola	20	0	10	0	113	0	0	342	500	744	0	1,729
Lampsilis ovata	0	0	345	150	300	203	0	16	200	80	0	1,294
Lemiox rimosus	0	0	0	0	0	0	0	0	0	63	0	63
Ligumia recta	0	0	50	50	100	50	0	0	0	0	0	250
Villosa iris	0	12	125	25	50	150	1,227	1,388	0	0	0	2,977
Total	20	12	530	225	568	438	1,227	2,548	2,074	1,888	1,423	10,953

Table 1.14. Total mussels of all ages released by FMCC from 2004 to 2019 for the Certus, Inc. and LMPI NRDAR cases in the Clinch and Powell rivers in Virginia and Tennessee.

Species (15)	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Dromus dromas	101	0	6	0	0	0	0	0	0	27	0	0	0	0	0	0	134
Epioblasma aureola	5,568	3,054	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8,622
Epioblasma brevidens	2,372	2,386	36,596	0	41	154	161	347	1,108	2,158	107	0	5	2,288	8,800	3,075	59,598
Epioblasma capsaeformis	11,637	2,463	35,240	178	742	56	50	1,107	10,066	6,549	2,208	0	627	230	0	4,342	75,495
Epioblasma triquetra	0	0	0	0	0	0	0	0	27	6	0	0	201	68	1,348	151	1,801
Lampsilis fasciola	0	10,889	0	0	0	0	9	3	2,500	134	77	7	0	1,404	251	676	15,950
Lampsilis ovata	0	6,635	100	100	602	1,400	894	15	0	0	0	0	0	0	396	1,655	11,797
Lemiox rimosus	67	0	0	0	0	0	0	0	0	0	0	0	11	0	0	22	100
Medionidus conradicus	0	0	0	0	0	0	0	75	4	50	1,462	49	3	0	1,149	8	2,800
Pleuronaia barnesiana	0	58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	58
Ptychobranchus fasciolaris	0	0	5	0	0	0	0	0	945	293	22	766	1	208	1	0	2,241
Ptychobranchus subtentus	0	1,397	72	0	0	0	0	0	0	250	785	586	0	0	105	0	3,195
Venustaconcha trabalis	2,102	938	0	428	0	0	5	0	0	0	0	0	0	0	0	0	3,473
Villosa iris	0	8,432	0	1,177	0	0	0	3,984	1,681	0	500	547	113	268	289	2,279	19,270
Villosa vanuxemensis	0	3,279	0	2,906	0	0	0	394	69	312	0	187	20	20	0	320	7,507
Total	21,847	39,531	72,019	4,789	1,385	1,610	1,119	5,925	16,400	9,779	5,161	2,142	981	4,486	12,339	12,528	212,041

Table 1.15. Total mussels >6 months old released by FMCC from 2005 to 2019 for the Certus, Inc. and LMPI NRDAR cases in the Clinch and Powell rivers in Virginia and Tennessee.

Species (13)	2005	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Dromus dromas	0	0	0	0	0	0	0	27	0	0	0	0	0	0	27
Epioblasma brevidens	0	0	0	154	161	347	1,108	2,158	107	0	5	2,288	8,800	3,075	18,203
Epioblasma capsaeformis	0	0	0	56	50	1,107	10,066	6,549	2,208	0	627	230	0	4,342	25,235
Epioblasma triquetra	0	0	0	0	0	0	27	6	0	0	201	68	1,348	151	1,801
Lampsilis fasciola	0	0	0	0	9	3	2,500	134	77	7	0	1,404	251	676	5,061
Lampsilis ovata	0	0	602	1,400	894	15	0	0	0	0	0	0	396	1,655	4,962
Lemiox rimosus	0	0	0	0	0	0	0	0	0	0	11	0	0	22	33
Medionidus conradicus	0	0	0	0	0	75	4	50	1,462	49	3	0	1,149	8	2,800
Ptychobranchus fasciolaris	0	0	0	0	0	0	945	293	22	766	1	208	1	0	2,236
Ptychobranchus subtentus	0	0	0	0	0	0	0	250	785	586	0	0	105	0	1,726
Venustaconcha trabalis	0	0	0	0	5	0	0	0	0	0	0	0	0	0	5
Villosa iris	200	1,077	0	0	0	3,984	1,681	0	500	547	113	268	289	2,279	10,938
Villosa vanuxemensis	0	2,906	0	0	0	394	69	312	0	187	20	20	0	320	4,228
Total	200	3,983	602	1,610	1,119	5,925	16,400	9,779	5,161	2,142	981	4,486	12,339	12,528	77,255

Table 1.16. Total mussels >6 months old released by FMCC from 2005 to 2019 for the Certus, Inc. NRDAR case in the Clinch River in Virginia.

Species (12)	2005	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Epioblasma brevidens	0	0	0	0	30	0	54	1,494	107	0	5	2,288	8,800	3,075	15,853
Epioblasma capsaeformis	0	0	0	24	0	60	3,826	4,450	2,208	0	627	230	0	4,342	15,767
Epioblasma triquetra	0	0	0	0	0	0	0	0	0	0	201	68	1,348	151	1,768
Lampsilis fasciola	0	0	0	0	9	3	2,500	134	77	7	0	1,404	251	676	5,061
Lampsilis ovata	0	0	302	1,200	894	15	0	0	0	0	0	0	396	1,655	4,462
Lemiox rimosus	0	0	0	0	0	0	0	0	0	0	11	0	0	22	33
Medionidus conradicus	0	0	0	0	0	75	4	50	1,462	49	3	0	1,149	8	2,800
Ptychobranchus fasciolaris	0	0	0	0	0	0	945	293	22	766	1	208	1	0	2,236
Ptychobranchus subtentus	0	0	0	0	0	0	0	50	785	586	0	0	105	0	1,526
Venustaconcha trabalis	0	0	0	0	5	0	0	0	0	0	0	0	0	0	5
Villosa iris	200	0	0	0	0	3,984	1,681	0	500	547	113	268	289	2,279	9,861
Villosa vanuxemensis	0	2,906	0	0	0	394	69	312	0	187	20	20	0	320	4,228
Total	200	2,906	302	1,224	938	4,531	9,079	6,783	5,161	2,142	981	4,486	12,339	12,528	63,600

Table 1.17. Total mussels >6 months old released by FMCC from 2007 to 2013 for the LMPI NRDAR case in the Powell River in Tennessee.

Species (7)	2007	2008	2009	2010	2011	2012	2013	Total
Dromus dromas	0	0	0	0	0	0	27	27
Epioblasma brevidens	0	0	154	131	347	1,054	664	2,350
Epioblasma capsaeformis	0	0	32	50	1,047	6,240	2,099	9,468
Epioblasma triquetra	0	0	0	0	0	27	6	33
Lampsilis ovata	0	300	200	0	0	0	0	500
Ptychobranchus subtentus	0	0	0	0	0	0	200	200
Villosa iris	1,077	0	0	0	0	0	0	1,077
Total	1,077	300	386	181	1,394	7,321	2,996	13,655

Table 1.18. Total mussels released >6 months old by AWCC and FMCC for the Certus, Inc. and LMPI NRDAR cases at each population restoration and monitoring site in the Clinch and Powell rivers, TN and VA, from 2004 to 2019. *An additional 410 individuals of *Epioblasma aureola* were released at several locations in Indian Creek, Cedar Bluff, VA, which are not included in the table. RDC = right descending channel and LDC = left descending channel. *Other sites include Nash Ford, Island at old Cleveland Elementary School, and releases from Nash Ford to Artrip. Site localities are given in Table 1.2

.	Clinch River, VA									Powell River, VA					
Species (24)	**Perry Property**	**Payne Property**	**Sycamore Lane**	**Bennett Property**	**Artrip**	**Whited Property**	**Cleveland Islands, LDC**	**Cleveland Islands, RDC**	**Other Sites**	**Rt. 833 Bridge**	**Fletcher Ford**	**Buchannan Ford**	**Upper Brooks Bridge**	**Lower Brooks Bridge**	**Oakley Property**
Actinonaias pectorosa	0	521	0	10	0	0	0	0	0	3	0	0	0	0	0
Cyprogenia stegaria	0	0	0	38	0	0	0	0	0	0	0	0	0	0	0
Dromus dromas	0	0	0	4	0	0	0	0	0	0	0	0	1	26	0
Epioblasma aureola	0	0	300	0	0	0	0	0	0	0	0	0	0	0	0
Epioblasma brevidens	0	0	0	11,979	5,131	0	4,194	3,587	5,732	0	2,204	18	1,194	1,120	18
Epioblasma capsaeformis	0	0	0	10,013	1,801	1,028	3,775	3,757	717	0	1,680	1,979	2,883	3,337	1,187
Epioblasma triquetra	0	0	0	1,764	0	0	272	0	0	0	231	0	33	0	0
Eurynia dilatata	0	371	0	356	119	0	0	0	0	0	0	0	0	0	0
Fusconaia cor	0	0	0	68	67	0	0	0	0	0	0	0	0	0	0
Lampsilis fasciola	100	3,135	3,317	1,686	1,243	200	1,284	0	1,465	143	1,086	0	0	0	0
Lampsilis ovata	0	1,355	3,175	265	300	0	677	0	75	400	683	0	0	0	0
Lasmigona costata	0	69	0	10	0	0	0	0	0	0	0	0	0	0	0
Lasmigona holstonia	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0
Lemiox rimosus	0	0	0	133	50	0	63	0	0	0	63	0	0	0	0
Ligumia recta	0	0	0	467	311	0	273	0	0	50	150	0	0	0	0
Medionidus conradicus	0	678	3,250	142	0	0	0	0	100	0	0	0	0	0	0
Plethobasus cyphyus	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Pleuronaia barnesiana	0	0	99	0	0	0	0	0	0	0	0	0	0	0	0
Pleuronaia dolabelloides	0	0	0	50	50	0	0	0	0	0	0	0	0	0	0
Ptychobranchus fasciolaris	0	741	1,584	196	0	0	0	0	0	0	0	0	0	0	0
Ptychobranchus subtentus	0	356	1,686	89	0	0	0	0	0	0	0	0	100	100	0
Venustaconcha trabalis	66	20	193	295	300	0	359	0	310	0	0	0	0	0	0
Villosa iris	204	3,699	5,963	0	0	0	0	0	0	1,110	1,867	0	0	0	0
Villosa vanuxemensis	0	4,369	1,829	970	1,694	69	1,344	0	879	0	0	0	0	0	0
Grand Total	370	15,314	21,417	28,538	11,066	1,297	12,241	7,344	9,278	1,706	7,964	1,997	4,211	4,583	1,205

Table 1.19. Percentage of mussels propagated at AWCC >6 months old released at restoration and monitoring sites from 2010 to 2019 for the Certus, Inc. and LMPI NRDAR cases. Total number (No.) of released mussels does not match previous tables as these do not include >6 month old mussels released before 2010. Mussel releases from other projects and the Certus, Inc. and LMPI NRDAR cases at AWCC are included under All Projects. Number of released mussels does not include mussels <6 months old.

		Certus and LMPI		All Projects	
Species (30)	No. Produced	No. Released	% Survival	No. Released	% Survival
Actinonaias pectorosa	88,958	708	0.8%	708	0.8%
Alasmidonta viridis	5,623	0	0.0%	82	1.5%
Cyprogenia stegaria	6,467	38	0.6%	129	2.0%
Dromus dromas	55,069	8	0.0%	21	0.0%
Epioblasma aureola	4,574	710	15.5%	710	15.5%
Epioblasma brevidens	482,472	23,236	4.8%	46,165	9.6%
Epioblasma capsaeformis	233,767	11,238	4.8%	23,246	9.9%
Epioblasma triquetra	19,764	620	3.1%	1,580	8.0%
Eurynia dilatata	7,069	909	12.9%	909	12.9%
Fusconaia cor	2,282	135	5.9%	273	12.0%
Fusconaia cuneolus	698	0	0.0%	29	4.2%
Hemistena lata	148	0	0.0%	0	0.0%
Lampsilis abrupta	427,172	0	0.0%	7,887	1.8%
Lampsilis fasciola	588,147	10,001	1.7%	14,649	2.5%
Lampsilis ovata	191,698	2,229	1.2%	3,323	1.7%
Lasmigona costata	66,390	82	0.1%	82	0.1%
Lasmigona holstonia	137,940	1,053	0.8%	3,334	2.4%
Lemiox rimosus	73,754	324	0.4%	1,418	1.9%
Ligumia recta	74,968	966	1.3%	1,969	2.6%
Medionidus conradicus	25,052	1,925	7.7%	3,059	12.2%
Plethobasus cyphyus	523	3	0.6%	3	0.6%
Pleuronaia barnesiana	1,171	99	8.5%	99	8.5%
Pleuronaia dolabelloides	457	100	21.9%	100	21.9%
Ptychobranchus fasciolaris	3,556	460	12.9%	460	12.9%
Ptychobranchus subtentus	16,888	472	2.8%	1,134	6.7%
Strophitus undulatus	5,617	0	0.0%	39	0.7%
Theliderma cylindrica	588	0	0.0%	0	0.0%
Venustaconcha trabalis	97,857	1,920	2.0%	3,772	3.9%
Villosa iris	49,519	2,873	5.8%	4,475	9.0%
Villosa vanuxemensis	88,339	9,942	11.3%	12,775	14.5%
Total	2,756,527	70,051	2.5%	132,430	4.8%

Table 1.20. Percentage of mussels propagated at FMCC >6 months old released at restoration and monitoring sites from 2010 to 2019 for the Certus, Inc. and LMPI NRDAR cases. Total number (No.) of released mussels does not match previous tables as these do not include >6 month old mussels released before 2010. Mussel releases from other projects and the Certus, Inc. and LMPI NRDAR cases at FMCC are included under All Projects. Number of released mussels does not include mussels <6 months old.

		Certus and LMPI		All Projects	
Species (16)	No. Produced	No. Released	% Survival	No. Released	% Survival
Cyprogenia stegaria	1,898	0	0.0%	0	0.0%
Dromus dromas	11,884	27	0.2%	27	0.2%
Epioblasma brevidens	250,176	18,049	7.2%	19,006	7.6%
Epioblasma capsaeformis	312,638	25,179	8.1%	30,852	9.9%
Epioblasma triquetra	19,150	1,801	9.4%	1,901	9.9%
Lampsilis fasciola	187,695	5,061	2.7%	5,852	3.1%
Lampsilis ovata	32,626	2,960	9.1%	2,960	9.1%
Lemiox rimosus	8,309	33	0.4%	33	0.4%
Ligumia recta	295	0	0.0%	0	0.0%
Medionidus conradicus	27,965	2,800	10.0%	2,800	10.0%
Potamilus alatus	7,634	0	0.0%	0	0.0%
Ptychobranchus fasciolaris	59,605	2,236	3.8%	2,236	3.8%
Ptychobranchus subtentus	55,757	1,726	3.1%	1,726	3.1%
Venustaconcha trabalis	10,869	5	0.0%	235	2.2%
Villosa iris	272,946	9,661	3.5%	9,977	3.7%
Villosa vanuxemensis	29,136	1,322	4.5%	1,322	4.5%
Total	1,288,583	70,860	5.5%	78,927	6.1%

Figures

(a) Spill of Octocure-554 Revised turned the Clinch River milky-white for 7 river miles from Cedar Bluff, VA, downstream to Richlands, VA.

(b) U.S. Fish and Wildlife Biologist Leroy Koch examining mussels killed during the Certus, Inc. chemical spill.

Figure 1.1. Photographs of the Certus, Inc. chemical spill that occurred in the Clinch River at Cedar Bluff, VA, on August 27, 1998.

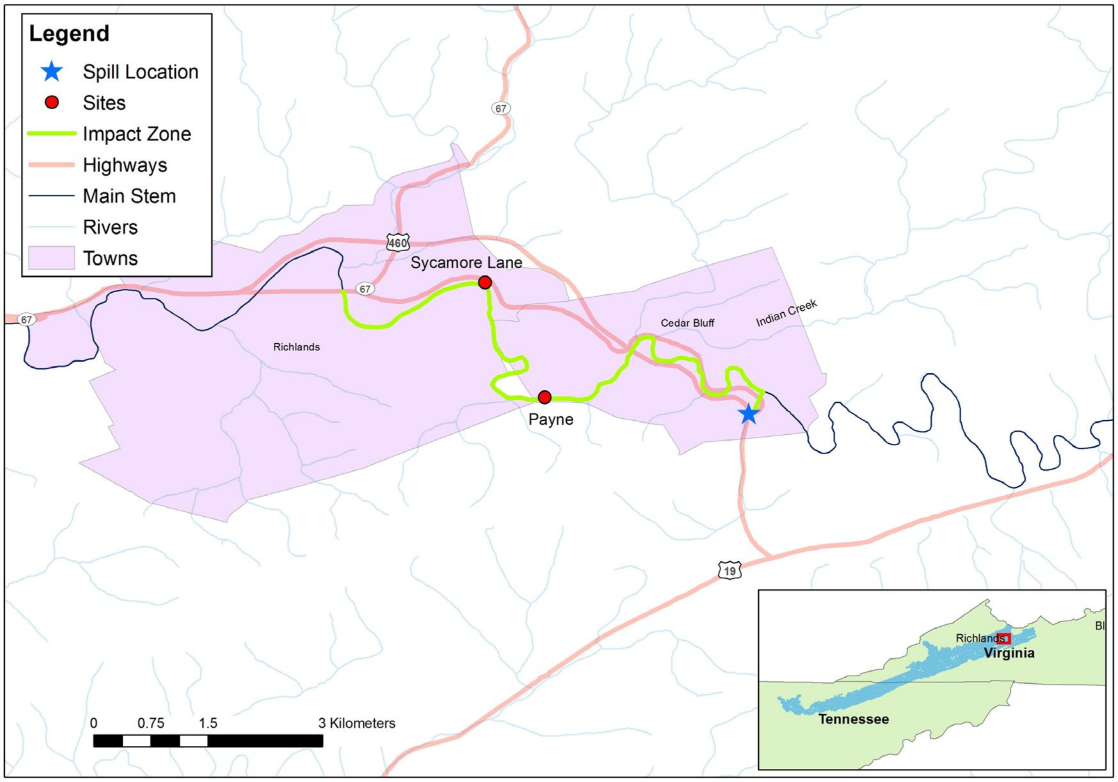
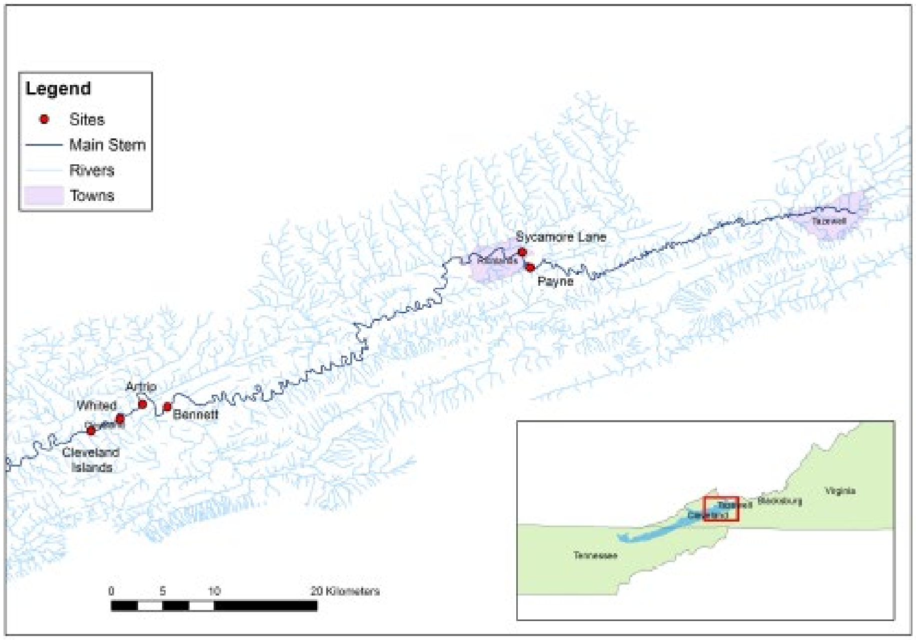
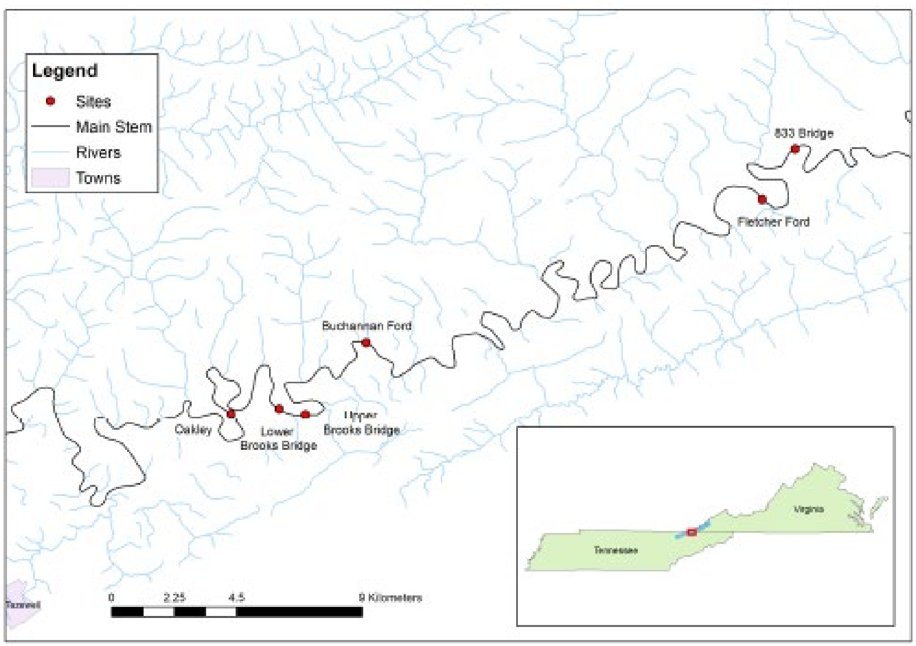


Figure 1.2. Impact zone of the Certus, Inc. chemical spill in the Clinch River, Tazewell County, Virginia on August 27, 1998. Blue star indicates location of chemical spill.



(a) Clinch River sites in Virginia.



(b) Powell River sites in Tennessee and Virginia.

Figure 1.3. Locations of restoration and monitoring sites in the Clinch and Powell Rivers for the Certus, Inc. and LMPI NRDAR cases.

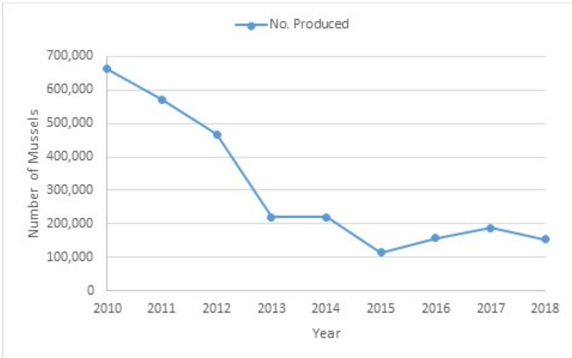
(a) *Epioblasma capsaeformis* released in Powell River, September 24, 2012.

(b) *Epioblasma triquetra* released at the Bennett Property in the Clinch River, VA.

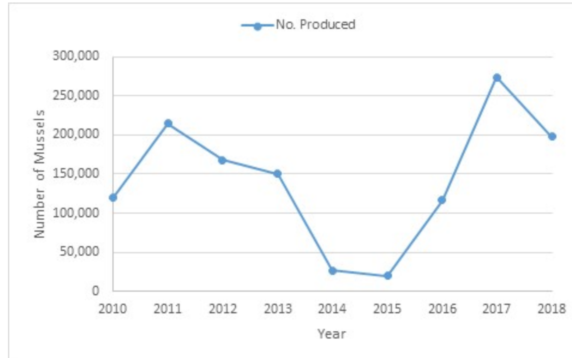
(c) *Lemiox rimosus* released at the Bennett Property in the Clinch River, VA.

(d) Release of *Ptychobranchus subtentus*, *Ptychobranchus fasciolaris*, *Villosa vanuxemensis* and *Lampsilis fasciola* in the Clinch River, VA, at Sycamore Lane, on September 26, 2014.

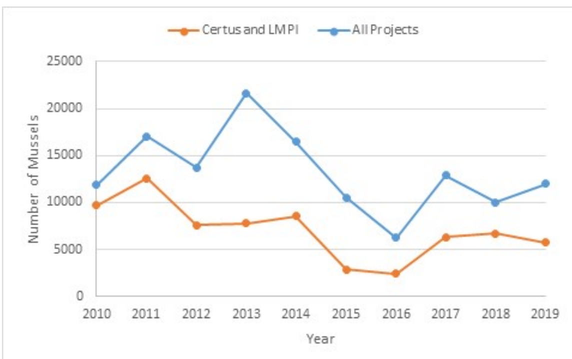
Figure 1.4. Photographs of juvenile mussels that were released for the Certus, Inc. and LMPI NRDAR cases in the Clinch and Powell rivers in Virginia and Tennessee.



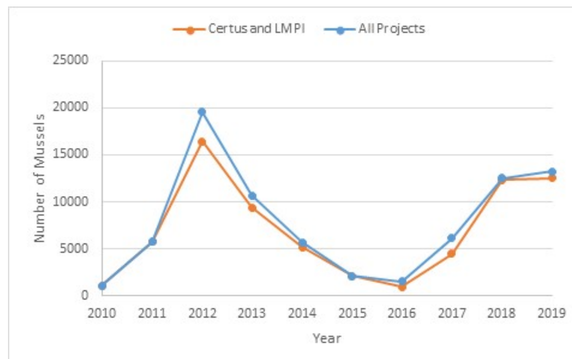
(a) AWCC Production



(b) FMCC Production



(c) AWCC Releases



(d) FMCC Releases

Figure 1.5. Numbers (No.) of mussels produced and numbers of >6 months old mussels released by AWCC (a and c) and FMCC (b and d) from 2010 to 2019.

Chapter 2

Monitoring Mussel Populations at Restoration Sites in the Clinch and Powell Rivers for the Certus, Inc. and Lone Mountain Processing, Inc. NRDAR Cases

Abstract

The Certus, Inc. and Lone Mountain Processing, Inc. Natural Resource Damage Assessment and Restoration (NRDAR) cases in the upper Tennessee River basin of Virginia are among the first and largest cases involving injury to freshwater mussels due to release of hazardous substances in the United States. The Certus, Inc. spill in 1998 released 1,350 gallons of Octocure-554 revised (a rubber accelerant) into the upper Clinch River, killing approximately 18,000 mussels, including individuals of three endangered species. The Lone Mountain Processing, Inc. spill occurred in the Powell River in 1996 and released 6,000,000 gallons of coal slurry, affecting mussels over a 65-mile section of river. Settlement money from these two cases was used to propagate and release mussels at population restoration sites in the upper Clinch River, VA and in the Powell River, TN and VA. We used mussel release data from Chapter 1 and a Leslie matrix model to estimate the expected mussel survival and abundance at two sites (Payne Property and Sycamore Lane) in the immediate impact zone of the Certus, Inc. spill. We compared the expected numbers of released mussels from 2004–2017 to density estimates from quadrat surveys and mark-recapture surveys at the same two sites from 2015 to 2017. Estimated mussel densities at these two monitoring sites in the Certus, Inc. spill impact zone were lower than expected based on number of mussels released and their expected annual survival and recruitment. Possible reasons for this lesser number include lower-than-expected survival of mussels at these sites, dispersal of released mussels downstream of the immediate release and monitoring areas, newly transformed juvenile's excysting from host fish outside the sites, or downward sampling bias. We also estimated densities at seven other monitoring sites from 2015 to 2017 using quadrat surveys. In all years, mussel population density was highest in the Clinch River at the Bennett Property and lowest at the Payne Property and Sycamore Lane. In the Powell River, density was highest at Lower Brooks Bridge in 2015 and 2016, and highest at the Oakley Property in 2017. Regardless, restoration efforts for the Certus, Inc. and LMPI NRDAR cases were successful in that species impacted by both spills have been restored to multiple sites in each river, including the endangered Golden Riffleshell (*Epioblasma aureola*) and Tennessee Bean (*Villosa trabalis*), and that populations of other *Epioblasma* species and numerous non-endangered species have been established ~40 miles downstream in the Clinch River in Russell County, VA, and in the Powell River in Claiborne County, TN, and Lee County, VA.

Introduction

Freshwater mussels (Unionidae) are among the most imperiled groups of freshwater organisms in North America (Vaughn and Taylor 1999). Of the approximately 300 recognized species, 88 are listed as federally endangered and 15 are listed as federally threatened under the Endangered Species Act (U.S. Fish and Wildlife Service 2018). Habitat alteration, especially river impoundment and channelization, is the leading cause of mussel decline in North America (Vaughn, Nichols, and Spooner 2008). Also included under habitat alteration are water pollution and water quality degradation (Downing, Van Meter, and Woolnough 2010). Due to their sessile nature, mussels are highly susceptible to releases of hazardous substances into the aquatic environment. Releases of contaminants into rivers can drastically reduce the diversity and abundance of local populations of freshwater mussels (Sheehan, Neves, and Kitchel 1989). Further, the limited dispersal capabilities of mussels make natural recolonization difficult and unlikely in the short term (~10–20 years).

The Certus, Inc. and Lone Mountain Processing, Inc. (LMPI) Natural Resource Damage Assessment and Restoration (NRDAR) cases in the upper Tennessee River basin of Virginia are among the first and largest cases involving injury to freshwater mussels due to release of hazardous substances in the United States. The Certus, Inc. chemical spill released 1,350 gallons of Octocure-554 revised, a rubber accelerant, into a tributary of the Clinch River when a tanker truck overturned in Tazewell County, VA, on August 27, 1998. An estimated 18,621 mussels, including 750 individuals of three endangered species (Golden Riffleshell, Tennessee Bean, and Rough Rabbitsfoot), were killed along a seven-mile section of stream as a result of the Certus incident.

The LMPI coal slurry spill was the result of a coal slurry holding pond failure at a processing plant in Lee County, VA, on October 24, 1996. The spill released 6,000,000 gallons of coal slurry into a series of tributaries of the Powell River. The resulting "blackwater" impacted a large section of the Powell River, and coal fines and sediment ultimately were deposited in Norris Reservoir, TN, 65 miles downstream from the release. While no dead mussels were found, coal fines later were detected in mussel gut tissues (U.S. Fish and Wildlife Service 2003). Additionally, at least 11,240 fish of various species, some of which are host fishes for the 15 federally endangered mussel species found in the impacted river reach, were killed. Coal fines and sediment also were deposited in the substrate throughout the affected length of the Powell River and likely continued to have chronic, sub-lethal impacts due to resuspension during high flow events in 1996 and into 1997. In contrast to the acute, lethal effects of the Certus, Inc. spill, the LMPI spill represented a chronic, sub-lethal effect on the mussel fauna in the impacted river reach.

The principle restoration goal for each case was "to restore the mussel assemblage and its supporting habitats to approximate baseline conditions" (U.S. Fish and Wildlife Service 2003; U.S. Fish and Wildlife Service 2004). Baseline condition for the Certus, Inc. NRDAR case was the estimated number of mussels (18,621) and respective species composition present in the impact zone before the spill. Consequently, many mussels released as part of restoration were at sites in the immediate impact zone of the Clinch River between Cedar Bluff, VA (RM 324) and Richlands, VA (RM 318). However, mussels also were released downstream at other reinforcement sites in the Clinch River in Russell County, VA (RM 270–277.5), to reduce the risk that released mussels would all be impacted by another single, catastrophic event or degradation of habitat in the urban areas of Cedar Bluff and Richlands. Further, the ability to propagate some affected species was limited. Notably, *Epioblasma capsaeformis* and *E. brevidens* were used as surrogates for the critically endangered *E. aureola* due to the greater availability of broodstock and ease of propagating these two species at mussel hatcheries. Specifically, these two *Epioblasma* species were used as surrogates to develop propagation, culture, and monitoring techniques for *E. aureola*. Baseline condition of the mussel assemblage was not quantified for the LMPI NRDAR case; however, the goal was to propagate a selected suite of the federally listed mussel species affected by the spill in the Powell River. Not all federally listed mussel species impacted by the spill were able to be propagated and restored due to technological limitations (e.g., undeveloped propagation techniques, such as unknown host fishes), thus restoration efforts in this case mainly focused on releasing *E. capsaeformis* and *E. brevidens*, as well as numerous non-endangered species, at sites in the Powell River to establish robust populations of these species and to restore their local populations and respective ecosystem services.

The objectives of this chapter were to: 1) estimate the expected number of mussels surviving at monitoring sites in the Clinch and Powell rivers based on mussels released per site from 2004—2017 using a Leslie matrix model, 2) estimate abundance and density using data from quadrat and mark-recapture surveys, 3) determine whether estimated abundance and density were higher or lower than expected at restoration sites, and 4) determine whether restoration goals were achieved for each case.

Methods

Study area

Mussels were released at six monitoring sites in the Clinch River (Figure 2.1) and three monitoring sites in the Powell River (Figure 2.2). In the Clinch River, the Bennett Property in Russell County had the highest number of released mussels >6 months old (28,538), most of which were *Epioblasma capsaeformis* and *E. brevidens* (Table 1.18). The Payne Property (15,314) and Sycamore Lane (21,417) in Tazewell County had the next highest number of released mussels. Both sites were in the immediate impact zone of the Certus, Inc. spill. At Artrip, a total of 11,066 mussels were released. A total of 1,297 were released at the Whited Property and 7,344 in the right-descending channel of Cleveland Islands, most of which were *E. capsaeformis*. In the Powell River, the majority of releases were at the Upper (4,211) and Lower Brooks Bridge (4,583) sites, although 1,205 mussels also were released at the Oakley Property. Almost all releases at the Powell sites were either *E. capsaeformis* or *E. brevidens*.

These nine monitoring sites in the Clinch and Powell Rivers were sampled from 2015 to 2017 (Table 2.1). Six sites were located in the Clinch River and three sites in the Powell River. We began monitoring the Oakley Property in the Powell River, TN in 2016, while the other eight sites were sampled during all three years. Local populations at two of the sites in the Clinch River, the Sycamore Lane site near Richlands, VA (RM 320), and the Payne Property site near Cedar Bluff, VA (RM 322.1), were impacted directly in 1998 by the Certus, Inc. chemical spill (Figure 2.3). The remaining Clinch River sites further downstream in Russell County were not directly impacted by the spill but were used as additional restoration sites for the Certus, Inc. NRDAR project to help reduce risk for potential impacts in the future to the two restoration sites located in the Certus, Inc. impact zone between Cedar Bluff and Richlands, VA. The Powell River monitoring sites were within the area affected by the LMPI coal slurry spill and were located in Claiborne County, TN, and Lee County, VA (RM 89.7–95.3).

Quadrat sampling

We used a systematic quadrat sampling design at all nine sites, where the location of the first quadrat of each systematic sample was determined randomly (Strayer and Smith 2003). All subsequent quadrats for each systematic sample were determined based on the first quadrat. A quadrat size of 0.25 m^2 was used because it is generally more accurate and precise than 1.0 m^2 quadrats when used to estimate abundance (Pooler and Smith 2005). We used three to four random starts (i.e., three to four systematic samples) at each site. The number of quadrats sampled at each site in 2015 depended on the expected density of mussel species and the desired level of precision. We determined expected densities using 2004–2014 mussel release data from the Freshwater Mollusk Conservation Center (FMCC) and the

Aquatic Wildlife Conservation Center (AWCC). A 95% annual survival rate was applied to each cohort to estimate the population density of each species released at each site (Jones, Neves, and Hallerman 2012). Recruitment from released mussels in the wild was assumed to be zero because released mussels were sub-adults. Assuming no recruitment also ensured that sufficient quadrats were sampled the first year because the density estimate was lower than if we had assumed recruitment (lower densities require more quadrats). We used the formula of Strayer, Claypool, and Sprague (1997) to determine the number of quadrats needed to achieve a given level of precision:

$$n = 2.6m^{-0.51}CV^{-1.82}$$

where *n* is the number of quadrats, *m* is the mean number of mussels expected per quadrat, and *CV* is the desired coefficient of variation (standard error/mean) (i.e., level of precision). We calculated *n* starting with the most common species at each site and added less common species until the number of quadrats became too high (e.g., >400 per site) to reasonably sample. These data were used to determine the target number of quadrats at each site in 2015. For 2016 and 2017, we used actual density estimates from 2015 quadrat sampling, rather than estimates based on past releases, to determine the target number of quadrats.

The distance between quadrats varied among sites and was determined using the formula:

$$d = \sqrt{\frac{L * W}{n/k}}$$

where *L* is the total length of a site, *W* is the mean width of a site, *n* is the target number of quadrats to be sampled, and *k* is the number of random starts (Strayer and Smith 2003). The distance between quadrats determined the size of the start area where the first quadrat for each systematic sample was placed. For example, a distance of 8 m resulted in an 8 x 8 m start area, and each random start was randomly placed in this box. Random starts at each site were determined using the RAND() function in Microsoft Excel 2015.

The upper and lower boundaries of each site were determined based on the location of past mussel releases and suitable habitat. River width was measured at 10-meter intervals along the length of each site using a laser rangefinder with 0.5 m precision. Area in each segment was calculated and used to convert population size estimates to densities per m^2 (See Appendix C for Google Earth photographic images of sites). These measurements also were used to calculate the distance between quadrats using the above formula.

The initial quadrat for each random start was placed, and then all subsequent quadrats were spaced at even intervals along a transect perpendicular to stream flow. The distance between each transect along the stream was the same as the interval between quadrats. Any distance between the last quadrat on a transect and the stream bank was subtracted from the distance between the bank and the first quadrat on the next transect. For example, an interval of 8 m would result in a distance of 8 m between each quadrat within a transect and a distance of 8 m between each transect. If there were 5 m between the last quadrat of one transect and the stream bank, the first quadrat on the next transect would be 3 m from the bank. Quadrats were excavated to an approximate depth of 20 cm or until bedrock or hardpan was reached. Mussels found in each quadrat were identified to species, identified as male/female (for dimorphic species), and measured (length only). Any mussels visible on the surface were recorded as “surface” while mussels not visible were recorded as “sub-surface”. The tag color and number, if any, also were recorded.

We used the data from the quadrat surveys to estimate abundance of each species by multiplying the mean number of individuals found in a systematic sample by the total number of possible systematic samples. Density was determined by dividing abundance by the area of the site sampled. We calculated 95% confidence intervals for abundance using the formula:

$$\exp\left(\log(\hat{N}) \pm 3.1825\sqrt{\frac{var(\hat{N})}{\hat{N}^2}}\right)$$

where $\hat{N}$ is the estimate of abundance and $var(\hat{N})$ is the estimate of the variance of the abundance estimate (Smith, Villella, and Lemarié 2001). The variance of the abundance estimate was calculated using the formula:

$$\widehat{var}(\hat{N}) = \frac{M(M-m)}{m} \times \frac{\sum_{i=1}^{m}(x_i - \bar{x})^2}{m-1}$$

where M is the number of possible systematic samples, m is the number of random starts, $\bar{x}$ is the mean number of mussels per systematic sample, and x_i is the number of mussels in random start i (Smith, Villella, and Lemarié 2001). Variance for density can be calculated by dividing $var(\hat{N})$ by the squared area. The same calculations were performed on the subset of mussels that were found on the surface of the substrate for comparison to mark recapture estimates.

Mark-recapture sampling

Because Sycamore Lane and Payne Property were in the impact zone of the Certus, Inc. chemical spill we decided to use an additional sampling method to independently estimate abundance and density. Thus, we used a mark-recapture approach at these two sites in addition to the quadrat sampling.

We used a robust design, mark-recapture framework (Pollock 1982) to sample the Sycamore Lane and Payne Property sites in the Clinch River during the late summer/early fall from 2015 to 2017. Each year's sampling represented a single primary period under the robust design framework. The population is assumed to be open to changes due to births, deaths, immigration, or emigration between primary periods (i.e., years). Each primary period consisted of two secondary sampling days as close to each other as possible (usually consecutive), when the population is assumed to be closed to changes due to births, deaths, immigration, or emigration between secondary periods (i.e., within each primary period). Each site was divided into 20-m wide transects oriented perpendicular to stream flow. Transects were divided into 1-m-wide lanes oriented parallel to flow to ensure full spatial coverage of the site. Each lane was sampled visually by snorkeling from the downstream to upstream end. In areas too shallow to snorkel, viewscopes or slowly walking through transect areas and visually inspecting for mussels were used instead. Substrate was not excavated during sampling. Each individual mussel was identified to species, sexed (for dimorphic species), and measured (length). We also noted the collector of each mussel. Mussels already tagged had their tag number and tag color recorded. Any untagged mussels were tagged using Hallprint® glue-on shellfish tags and cyanoacrylate glue. After processing, mussels were returned to the location from which they were sampled.

A set of eight candidate models was developed for estimating abundance. These models contained the following parameters:

S_i = Apparent survival during primary period *i*

γ′ = probability of not being available for capture during primary period *i*, given that an individual was not available for capture during primary period

$i - 1$ (i.e., the probability of not immigrating back into study area)
γ'' = probability of not being available for capture during primary period i, given that an individual was available for capture during period $i - 1$ (i.e., the probability of temporarily emigrating)
p_{ij} = probability of being captured during secondary sampling occasion j of primary period i
c_{ij} = probability of being recaptured during secondary sampling occasion j of primary period i

All models assumed that capture probability was constant within a primary period (i.e., across the 2 secondary surveys), but could vary from one primary period to another {i.e., $(p_{11} = p_{12})$ 6= $(p_{21} = p_{22})$}. Temporary emigration was assumed to be constant and random {i.e., $\gamma'(.) = \gamma''(.)$}.

We created various *a priori* models as follows: Model 1 was the most general model, allowing both initial capture (*p*) and recapture (*c*) probabilities to vary with time between primary periods (interval between primary sampling period) and not be equal to each other between secondary sampling occasions within each primary period (i.e., a behavior response to being captured initially). Model 2 still allowed capture and recapture probabilities to vary with time (interval between primary sampling periods) but they were equal between secondary sampling occasions within each primary period (i.e., no behavior response). Capture and recapture were constant between primary sampling periods in models 3 and 4, but model 3 had no behavior response while model 4 had a behavior response. Survival varied with time between primary periods for all four models. Models 5 – 8 were equivalent to models 1 – 4 except that survival was constant.

We analyzed our candidate model set using Program MARK (White and Burnham 1999) to determine the model with the highest likelihood (Villella, Smith, and Lemarié 2004; Meador, Peterson, and Wisniewski 2011). Likelihood estimates were based on Akaike's Information Criterion (AIC) (Akaike 1973) modified for small sample sizes (AICc) (Sugiura 1978):

$$AIC_c = -2\log\left(L(\hat{\theta})\right) + \frac{2K(K+1)}{n-K-1}$$

where $L(\hat{\vartheta})$ is the likelihood of the parameter estimates, given the data, K is the number of parameters, and n is the sample size. We considered the best model as the one with the lowest AIC score and models were considered competing if ΔAIC <2.0. To estimate the abundance of both the total mussel assemblage and the population of *Villosa iris* at the Payne Property, we used the top model in each case.

Due to low recapture rates, we were not able to use the robust design model to estimate abundance at Sycamore Lane although it was used to estimate abundance for both *V. iris* and the total mussel assemblage at the Payne Property. Therefore, at the Sycamore Lane site, we used the modified Lincoln-Petersen estimator (also known as the Chapman Estimator) to estimate mussel abundance. The formula used was:

$$\hat{N} = \frac{(n_1+1)(n_2+1)}{(m_2+1)} + 1$$

where $\hat{N}$ is the estimated abundance, n_1 is the number of individuals caught on the first occasion, n_2 is the number caught on the second, and m_2 is the number of marked individuals caught on the second occasion (Chapman 1951). Standard error was calculated using the formula:

$$\widehat{SE} = \sqrt{\frac{(n_1+1)(n_2+1)(n_1-m_2)(n_2-m_2)}{(m_2+1)^2(m_2+2)}}$$

from Pollock et al. (1990). Estimates from the Lincoln-Petersen estimator and the robust design model (in cases where we used it) were compared to quadrat estimates of all mussels found in quadrats (combined surface and subsurface) as well as quadrat estimates based only mussels found at the surface.

Expected vs. estimated mussel abundance

We used a Leslie matrix model that was developed in collaboration with U.S. Department of the Interior economist Kristin Skrabis to estimate the expected number of total mussels at all nine restoration and monitoring sites in 2017. For the model, we assumed all mussels released at these sites could achieve a maximum age of 40 years, began breeding at 5 years old, and had an annual recruitment rate of 7.6% per year. Annual survival was set as 95% until age class 30 when survival began to decrease annually to a survival rate of 60% to the final age class (based on Jones, Neves, and Hallerman 2012). We assumed all mussels died after reaching 40 years of age.

We used the mussel release data compiled in Chapter 1 as input for the model. Only mussels >6 months old at time of release were included in the analysis. We set mussels at 1-year-old at time of release (i.e., in the 1-2 year age class). We included all mussel species released at the Payne Property and Sycamore Lane sites in the model. At the remaining monitoring sites, we included only those species released at the site that did not occur at those sites prior to restoration (Table 2.2). Hence, the natural mussel assemblage at sites in the Clinch River in Russell County, VA, and in the Powell River, TN was not included in our analysis of expected versus estimated mussel abundance. We compared the expected number of mussels at all sites in 2015, 2016, and 2017 with actual abundance estimates based on quadrat and mark-recapture estimates and calculated the percentage of expected mussels not found during monitoring.

Mussel length and growth rates

The shell growth rate of each tagged mussel sampled more than once was calculated using the following formula:

$$G = 100\frac{M_f - M_i}{M_f}$$

where M_f is the final measurement and M_i is the initial measurement. When an individual was sampled more than twice, *G* was calculated for each interval. In cases where the later measurement was less than the first measurement, we set the growth rate to zero rather than negative and included the zero in the calculation of the mean and standard deviation.

We calculated mean lengths of tagged mussels released in 2013 at the Payne and Sycamore Lane sites for *Lampsilis fasciola*, *Ptychobranchus fasciolaris*, and *Villosa vanuxemensis*. Individuals from the 2013 cohort that were sampled from 2015–2017 during our quadrat and mark recapture sampling were measured and mean lengths calculated for each year. We also calculated mean lengths of *V. iris* tagged during our 2015 mark recapture sampling and tracked the mean lengths of this cohort in 2016 and 2017.

Results

Quadrat monitoring data

Across all nine monitoring sites, mussel densities and abundance were generally higher in 2017 compared to 2016 but lower than the first year of monitoring in 2015 (Figure 2.4 and Figure 2.5). In the Clinch River, the Bennett Property had the highest abundances (39,974, 22,919, and 38,750) and densities (4.76, 2.73, and 4.61/m^2) of all sites in all three monitoring years. In the Powell River, Lower Brooks Bridge had the highest abundances (14,364, 8,861, and 9,443) across all sites and years and the highest densities (2.03 and 1.25/m^2) in 2015 and 2016. The Oakley Property had the highest density of the Powell Rivers sites in 2017 (1.45/m^2).

Clinch River, VA

Abundance of the total mussel assemblage at the Payne Property ranged from 1,257 individuals in 2016 to 2,539 in 2015 (Table 2.3), and density ranged from 0.36/m^2 in 2016 to 0.72/m^2 in 2015 (Table 2.4). Estimated mussel abundance at the Payne Property in 2015 ranged from 46 individuals of *Ptychobranchus subtentus*, and *Villosa vanuxemensis* to 1,292 individuals of *V. iris* and density ranged from 0.01 to 0.37/m^2 for these species, respectively. In 2016, abundance ranged from 126 individuals of *P. fasciolaris* to 838 individuals of *V. iris*, while density ranged from 0.04 to 0.24/m^2, respectively. In 2017 abundance ranged from 54 individuals of *Pleuronaia barnesiana* to 1,141 individuals of *V. iris*, and density ranged from 0.02 to 0.32/m^2, respectively. The most abundant species was *Villosa iris*, followed by *Lampsilis fasciola* and *Ptychobranchus fasciolaris*.

Abundance of the total mussel assemblage at Sycamore Lane ranged from 1,590 individuals in 2016 to 2,835 in 2017, and density ranged from 0.33/m^2 in 2016 to 0.60/m^2 in 2017. In 2015, abundance ranged from 64 individuals of *Lasmigona costata* and *V. vanuxemensis* to 827 individuals of *V. iris*, while density ranged from 0.01 to 0.17/m^2 for these species, respectively. In 2016, abundance ranged from 55 individuals of *Lampsilis ovata*, *Lasmigona costata*, *Medionidus conradicus*, and *V. vanuxemensis* to 877 individuals of *V. iris*, while density ranged from 0.01 to 0.18/m^2, respectively. In 2017, abundance ranged from 75 individuals of *M. conradicus* to 1,418 individuals of *V. iris*, while density ranged from 0.02 to 0.30/m^2, respectively. The most common species was *V. iris* followed by *P. fasciolaris* and *P. subtentus*.

Abundance of the total mussel assemblage at the Bennett Property ranged from 22,919 individuals in 2016 to 39,974 in 2015, and density ranged from 2.73/m^2 in 2016 to 4.76/m^2 in 2015. In 2015, abundance ranged from 172 individuals of *L. ovata* and *L. costata* to 16,985 individuals of *Actinonaias pectorosa*, while density ranged from 0.02 to 2.02/m^2 for these species, respectively. In 2016, abundance ranged from 167 individuals of *Lampsilis fasciola* and *Theliderma cylindrica* to 14,053 individuals of *A. pectorosa*, while density ranged from 0.02 to 1.67/m^2, respectively. In 2017, abundance ranged from 160 individuals of *Fusconaia cor*, *L. costata*, *Lemiox rimosus*, *Ligumia recta*, *P. subtentus*, and *V. vanuxemensis* to 18,094 individuals of *A. pectorosa*, while density ranged from 0.02 to 2.15/m^2, respectively. The most common species at Bennett was *Actinonaias pectorosa*. *Epioblasma capsaeformis* and *E. brevidens*, which were not present at the site before being restored there, were the third and fourth most abundant species, respectively.

Abundance of the total mussel assemblage at Artrip ranged from 4,422 individuals in 2016 to 11,359 in 2015, and density ranged from 1.01/m^2 in 2016 to 2.59/m^2 in 2015. In 2015, abundance ranged from 48 individuals of *F. subrotunda* to 3,369 individuals of *A. pectorosa*, while density ranged from 0.01 to 0.77/m^2 for these species, respectively. In 2016, abundance ranged from 56 individuals of *P. subtentus* and *V. vanuxemensis* to 1,847 individuals of *A. pectorosa*, while density ranged from 0.01 to 0.42/m^2, respectively. In 2017 abundance ranged from 55 individuals of *F. cor* and *Pleuronaia dolabelloides* to 1,692 individuals of *A. pectorosa*, while density ranged from 0.01 to 0.39/m^2, respectively. The most common species in all years was *A. pectorosa*.

Abundance of the total mussel assemblage at the Whited Property ranged from 5,789 individuals in 2016 to 14,458 in 2015, and density ranged from 1.04/m^2 in 2016 to 2.59/m^2 in 2015. In 2015, abundance ranged from 65 individuals of *F. cor*, *F. subrotunda*, *L. ovata*, *L. costata*, and *Pleuronaia barnesiana* to 6,934 individuals of *A. pectorosa* with densities ranging from 0.01 to 1.24/m^2 for these species, respectively. In 2016 abundance ranged from 71 individuals of *F. cor* and *L. fasciola* to 2,965 individuals of *A. pectorosa* with densities ranging from 0.01 to 0.53/m^2, respectively. In 2017, abundance ranged from 73 individuals of *Lemiox rimosus* to 8,138 individuals of *A. pectorosa* with densities ranging from 0.01 to 1.46/m^2, respectively. *Actinonaias pectorosa* also was the most common species at the site.

Abundance of the total mussel assemblage at Cleveland Islands in the right descending channel ranged from 2,422 individuals in 2016 to 8,528 in 2015, and density ranged from 0.55/m^2 in 2016 to 1.92/m^2 in 2015. In 2015, the estimated abundance ranged from 88 individuals of *L. fasciola* to 2,638 individuals of *A. pectorosa* with densities ranging from 0.02 to 0.59/m^2 for these species, respectively. In 2016, abundance ranged from 67 individuals of *Amblema plicata*, *Cyclonaias tuberculata*, *F. subrotunda*, and *V. iris* to 875 individuals of *A. pectorosa* with densities ranging from 0.02 to 0.20/m^2, respectively. In 2017, abundance ranged from 70 individuals of *C. tuberculata*, *L. fasciola*, *P. dolabelloides*, and *V. iris* to 1,468 individuals of *A. pectorosa* with densities ranging from 0.02 to 0.33/m^2, respectively. The most common species was again *A. pectorosa*, followed by *Eurynia dilatata* and *Pleuronaia* sp.

Powell River, TN

Abundance of the total mussel assemblage at Upper Brooks Bridge ranged from 3,229 individuals in 2016 to 8,391 in 2015, and density ranged from 0.64/m^2 in 2016 to 1.67/m^2 in 2015. In 2015 estimated abundance ranged from 64 individuals of *Dromus dromas* and *Ptychobranchus subtentus* to 2,479 individuals of *A. pectorosa* (Table 2.5) with densities ranging from 0.01 to 0.49/m^2 for these species, respectively (Table 2.6). In 2016, abundance ranged from 65 individuals of *Epioblasma brevidens*, *Eurynia dilatata*, *Lampsilis fasciola*, *L. ovata*, and *Ligumia recta* to 1,098 individuals of *A. pectorosa* with densities ranging from 0.01 to 0.22/m^2, respectively. In 2017, abundance ranged from 66 individuals of *L. ovata* to 2,173 individuals of *A. pectorosa* with densities ranging from 0.01 to 0.43/m^2, respectively.

Lower Brooks Bridge had the highest abundance of the Powell River sites across all years. Abundance of the total mussel assemblage ranged from 8,861 individuals in 2016 to 14,364 in 2015, and density ranged from 1.25/m^2 in 2016 to 2.03/m^2 in 2015. In 2015, abundance ranged from 120 individuals of *D. dromas*, *E. dilatata*, *L. ovata*, *L. costata*, *P. subtentus*, and *V. vanuxemensis* to 4,668 individuals of *A. pectorosa* with densities ranging from 0.02 to 0.66/m^2 for these species, respectively. In 2016, abundance ranged from 88 individuals of *D. dromas*, *L. costata*, *P. subtentus*, *V. iris*, and *V. vanuxemensis* to 3,509 individuals of *A. pectorosa* with densities ranging from 0.01 to 0.50/m^2, respectively. In 2017, abundance ranged from 79 individuals of *D. dromas*, *T. intermedia*, and *V. iris* to 3,226 individuals of *A. pectorosa* with densities ranging from 0.01 to 0.46/m^2, respectively. *Actinonaias pectorosa* was the most common species at both Brooks Bridge sites.

The Oakley site had the lowest abundance of the Powell River sites across all years but had the highest density in 2017 and the second highest in 2016. Abundance of the total mussel assemblage ranged from 907 individuals in 2016 to 1,673 in 2017, and density ranged from 0.79/m^2 in 2016 to 1.45/m^2 in 2017. In 2016, abundance ranged from 32 individuals of *C. tuberculata*, *L. fasciola*, *L. ovata*, and *P. fasciolaris* to 259 individuals of *Epioblasma capsaeformis* with densities ranging from 0.03 to 0.23/m^2 for these species, respectively. In 2017, abundance ranged from 21 individuals of *E. brevidens* to 418 individuals of *E. capsaeformis* with densities ranging from 0.02 to 0.36/m^2, respectively. *Epioblasma capsaeformis* was the most common species at this site.

Mark-recapture monitoring data

During mark-recapture sampling in 2015, 111 untagged mussels were sampled and tagged in the Clinch River at the Payne Property (White tags G677–G787). We also sampled mussels that were already tagged from previous releases for a total of 137 mussels sampled at the Payne Property (Table 2.7). During mark recapture sampling in 2016, 92 untagged mussels were sampled and tagged at the Payne Property (Gray tags A000–A093). Including sampled mussels that were already tagged, a total of 147 mussels were sampled. Of these, only 11 were recaptures from 2015. In 2017, 99 untagged mussels were tagged at the Payne Property (Gray tags B264–B361). A total of 141 mussels were sampled, of which 18 were recaptures from 2015 and 2016. One individual of *Epioblasma capsaeformis* collected at the Payne Property in 2015 was likely an inadvertent release from a past study or from hatchery produced sources and the individual was removed from the site.

During mark-recapture sampling in 2015, 101 untagged mussels were sampled and tagged in the Clinch River at the Sycamore Lane site (White tags G788–G898). We also sampled mussels that were already tagged from previous releases/studies for a total of 194 mussels sampled at Sycamore Lane (Table 2.7). During mark-recapture sampling in 2016, 184 untagged mussels were sampled and tagged at Sycamore Lane (Gray tags A094–A277). Including sampled mussels that were already tagged, a total of 418 mussels were sampled at Sycamore Lane. Of these, only 13 were recaptures from 2015. During mark-recapture sampling in 2017, 263 untagged mussels were sampled and tagged at the Sycamore Lane site (Gray tags B000–B263). A total of 644 mussels were sampled at Sycamore Lane, of which 49 were recaptures from 2015 and 2016. One individual of *E. brevidens* collected at the Sycamore Lane site in 2016 was likely an inadvertent release from a past study or from hatchery produced sources and was also removed from the site.

Due to low recapture rates of our tagged mussels, we were not able to estimate mussel abundance at Sycamore Lane using the robust design model. Estimates using the Lincoln-Petersen estimator ranged from 976 to 1,872 individuals comprising the total mussel assemblage at this site. These estimates were generally higher than the quadrat abundance estimates calculated using only mussels found at the substrate surface during quadrat sampling but not higher than quadrat estimates using combined surface and subsurface mussels (Figure 2.6). For *Villosa iris* at Sycamore Lane, Lincoln-Petersen estimates ranged from 357 to 914 and also were generally higher than surface quadrat estimates but lower than combined quadrat estimates (Figure 2.7). We were unable to estimate apparent survival at Sycamore Lane for either the total assemblage or *V. iris*.

The top model for the total mussel assemblage at the Payne Property was Model 5, suggesting that detectability varied among years, and recapture rates of individuals marked on the first sampling day of each year were lower the next day (behavior response). Abundance estimates for the total assemblage at the Payne Property ranged from 155 to 186 individuals and the estimate for apparent survival was 86%, (95% CI [5%, 99%]). For *Villosa iris* at the Payne property, the top model was Model 8, suggesting that detectability was similar among years and recapture rates were lower on the second day of sampling. Abundance estimates for *V. iris* at the Payne Property ranged from 113 to 135 individuals and the estimate for apparent survival was 96%, (95% CI [0%, 100%]). The lowest estimates of abundance for *V. iris* and the total assemblage at the Payne Property were calculated using the robust design model (Figure 2.8 and Figure 2.9). Estimates of abundance based on surface quadrat data and the Lincoln-Petersen estimator of our mark recapture data were similar for the total assemblage, while the Lincoln-Petersen estimate of abundance was slightly higher for *V. iris*. Estimates of abundance from combined quadrat data (surface and subsurface mussels) were higher for both the total assemblage and *V. iris* at the Payne Property.

Expected vs. estimated mussel abundance

Based on the Leslie matrix analysis of the mussel release data for the impact zone of the Clinch River, the expected numbers of surviving mussels at the Payne Property were 13,052, 13,005, and 13,233 individuals in 2015, 2016, and 2017, respectively. The estimated abundances at this site based on quadrat sampling were 2,598, 1,243, and 1,450 individuals in 2015, 2016, and 2017, respectively (Table 2.3). The expected numbers of mussels at Sycamore Lane were 9,171, 9,087, and 11,526 individuals in 2015, 2016, and 2017, respectively. The estimated abundances of surviving mussels at Sycamore Lane were 1,973, 1,567, and 2,054 individuals in 2015, 2016, and 2017, respectively. Estimated abundance was much lower than expected abundance across all years at all sites, although this effect was especially pronounced at the Payne Property and Sycamore Lane sites (Figure 2.10). Overall, the percentage of expected mussels not found during quadrat monitoring across sites and years ranged from 42.6 % to 94.8%, with a mean of 74.5% (Table 2.8). The Payne Property had the highest discrepancy (85.5%), followed by Cleveland Islands RDC (85.3%) and Sycamore Lane (81.1%).

Mussel length and growth rates

Shell growth rates of mussels sampled during mark recapture surveys were only calculated for *Villosa iris* at the Payne Property and Sycamore Lane sites due to low recapture rates of other species. From 2015–2017, this species grew 1.34 mm, or 3.7%, with a mean growth of 0.67 mm (1.85%) per year (Table 2.9).

The mean lengths of *L. fasciola*, *P. fasciolaris*, and *V. vanuxemensis* from the 2013 release cohort all increased substantially from 2013 to 2015 (19.5 mm, 21.1 mm, and 15.8 mm, respectively) and with a much slower increase from 2015 to 2017 (5.73 mm, 7.5 mm, and 4.75 mm, respectively) (Figure 2.11, Figure 2.12, and Figure 2.13). Growth rates of *V. iris* from the 2015 mark recapture cohort were similar to the other three species from 2015 to 2017 (3.8 mm) (Figure 2.14).

Mean lengths of *Ptychobranchus fasciolaris* were significantly lower at the Payne Property and Sycamore Lanes sites than almost all other monitored sites. Only the Payne Property and Upper Brooks Bridge had similar mean lengths (55.29 mm vs. 64.28 mm, respectively) (Table 2.10).

Discussion

At the Payne Property and Sycamore Lane sites in the Upper Clinch River, our quadrat density and abundance estimates were much lower than we would expect based on past releases and expected survival rates (Compare Table 1.18 with Tables 2.3 and 2.5; also see (Figure 2.10)). Both sites are in the immediate impact zone of the Certus, Inc. spill, and many of the released mussels for this restoration project occurred at these two sites. Further, at all of the other sites, estimated abundance was much lower than expected abundance (Table 2.8). There are several potential causes of lower estimated abundance relative to expected abundance (Figure 2.15). First, it is possible that survival is lower than we are currently assuming in the Leslie matrix model (e.g., 95% per year). It is possible that the release of propagated individuals into the wild results in a higher than expected mortality. However, estimates of apparent survival from our mark-recapture survey suggest survival is relatively high at the Payne Property (86%–96%). Further, freshwater mussels typically have high annual survival rates. A study of

Amblema plicata in the Mississippi and Otter Tail Rivers found that annual survival was greater than 97% in natural habitats (Hart et al. 2001). Meador, Peterson, and Wisniewski (2011) found high annual survival of mussels in slackwater and pool habitats (>90%) in the Altamaha River, GA, from 2006 to 2007, although mussels in swiftwater habitats had somewhat lower survival (75%). Villella, Smith, and Lemarié (2004) found annual survival was >90% for three species of adult mussels (*Elliptio complanata*, *E. fisheriana*, and *Lampsilis cariosa*) in the Cacapon River, WV. Carey et al. (2015) found that 65-70% of laboratory-propagated *Epioblasma capsaeformis* released in 2010 and 2011 in the Clinch River at Cleveland Islands survived when sampled in 2011 and 2012. A recovery survival rate of 82% also was observed a year after release of lab propagated *Epioblasma brevidens* into cages in the Powell River, TN (Hua et al. 2011). Thus, available data suggest lower than expected annual survival is not the major contributor to the lower than expected abundance found at our sites. However, an initial high mortality "spike" upon release could occur.

Another possibility is that mussels released at restoration sites are dispersing downstream of the immediate release and monitoring areas. For example, out of 100 mussels relocated in the Kishwaukee River, 20 were detected outside of the relocation area over the course of three years, one of which moved approximately 50 m downstream over two months (Tiemann et al. 2016). However, other studies have found limited downstream movement. Balfour and Smock (1995) found that the mean net movement downstream of 84 *Elliptio complanata* in a first-order stream in Virginia over the course of a year was 27 cm, although three mussels (i.e., outliers) moved much further than 27 cm (12.5 m upstream, 25.5 m upstream, and 46.2 m downstream). Another study found downstream movement rates were less than 1% over a period of four years with most movement within 40 m (Villella, Smith, and Lemarié 2004). However, Tiemann et al. (2016) only included a buffer zone of 75 m downstream of their immediate sampling area, and none of these studies were explicitly examining downstream dispersal. Further, both Balfour and Smock (1995) and Villella (2004) were examining natural populations of mussels. It is possible that propagated mussels released into the wild or translocated mussels released at a different site have higher dispersal than natural populations. We also found some evidence of downstream dispersal in our study. Two tagged mussels were found at least a kilometer downstream from where they were released at the Sycamore Lane site (one *Villosa iris* and one *Actinonaias pectorosa*), and we observed a dead, tagged *Lampsilis fasciola* ~150 m downstream of its release location at the Payne Property. Finally, in 2015 we observed a tagged (Gray C284) *Lasmigona costata* in the downstream section of Sycamore Lane, which was released at the Payne Property in 2009, approximately 2.5 km upstream.

It is also possible that a high proportion of newly transformed juvenile mussels are excysting from host fish (i.e., fish that were infected with glochidia from mussels released at these restoration sites) outside of the monitoring areas. For example, setting recruitment to zero in our Leslie matrix model decreases the expected number of mussels in 2017 at the Payne Property to 10,800 individuals and at Sycamore Lane to 10,996 individuals. However, zero recruitment alone cannot account for the large discrepancies between our expected densities and estimated densities from quadrat samples, especially at these two sites.

Another possibility is that we found fewer mussels than what are actually present at our sites. For example, Balfour and Smock (1995) found that most mussels less than 3 years of age remained buried in the sediment year round. Amyot and Downing (1991) found that mussels that were buried in mid-summer tended to be smaller and were likely juveniles. The expected age distribution of mussels at the

Payne Property and Sycamore Lane sites in 2017 suggests that 35% of the mussels might be less than five years of age. This might have caused a negative bias in our mark recapture estimates, given that we were only searching on the surface. However, if detectability was near 100% in our quadrat survey, buried juveniles would have been detected and thus not have affected our abundance estimates. At the same time, collector experience can affect detectability (Wisniewski et al. 2014), suggesting differences in the experience of collectors may have affected our quadrat surveys. For example, there was a consistent decline in estimated abundance across all sites in 2016 compared to 2015 (Figure 2.5). While this decrease may be partly due to a real decrease in abundance, it seems unlikely that such a consistent decrease in estimated abundance would be entirely a result of an actual decrease in abundance, given that our sites were in two different watersheds.

Taken together, the reasons for this discrepancy between expected and estimated abundance have substantial implications for future planning of mussel restoration via propagation. Based on our Leslie matrix analysis and monitoring, up to 85% of the expected number of mussels (based on number released and expected survival) are unaccounted for. Mussels that emigrate downstream from the release site and are alive should still be credited towards restoration even if they are no longer at the immediate restoration site. However, mussels that have died because of higher than expected mortality should not be credited. Knowing what proportion of this discrepancy is due to higher than expected mortality rather than emigration is important for planning, as it would allow a more realistic estimate of the necessary yearly production to result in the targeted abundance.

The large mussel assemblage present in the Clinch River at the Bennett Property is mostly due to one species (*Actinonaias pectorosa*) that was already naturally present at the site and was not released there as part of ongoing propagation efforts. However, *Epioblasma capsaeformis*, *E. brevidens*, *E. triquetra* and several other mussel species listed as endangered were not present at the Bennett Property prior to propagation efforts, and they are now among the most common species at this site. The only species released in the Clinch River at the Whited Property was *E. capsaeformis*, which was not detected in quadrat samples in 2016 or 2017; although, a few individuals were sampled there in 2015. Similarly, *E. brevidens* was only detected in 2017 in the right descending channel of Cleveland Islands in the Clinch River, although *E. capsaeformis* was sampled in this channel at a higher density and abundance in 2015 compared to 2016 and 2017. In the Powell River, at the Upper and Lower Brooks Bridge sites, as well as at the Oakley Property, a high proportion of *E. capsaeformis* and *E. brevidens* were sampled at these three sites relative to the number of mussels released, suggesting recruitment and/or survival were higher than expected at these sites.

Estimates of abundance using a robust design model tended to underestimate abundance compared to quadrat sampling and Lincoln-Petersen estimates. This is likely due to the very low recapture rate, both within and among primary periods, making modeling difficult to conduct. For example, of the 39 *Villosa iris* sampled in 2015 on the first sampling day at Sycamore Lane, only 6 were recaptured the next day. Of the 88 *V. iris* that were sampled on both days that year, only 8 were sampled again in 2016, while 183 were sampled for the first time. Thus, we should expect actual abundance to be much higher than the number sampled and likely higher than the estimates from the robust design model. It is also possible that smaller individuals were buried in the sediment and unavailable for capture during our mark-recapture survey. This would also result in an underestimate of abundance compared to expected abundance.

The Lincoln-Petersen estimator provided a better, lower-bound estimate of abundance compared to the robust design model, even though it does not account for imperfect detectability. The Lincoln-Petersen estimator generally had higher estimates of abundance compared to estimates using the surface quadrat data. Both estimates only accounted for mussels found on the surface of the substrate. However, the mark recapture surveys were typically conducted during the early fall when detectability at the substrate surface was expected to be higher, while the quadrat surveys were conducted in mid-to-late summer, when detectability at the substrate surface was likely lower.

The mean length of *Ptychobranchus fasciolaris* was significantly lower at the Payne Property and Sycamore Lane sites when compared to the other restoration and monitoring sites. Physiochemical factors, such as habitat, temperature, and degree of eutrophication, can affect the growth rates and sizes of freshwater mussels (Bauer 1992). However, the majority of *P. fasciolaris* released during restoration was at the Payne Property and Sycamore Lane sites (741 and 1,606, respectively). Only 196 were released at the Bennett Property and none were released at the other 6 restoration and monitoring sites. Many of the mussels released at the Payne Property and Sycamore Lane sites also were released before 2013, and because these sites were in the impact zone of the Certus, Inc. chemical spill, there was no population of these species present before releases. The smaller size of the *P. fasciolaris* populations at these sites is likely because the populations there are much younger than populations at other restoration sites. Further, the mean length of *P. fasciolaris*, *Lampsilis fasciola*, and *Villosa vanuxemensis* in the impact zone sites increased 20–28 mm from 2013 to 2017. Growth of *V. iris* from 2015 to 2017 was only 3.7 mm, but this was not much lower than the 4.8–7.4 mm that the other three species grew during the same time period. As most of these four species were released before 2013, it is likely that the much lower growth from 2015 to 2017 is a result of mussels reaching an age where overall growth rate slows.

Growth rates of *V. iris* at the Payne Property and Sycamore Lane sites were similar to comparable sized *V. iris* sampled at three sites in the Clinch River from 1988 to 1993 (Scott 1994). Growth rates of *L. fasciola* also were similar to comparably sized *L. fasciola* at four sites in the Clinch River from 1986 to 1993 in the same study. This suggests that growth is not negatively impacted at the Payne Property and Sycamore Lane sites in the impact zone of the Certus, Inc. chemical spill.

Overall, mussel restoration efforts associated with the Certus, Inc. NRDAR case were successful based on the number of species and juvenile mussels produced and released at restoration sites. While abundance at the two monitoring sites in the immediate impact zone is lower than expected based on expected survival, there are still populations of numerous species that have low to medium densities at these sites. However, 37,101 mussels >6 months old, representing 14 species, were released in the impact zone (Table 1.18). A further 60,486 mussels representing 20 species have been released at restoration and monitoring sites downstream in the Clinch River in Russell County (Table 1.18). Together, this is far greater than the estimated 18,621 mussels killed during the spill. Further, the estimated kill was calculated by multiplying dead mussels by three to account for mussels buried in the substrate. If a significant number (e.g. 80%) of mussels migrated to the surface before dying as a result of the spill, then a 3x multiplier applied to dead mussels found on the surface would overestimate the injury. However, no quantitative sampling was done after the spill to validate the use of the 3x multiplier. Future spills should include some quantitative sampling, such as excavation of quadrats, to more accurately determine the multiplier that should be used to estimate injury. While it is unknown why estimated abundance is lower than expected in the impact zone, if released mussels are migrating downstream, then they should still be counted toward restoration for the Certus, Inc. NRDAR case. From 2016 to 2017, a total of 731 *Epioblasma aureola* were reintroduced in the Clinch River, 300 of which were released at Sycamore Lane in the impact zone of the Certus, Inc. spill. Further, their surrogates, *E. capsaeformis* and *E. brevidens* have been well established at other augmentation sites in the Clinch River, VA. In particular, these two species are now the second and third most common species at the Bennett Property, even though they did not occur there before restoration. Restoration success for the LMPI NRDAR case is harder to measure as the impacts to mussels were potentially chronic and sub-lethal. Nevertheless, *E. capsaeformis* and *E. brevidens*, the primary species that were released in the Powell River, TN, as part of restoration efforts, are currently found at low to moderate densities at several restoration and monitoring sites in the river.

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Tables

Table 2.1. Location information, survey methods, and sample sizes (N) for sites quantitatively sampled in 2015, 2016, and 2017 for the Certus, Inc. and LMPI NRDAR mussel restoration cases in the Clinch and Powell rivers, Tennessee and Virginia. Site length and mean stream width were rounded to nearest whole number, whereas site *area was calculated using the unrounded site length and stream width. Sample sizes for quadrat surveys are number of quadrats sampled and sample sizes for mark-recapture surveys are number of individuals sampled. - Site was not sampled in 2015.

								Sample Size (N)		
Site	**River**	**River Mile**	**Length (m)**	**Mean Width (m)**	***Area (m^2)**	**Latitude/Longitude**	**Survey Method**	**2015**	**2016**	**2017**
Payne Property, VA	Clinch	322.1	151	23	3,531	37.081642°, -81.778816°	Quadrat	306	337	260
							Mark-recapture	137	147	141
Sycamore Lane, VA	Clinch	320	245	20	4,756	37.095162°, -81.785898°	Quadrat	299	347	255
							Mark-recapture	194	418	644
Bennett Property, VA	Clinch	277.5	235	36	8,407	36.959511°, -82.097550°	Quadrat	196	201	210
Artrip, VA	Clinch	274.5	210	21	4,380	36.961647°, -82.119429°	Quadrat	364	313	321
Whited Property, VA	Clinch	272.7	146	38	5,577	36.948771°, -82.139325°	Quadrat	341	316	307
Cleveland Islands, VA	Clinch	270	240	18	4,440	36.938084°, -82.164613°	Quadrat	202	264	254
Upper Brooks Bridge, TN	Powell	95.3	150	34	5,038	36.534982°, -83.442999°	Quadrat	317	312	306
Lower Brooks Bridge, TN	Powell	94.7	184	38	7063	36.536824°, -83.451406°	Quadrat	236	322	359
Oakley Property, TN	Powell	89.7	58	20	1,150	36.535212°, -83.467035°	Quadrat	-	142	220

Table 2.2. Mussel species that were assessed for expected abundance and density in 2015, 2016, and 2017 at sites outside of the impact zone of the Certus, Inc. chemical spill in the Clinch River, VA, and Powell River, TN. These six species did not occur at restoration and monitoring sites before being released, or occurred at these sites at very low densities.

	Clinch River, VA				Powell River, TN		
Species (6)	**Bennett**	**Artrip**	**Whited Property**	**Cleveland Islands - RDC**	**Upper Brooks Bridge**	**Lower Brooks Bridge**	**Oakley Property**
Epioblasma brevidens	X	X		X	X	X	X
Epioblasma capsaeformis	X	X	X	X	X	X	X
Epioblasma triquetra	X				X	X	X
Lemiox rimosus	X						
Ligumia recta	X	X					
Venustaconcha trabalis	X						

Table 2.3. Estimated abundances of freshwater mussels at population restoration and monitoring sites based on quadrat sampling in the Clinch River, VA from 2015 to 2017. Cleveland Islands is the right-descending channel site. RM = River Mile.

	Payne Property (RM 322.1)			Sycamore Lane (RM 320)			Bennett (RM 277.5)			Artrip (RM 274.5)			Whited Property (RM 272.7)			Cleveland Islands (RM 270)		
Species (22)	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017
Actinonaias pectorosa	92	0	0	0	0	0	16,985	14,053	18,094	3,369	1,847	1,692	6,934	2,965	8,138	2,638	875	1,468
Amblema plicata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67	0
Cyclonaias tuberculata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67	70
Epioblasma brevidens	0	0	0	0	0	0	3,603	1,338	3,203	915	224	819	0	0	0	0	0	140
Epioblasma capsaeformis	0	0	0	0	0	0	3,774	1,506	3,683	578	112	491	327	0	0	1,495	202	559
Eurynia dilatata	0	0	0	0	0	0	686	0	0	1,059	392	710	1,897	1,059	1,599	1,670	269	1,259
Fusconaia cor	0	0	0	0	0	0	686	0	160	144	0	55	65	71	145	176	135	350
Fusconaia subrotunda	0	0	0	0	0	0	0	0	0	48	0	0	65	0	0	0	67	0
Lampsilis fasciola	692	293	380	255	219	224	858	167	480	385	168	164	654	71	218	88	135	70
Lampsilis ovata	0	0	0	0	55	0	172	0	0	0	0	0	65	0	0	0	0	0
Lasmigona costata	0	0	0	64	55	0	172	0	160	144	0	0	65	0	0	0	0	0
Lemiox rimosus	0	0	0	0	0	0	0	0	160	0	0	0	0	0	73	0	0	0
Ligumia recta	0	0	0	0	0	0	0	0	160	0	0	0	0	0	0	0	0	0
Medionidus conradicus	0	0	0	255	55	75	7,892	2,677	6,565	1,444	448	273	1,439	494	581	967	135	210
Pleuronaia barnesiana	0	0	54	0	0	0	0	0	0	0	0	0	65	0	0	0	0	0
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	0	0	0	55	0	0	0	0	0	70
Pleuronaia spp.	0	0	0	0	0	0	858	335	1,601	578	504	327	1,308	494	799	879	202	979
Ptychobranchus fasciolaris	369	126	217	255	164	746	2,059	1,506	2,882	1,059	112	491	981	494	581	264	202	420
Ptychobranchus subtentus	46	0	0	255	110	373	1,029	502	160	0	56	0	0	0	0	0	0	0
Theliderma cylindrica	0	0	0	0	0	0	0	167	0	96	0	0	0	0	0	0	0	0
Villosa iris	1,292	838	1,141	827	877	1,418	858	669	1,281	1,540	504	655	589	141	145	352	67	70
Villosa vanuxemensis	46	0	109	64	55	0	343	0	160	0	56	164	0	0	0	0	0	0
Grand Total	2,539	1,257	1,901	1,973	1,590	2,835	39,974	22,919	38,750	11,359	4,422	5,895	14,458	5,789	12,280	8,528	2,422	5,664

Table 2.4. Estimated densities of freshwater mussels at population restoration and monitoring sites based on quadrat sampling in the Clinch River, VA from 2015 to 2017. Density is reported as individuals per m^2. Cleveland Islands is the right-descending channel site. RM = River Mile.

	Payne Property (RM 322.1)			Sycamore Lane (RM 320)			Bennett (RM 277.5)			Artrip (RM 274.5)			Whited Property (RM 272.7)			Cleveland Islands (RM 270)		
Species (22)	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017	2015	2016	2017
Actinonaias pectorosa	0.03	0	0	0	0	0	2.02	1.67	2.15	0.77	0.42	0.39	1.24	0.53	1.46	0.59	0.20	0.33
Amblema plicata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.02	0
Cyclonaias tuberculata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.02	0.02
Epioblasma brevidens	0	0	0	0	0	0	0.43	0.16	0.38	0.21	0.05	0.19	0	0	0	0	0	0.03
Epioblasma capsaeformis	0	0	0	0	0	0	0.45	0.18	0.44	0.13	0.03	0.11	0.06	0	0	0.34	0.05	0.13
Eurynia dilatata	0	0	0	0	0	0	0.08	0	0	0.24	0.09	0.16	0.34	0.19	0.29	0.38	0.06	0.28
Fusconaia cor	0	0	0	0	0	0	0.08	0	0.02	0.03	0	0.01	0.01	0.01	0.03	0.04	0.03	0.08
Fusconaia subrotunda	0	0	0	0	0	0	0	0	0	0.01	0	0	0.01	0	0	0	0.02	0
Lampsilis fasciola	0.20	0.08	0.11	0.05	0.05	0.05	0.10	0.02	0.06	0.09	0.04	0.04	0.12	0.01	0.04	0.02	0.03	0.02
Lampsilis ovata	0	0	0	0	0.01	0	0.02	0	0	0	0	0	0.01	0	0	0	0	0
Lasmigona costata	0	0	0	0.01	0.01	0	0.02	0	0.02	0.03	0	0	0.01	0	0	0	0	0
Lemiox rimosus	0	0	0	0	0	0	0	0	0.02	0	0	0	0	0	0.01	0	0	0
Ligumia recta	0	0	0	0	0	0	0	0	0.02	0	0	0	0	0	0	0	0	0
Medionidus conradicus	0	0	0	0.05	0.01	0.02	0.94	0.32	0.78	0.33	0.10	0.06	0.26	0.09	0.10	0.22	0.03	0.05
Pleuronaia barnesiana	0	0	0.02	0	0	0	0	0	0	0	0	0	0.01	0	0	0	0	0
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	0	0	0	0.01	0	0	0	0	0	0.02
Pleuronaia spp.	0	0	0	0	0	0	0.10	0.04	0.19	0.13	0.12	0.07	0.23	0.09	0.14	0.20	0.05	0.22
Ptychobranchus fasciolaris	0.10	0.04	0.06	0.05	0.03	0.16	0.24	0.18	0.34	0.24	0.03	0.11	0.18	0.09	0.10	0.06	0.05	0.09
Ptychobranchus subtentus	0.01	0	0	0.05	0.02	0.08	0.12	0.06	0.02	0	0.01	0	0	0	0	0	0	0
Theliderma cylindrica	0	0	0	0	0	0	0	0.02	0	0.02	0	0	0	0	0	0	0	0
Villosa iris	0.37	0.24	0.32	0.17	0.18	0.30	0.10	0.08	0.15	0.35	0.12	0.15	0.11	0.03	0.03	0.08	0.02	0.02
Villosa vanuxemensis	0.01	0	0.03	0.01	0.01	0	0.04	0	0.02	0	0.01	0.04	0	0	0	0	0	0
Grand Total	0.72	0.36	0.54	0.41	0.33	0.60	4.76	2.73	4.61	2.59	1.01	1.35	2.59	1.04	2.20	1.92	0.55	1.28

Table 2.5. Estimated abundances of freshwater mussels at population restoration and monitoring sites based on quadrat sampling in the Powell River, TN from 2015 to 2017. Cleveland Islands is the right-descending channel site. RM = River Mile.

	Upper Brooks Bridge (RM 95.3)			Lower Brooks Bridge (RM 94.7)			Oakley (RM 89.7)	
Species (19)	2015	2016	2017	2015	2016	2017	2016	2017
Actinonaias ligamentina	1,526	775	593	3,352	2,807	2,439	97	167
Actinonaias pectorosa	2,479	1,098	2,173	4,668	3,509	3,226	130	314
Amblema plicata	127	0	132	479	351	472	0	42
Cyclonaias tuberculata	445	0	132	239	0	236	32	42
Dromus dromas	64	0	0	120	88	79	0	0
Epioblasma brevidens	572	65	263	599	263	708	65	21
Epioblasma capsaeformis	763	388	790	1,197	351	236	259	418
Eurynia dilatata	0	65	0	120	175	315	97	188
Lampsilis fasciola	381	65	132	599	263	157	32	188
Lampsilis ovata	127	65	66	120	0	0	32	42
Lasmigona costata	0	0	0	120	88	0	0	0
Ligumia recta	0	65	0	0	0	0	0	0
Medionidus conradicus	1,462	517	527	1,317	526	787	65	63
Plethobasus cyphyus	0	0	0	239	0	0	0	0
Ptychobranchus fasciolaris	191	129	263	958	175	630	32	146
Ptychobranchus subtentus	64	0	0	120	88	0	0	0
Theliderma intermedia	0	0	0	0	0	79	0	0
Villosa iris	191	0	132	0	88	79	65	42
Villosa vanuxemensis	0	0	0	120	88	0	0	0
Grand Total	8,391	3,229	5,203	14,364	8,861	9,443	907	1,673

Table 2.6. Estimated densities of freshwater mussels at population restoration and monitoring sites based on quadrat sampling in the Powell River, TN from 2015 to 2017. Density is reported as individuals per m^2. Cleveland Islands is the right-descending channel site. RM = River Mile.

	Upper Brooks Bridge (RM 95.3)			Lower Brooks Bridge (RM 94.7)			Oakley (RM 89.7)	
Species (19)	2015	2016	2017	2015	2016	2017	2016	2017
Actinonaias ligamentina	0.30	0.15	0.12	0.47	0.40	0.35	0.08	0.15
Actinonaias pectorosa	0.49	0.22	0.43	0.66	0.50	0.46	0.11	0.27
Amblema plicata	0.03	0	0.03	0.07	0.05	0.07	0	0.04
Cyclonaias tuberculata	0.09	0	0.03	0.03	0	0.03	0.03	0.04
Dromus dromas	0.01	0	0	0.02	0.01	0.01	0	0
Epioblasma brevidens	0.11	0.01	0.05	0.08	0.04	0.10	0.06	0.02
Epioblasma capsaeformis	0.15	0.08	0.16	0.17	0.05	0.03	0.23	0.36
Eurynia dilatata	0	0.01	0	0.02	0.02	0.04	0.08	0.16
Lampsilis fasciola	0.08	0.01	0.03	0.08	0.04	0.02	0.03	0.16
Lampsilis ovata	0.03	0.01	0.01	0.02	0	0	0.03	0.04
Lasmigona costata	0	0	0	0.02	0.01	0	0	0
Ligumia recta	0	0.01	0	0	0	0	0	0
Medionidus conradicus	0.29	0.10	0.10	0.19	0.07	0.11	0.06	0.05
Plethobasus cyphyus	0	0	0	0.03	0	0	0	0
Ptychobranchus fasciolaris	0.04	0.03	0.05	0.14	0.02	0.09	0.03	0.13
Ptychobranchus subtentus	0.01	0	0	0.02	0.01	0	0	0
Theliderma intermedia	0	0	0	0	0	0.01	0	0
Villosa iris	0.04	0	0.03	0	0.01	0.01	0.06	0.04
Villosa vanuxemensis	0	0	0	0.02	0.01	0	0	0
Grand Total	1.67	0.64	1.03	2.03	1.25	1.34	0.79	1.45

Table 2.7. Numbers of mussels sampled at the two population restoration and monitoring sites in the impact zone for the Certus Inc. NRDAR case in the Clinch River, Tazewell County, VA, using transect sampling for mark-recapture from 2015 to 2017. *Inadvertent release of individuals that were removed from site.

		2015			2016			2017		
Site	Species	Pass #1	Pass #2	Total	Pass #1	Pass #2	Total	Pass #1	Pass #2	Total
Payne Property	*Actinonaias ligamentina*	0	0	0	1	0	1	0	2	2
	Actinonaias pectorosa	5	2	7	6	7	13	5	2	7
	*Epioblasma capsaeformis**	1	0	1	0	0	0	0	0	0
	Eurynia dilatata	0	0	0	0	0	0	0	1	1
	Lampsilis fasciola	6	2	8	8	5	13	6	3	9
	Lasmigona costata	0	0	0	1	2	3	0	0	0
	Medionidus conradicus	1	0	1	3	2	5	1	0	1
	Pleurobema oviforme	0	1	1	0	0	0	0	0	0
	Pleuronaia spp.	0	1	1	0	2	2	1	0	1
	Ptychobranchus fasciolaris	9	3	12	4	7	11	3	0	3
	Ptychobranchus subtentus	0	0	0	3	0	3	1	1	2
	Villosa iris	71	29	100	56	36	92	66	47	113
	Villosa vanuxemensis	4	2	6	3	1	4	1	1	2
	Total Assemblage	**97**	**40**	**137**	**85**	**62**	**147**	**84**	**57**	**141**
Sycamore Lane	*Actinonaias pectorosa*	0	1	1	0	0	0	0	0	0
	*Epioblasma brevidens**	0	0	0	1	0	1	0	0	0
	Eurynia dilatata	0	0	0	1	0	1	0	0	0
	Lampsilis fasciola	8	13	21	27	11	38	25	29	54
	Lampsilis ovata	1	1	2	0	2	2	1	0	1
	Lasmigona costata	0	0	0	3	1	4	0	1	1
	Medionidus conradicus	7	17	24	20	7	27	20	24	44
	Pleurobema oviforme	1	1	2	0	0	0	0	0	0
	Pleuronaia barnesiana	0	1	1	0	0	0	0	0	0
	Pleuronaia spp.	1	0	1	3	0	3	5	4	9
	Ptychobranchus fasciolaris	11	12	23	34	19	53	34	43	77
	Ptychobranchus subtentus	3	16	19	43	24	67	55	74	129
	Villosa iris	39	55	94	117	86	203	141	163	304
	Villosa vanuxemensis	1	5	6	10	9	19	14	11	25
	Total Assemblage	**72**	**122**	**194**	**259**	**159**	**418**	**295**	**349**	**644**

Table 2.8. Percentages of expected mussels not accounted for in quadrat estimates at each restoration and monitoring site in the Clinch and Powell rivers in Tennessee and Virginia. Percentage unaccounted mussels are likely a function of both emigration and additional mortality. Cleveland Islands is the right-descending channel site.

	Clinch River, VA						Powell River, TN		
	Payne Property	Sycamore Lane	Bennett Property	Artrip	Whited Property	Cleveland Islands	Upper Brooks Bridge	Lower Brooks Bridge	Oakley Property
2015	80.5%	78.5%	42.6%	70.7%	63.8%	63.2%	63.3%	54.7%	
2016	90.3%	82.8%	79.0%	93.1%	-	94.8%	87.2%	85.5%-	69.9%
2017	85.6%	82.2%	50.5%	71.8%	-	86.0%	70.1%	82.3%	60.1%
Mean	85.5%	81.1%	57.4%	78.5%	63.8%	81.3%	73.5%	74.2%	65.0%

Table 2.9. Mean growth rates of tagged *Villosa iris* at the Payne and Sycamore Lane sites, Clinch River, VA, collected during mark-recapture sampling from 2015 to 2017.

	2015-2016	2016-2017	2015-2017
Mean (mm)	1.28	2.6	
SD	3.52	2.37	7.26
N	21	36	17

Table 2.10. Tukey pairwise comparison of mean lengths of *Ptychobranchus fasciolaris* by site.

Site	**N**	**Mean (mm)**	**Grouping**			
Bennett Property	39	79.82	A			
Brooks Bridge, Lower	18	75.38	A	B		
Whited Property	30	75.26	A	B		
Oakley Property	8	70.75	A	B		
Artrip	32	70.53		B		
Cleveland Islands, RDC	12	69.89	A	B		
Brooks Bridge, Upper	9	64.28		B	C	
Payne Property	41	55.29			C	D
Sycamore Lane	166	50.967				D

Figures

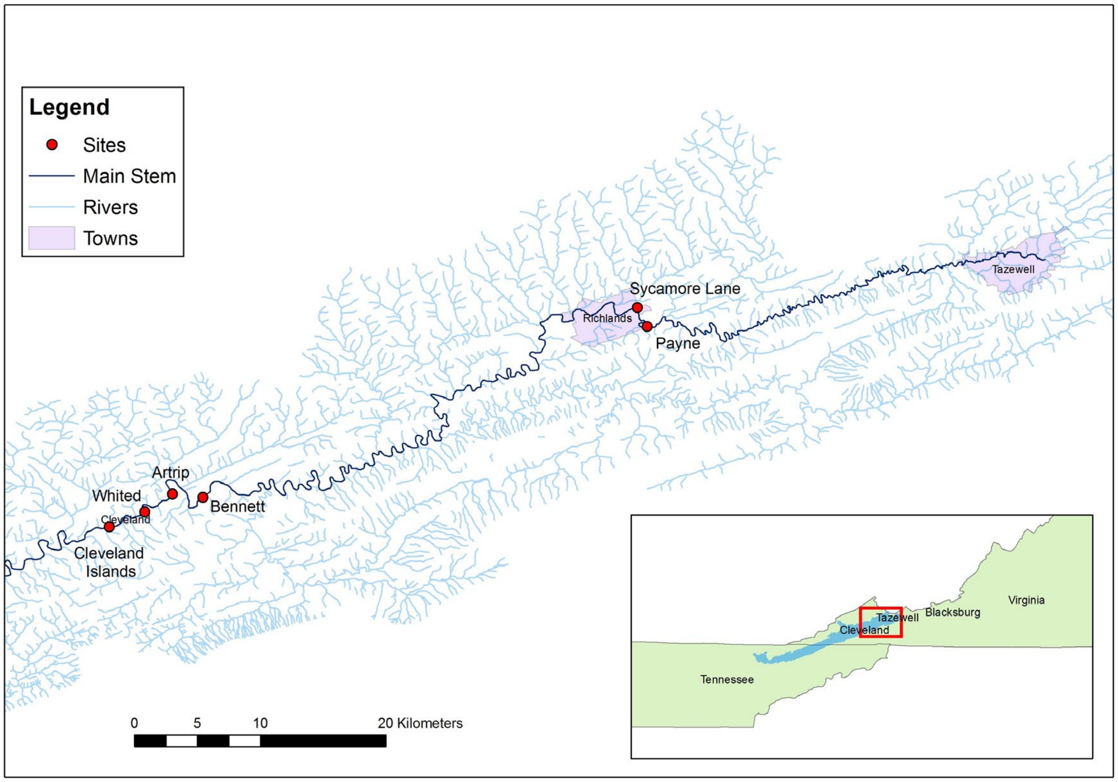


Figure 2.1. Locations of mussel population restoration and monitoring sites in the Upper Clinch River, Russell and Tazewell counties, VA for the Certus, Inc. NRDAR case.

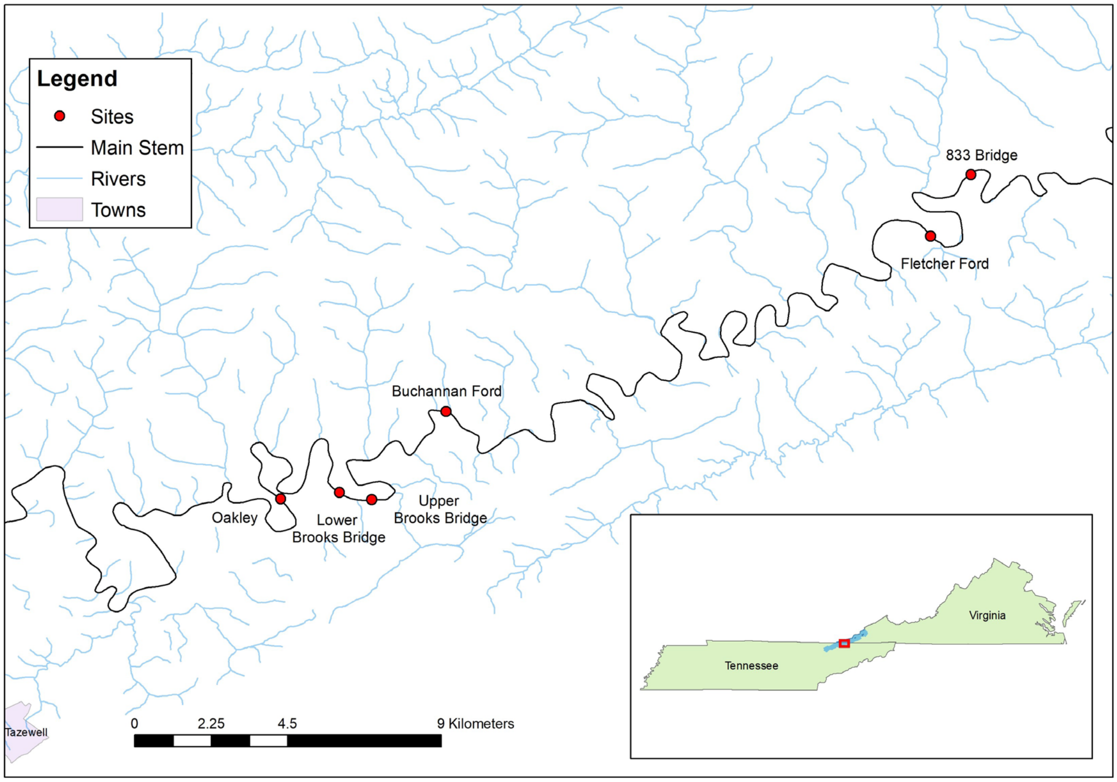


Figure 2.2. Locations of mussel population restoration and monitoring sites in the Powell River, Claiborne County, TN, and Lee County, VA, for the LMPI NRDAR case.

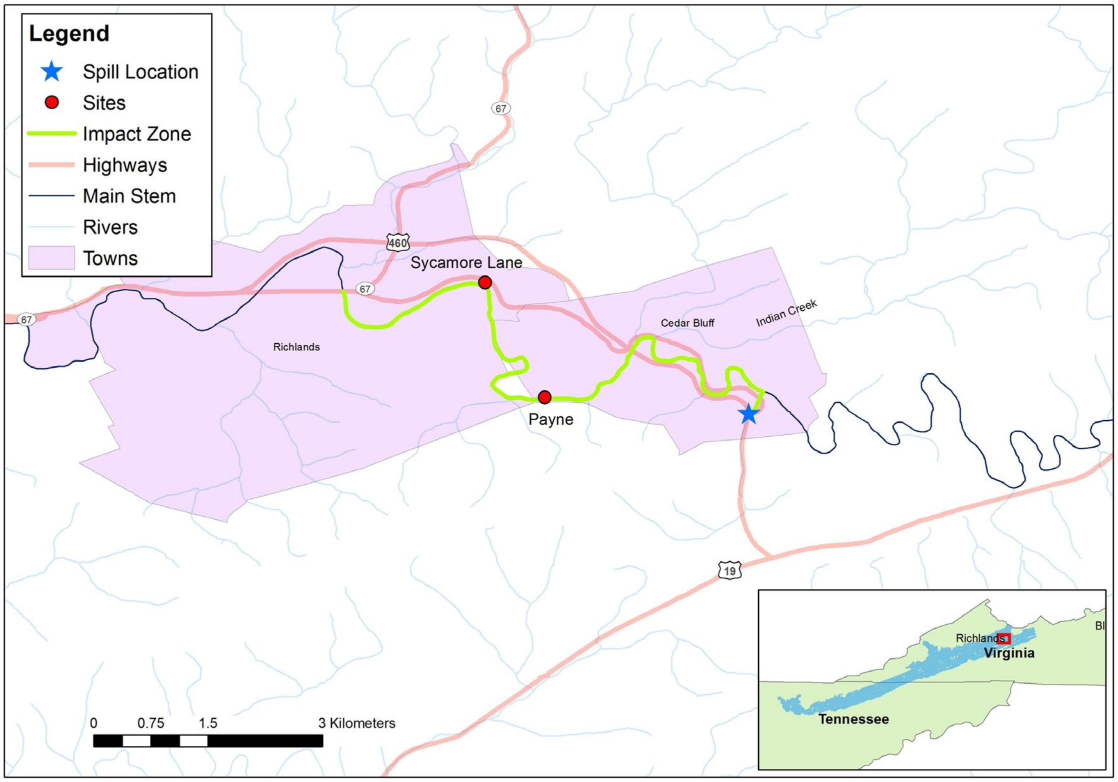


Figure 2.3. The Certus, Inc., chemical spill impact zone in the upper Clinch River at Cedar Bluff, Tazewell County, VA, showing the two main population restoration and monitoring sites, Sycamore Lane (River Mile 320) and Payne Property (River Mile 322.1).

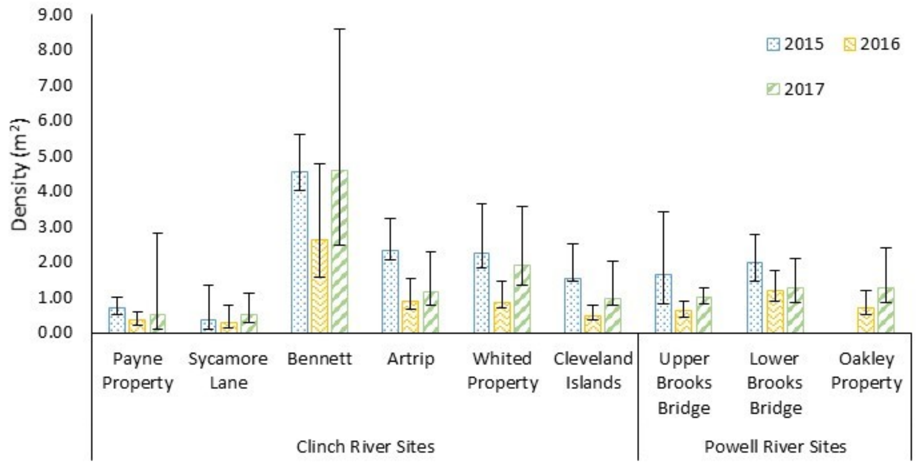


Figure 2.4. Estimated densities of freshwater mussels at population restoration and monitoring sites in the Clinch and Powell Rivers, Virginia and Tennessee based on quadrat sampling conducted from 2015 to 2017. Sites are ordered from upstream to downstream within each river and error bars represent 95% confidence intervals.

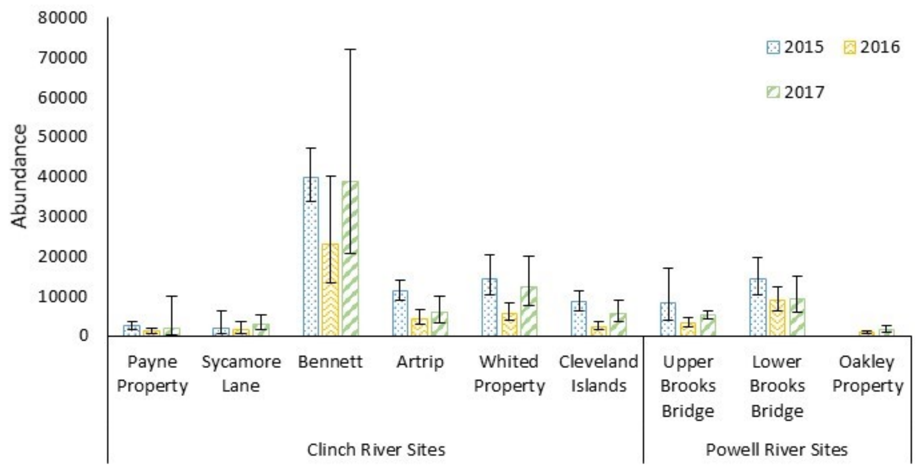


Figure 2.5. Estimated abundance of freshwater mussels at population restoration and monitoring sites in the Clinch and Powell Rivers, Virginia and Tennessee based on quadrat sampling conducted from 2015 to 2017. Sites are ordered from upstream to downstream within each river and error bars represent 95% confidence intervals.

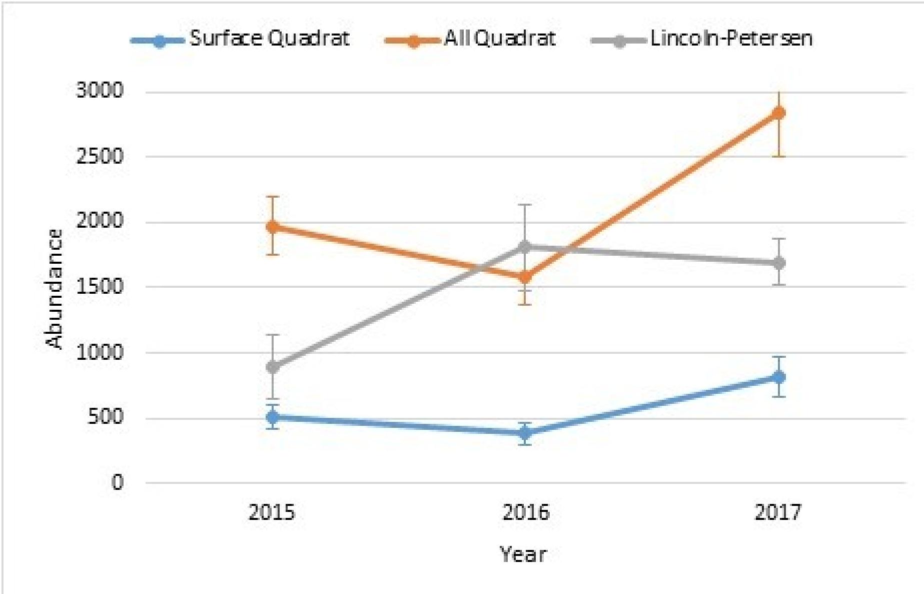


Figure 2.6. Abundance estimates for the total mussel assemblage at Sycamore Lane, Clinch River, VA from 2015 to 2017. Surface quadrat abundance was calculated using only mussels found on the surface of the substrate during quadrat surveys. All quadrat abundance includes surface and subsurface mussels. The modified Lincoln-Petersen estimator was used to estimate abundance on data collected from mark-recapture surveys. Error bars represent standard error.

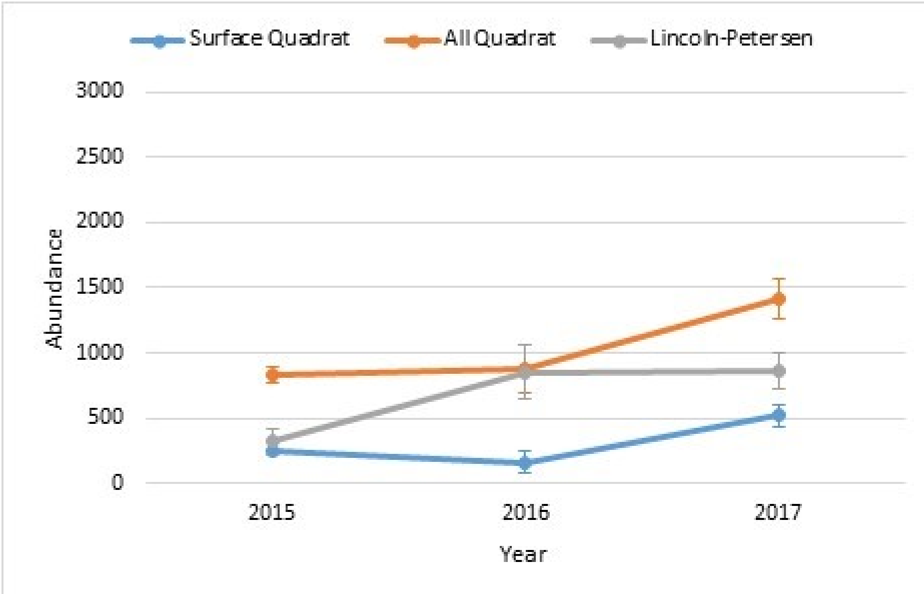


Figure 2.7. Abundance estimates for *Villosa iris* at Sycamore Lane, Clinch River, VA from 2015 to 2017. Surface quadrat abundance was calculated using only mussels found on the surface of the substrate during quadrat surveys. All quadrat abundance includes surface and subsurface mussels. The modified Lincoln-Petersen estimator was used to estimate abundance on data collected from mark-recapture surveys. Error bars represent standard error.

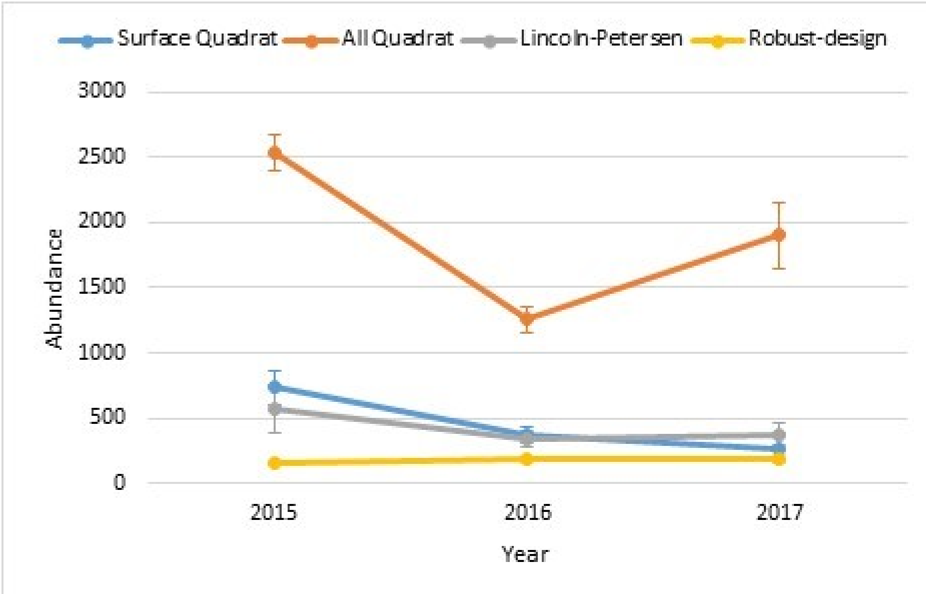


Figure 2.8. Abundance estimates for the total mussel assemblage at the Payne Property, Clinch River, VA from 2015 to 2017. Surface quadrat abundance was calculated using only mussels found on the surface of the substrate during quadrat surveys. All quadrat abundance includes surface and subsurface mussels. Both the modified Lincoln Petersen estimator and robust design model were used to estimate abundance on data collected from mark-recapture surveys. Error bars represent standard error.

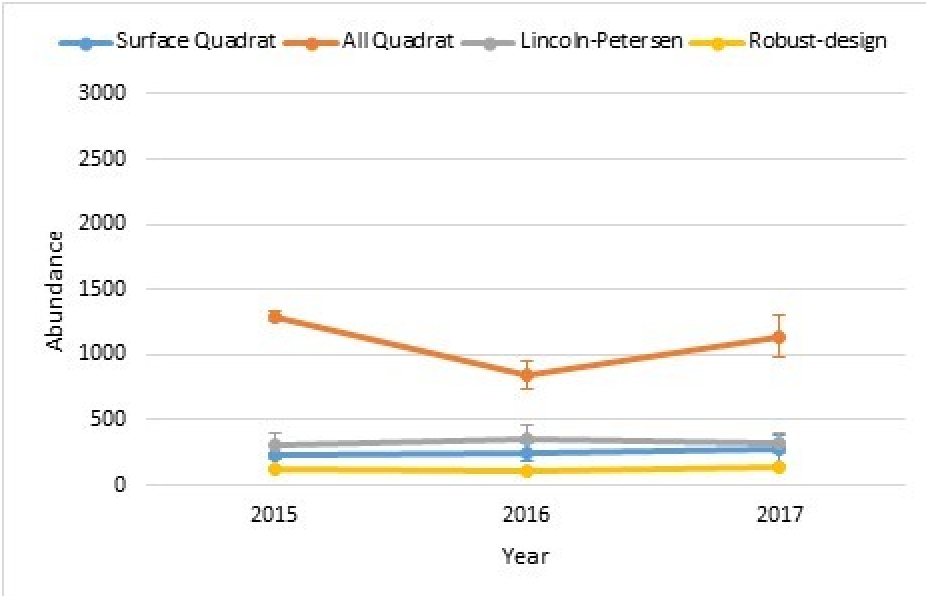
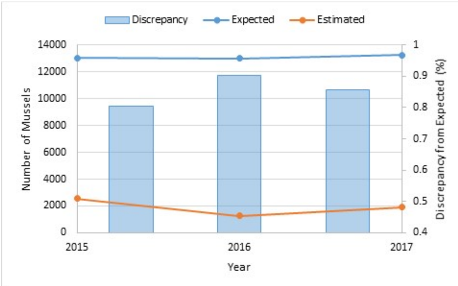
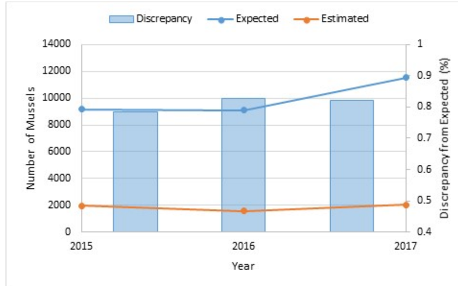


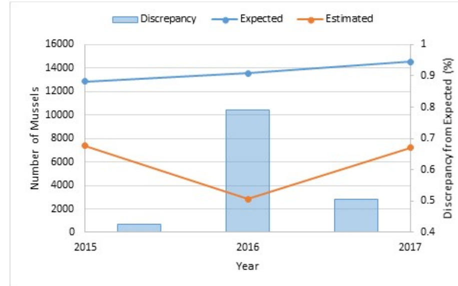
Figure 2.9. Abundance estimates for *Villosa iris* at the Payne Property, Clinch River, VA from 2015 to 2017. Surface quadrat abundance was calculated using only mussels found on the surface of the substrate during quadrat surveys. All quadrat abundance includes surface and subsurface mussels. Both the modified Lincoln-Petersen estimator and robust design model were used to estimate abundance on data collected from mark-recapture surveys. Error bars represent standard error.



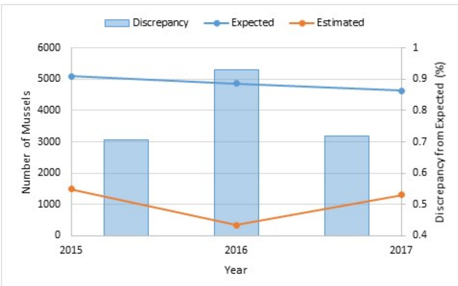
(a) Payne Property



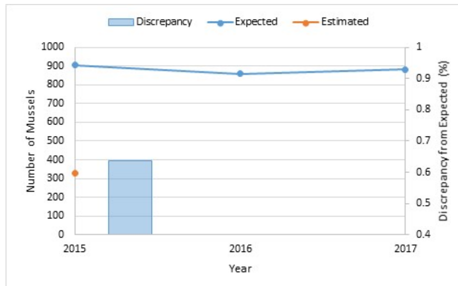
(b) Sycamore Lane



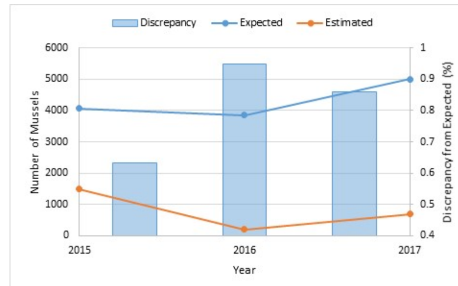
(c) Bennett Property



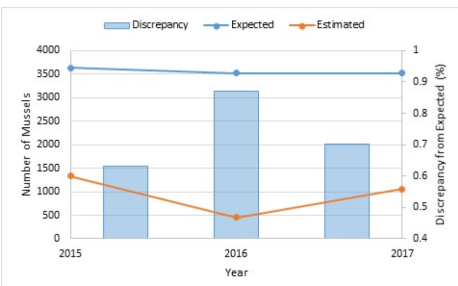
(d) Artrip



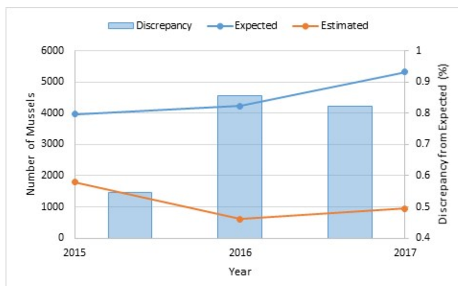
(e) Whited Property



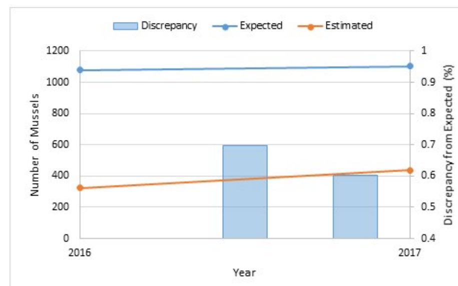
(f) Cleveland Islands, RDC



(g) Upper Brooks Bridge



(h) Lower Brooks Bridge



(i) Oakley Property

Figure 2.10. Comparison of expected abundance to estimated abundance of released mussels at nine monitoring sites in the Clinch and Powell rivers, VA and TN. Expected abundance was determined using release data inputted to a Leslie matrix model assuming 95% survival, and estimated abundance was determined from quadrat sampling data. Bars represent discrepancy (in percentage) from expected abundance. Abundance was not estimated for the Whited Property in 2016 and 2017 because the species released at the sites were not detected in those years.

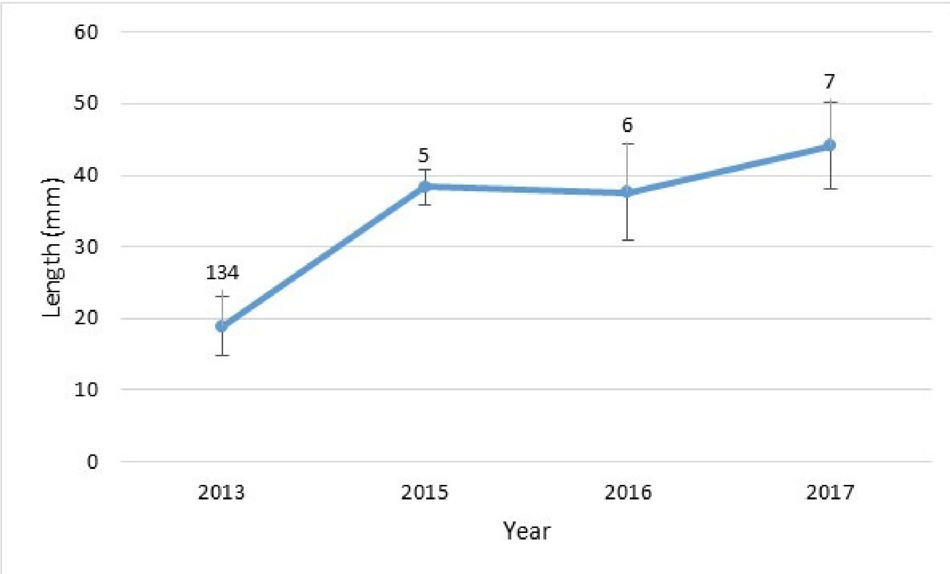


Figure 2.11. Mean lengths of the 2013 cohort of *Lampsilis fasciola* from 2013 to 2017 in the Clinch River, VA, at the Payne Property and Sycamore Lane. Numbers above means represent sample size.

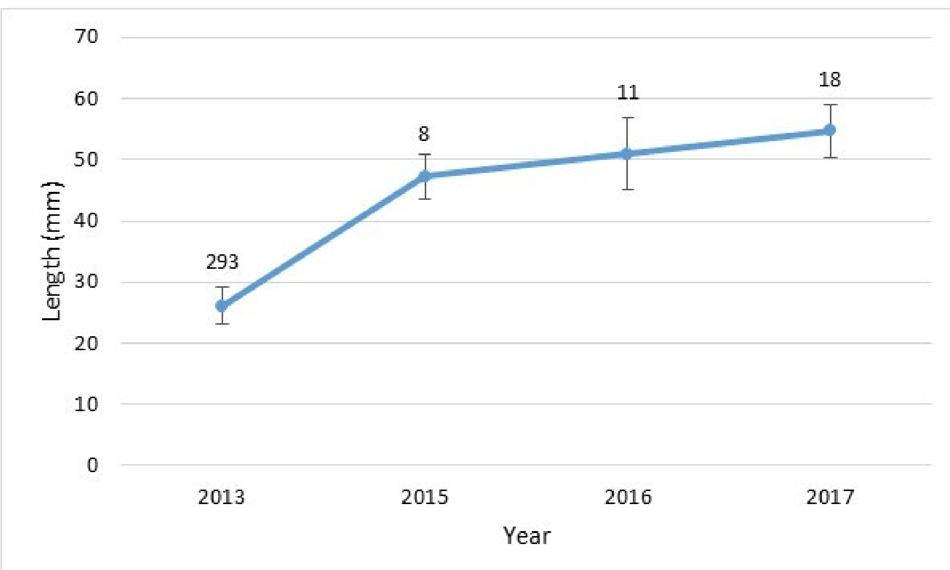


Figure 2.12. Mean lengths of the 2013 cohort of *Ptychobranchus fasciolaris* from 2013 to 2017 in the Clinch River, VA, at the Payne Property and Sycamore Lane. Numbers above means represent sample size.

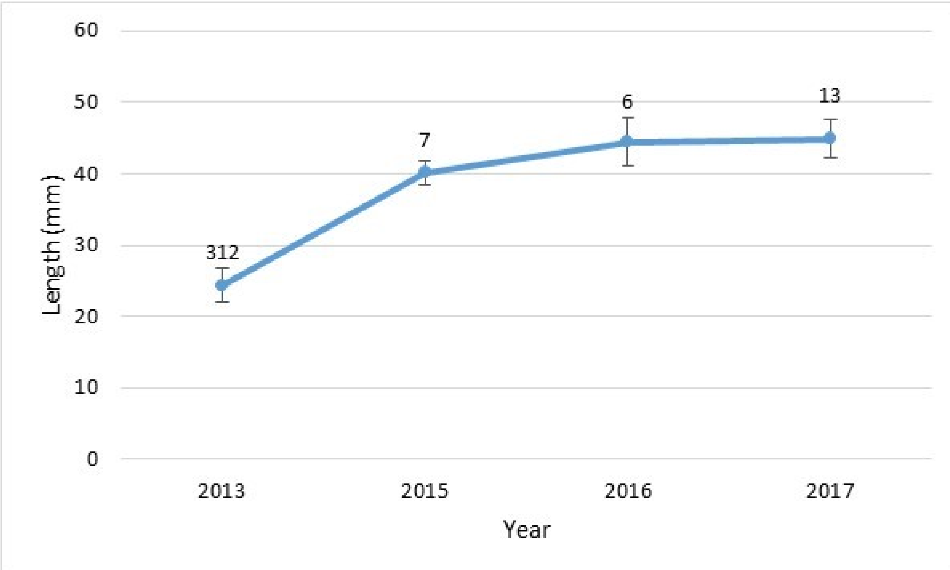


Figure 2.13. Mean lengths of the 2013 cohort of *Villosa vanuxemensis* from 2013 to 2017 in the Clinch River, VA, at the Payne Property and Sycamore Lane. Numbers above means represent sample size.

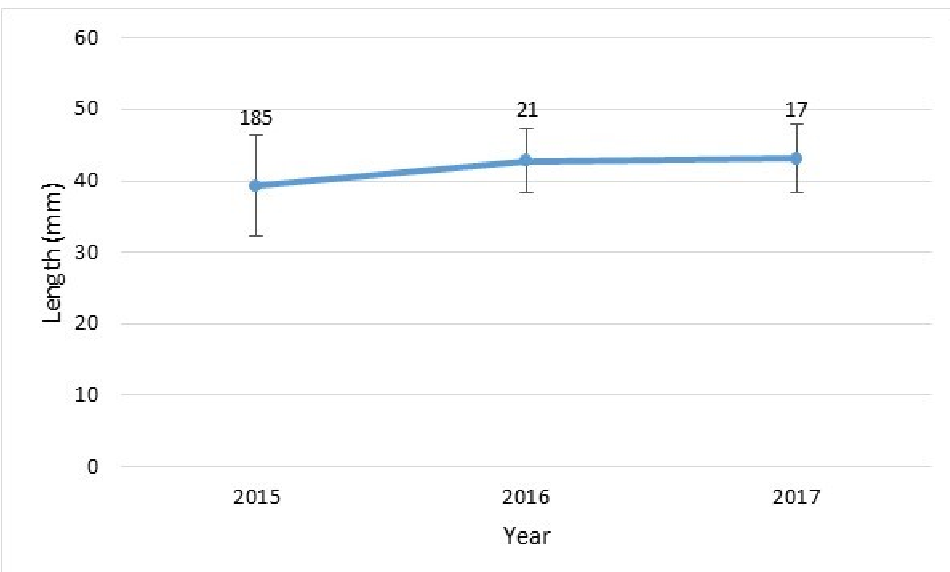


Figure 2.14. Mean lengths of *Villosa iris* sampled during the 2015 mark recapture survey conducted from 2015 to 2017 in the Clinch River, VA, at the Payne Property and Sycamore Lane. Numbers above means represent sample size.

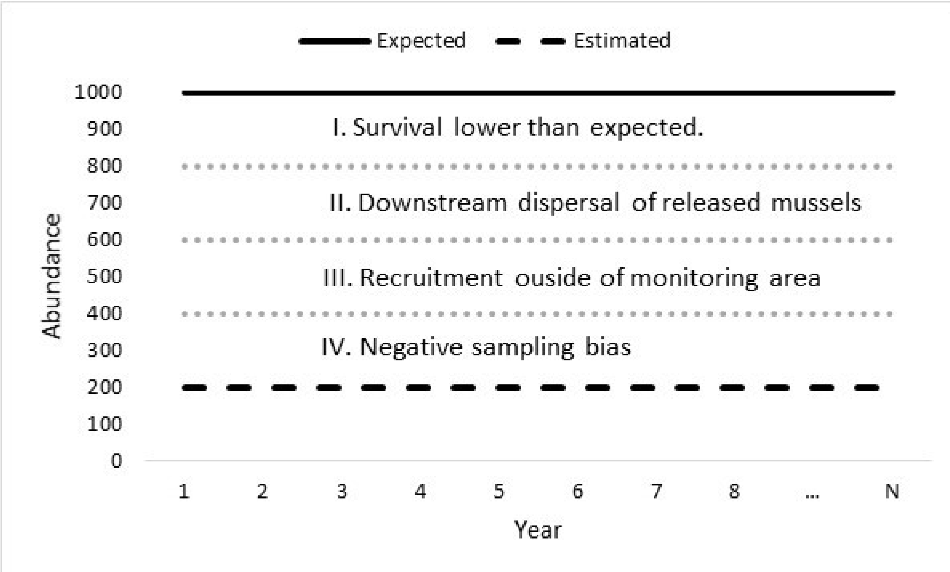


Figure 2.15. Conceptual diagram illustrating why estimated abundance was lower than expected abundance, which was based on expected survival of released mussels as determined from a Leslie matrix model.

Chapter 3

Resource Equivalency Analysis (REA) of freshwater mussel populations injured by the Certus, Inc. and Lone Mountain Processing, Inc. toxic spills in the Clinch and Powell Rivers, VA

Abstract

The Certus, Inc. and Lone Mountain Processing, Inc. (LMPI) Natural Resource Damage Assessment and Restoration (NRDAR) cases represent two of the earliest and largest cases involving injury to freshwater mussels in the United States. The Certus, Inc. case occurred in 1998 in the upper Clinch River at Cedar Bluff, Virginia (VA) in Tazewell County and involved a tanker-truck chemical spill that killed an estimated 18,000 mussels of 13 species, including hundreds of individuals of three species listed as federally endangered. The LMPI case occurred in 1996 in the Powell River and involved a failure of a holding pond in Lee County, VA, which released 6,000,000 gallons of coal slurry, affecting mussel populations over a 65-mile section of river. At the time of each incident, the approach to injury assessment for freshwater mussels was poorly developed. Currently, Habitat/Resource Equivalency Analysis (HEA/REA) are the most common approaches used in NRDAR cases for determining the scale of restoration to restore or replace natural resources and/or ecological services lost due to an injury. We developed a REA to retrospectively analyze injury to mussel populations in terms of discounted mussel-years (DMYs) lost for the Certus, Inc. NRDAR case. We also calculated expected gains as DMYs for hatchery-reared mussels released from 2003 to 2019 to restore populations lost due to the spill. Two REA restoration scenarios were considered for the case. The full-establishment scenario (Full Scenario) assumed that all released mussels immediately established on site and thereafter experienced normal age-specific survival and recruitment rates. The reduced-establishment scenario (Reduced Scenario) assumed that only 25% of released mussels established on site and then experienced normal age-specific survival and recruitment rates. The Reduced Scenario was used to assess restoration requirements for higher-than-normal mortality upon the initial release of mussels. Injury for the LMPI case was more difficult to quantify. It covered a very large area where mussels more often experienced sub-lethal, chronic effects compared to the Certus case, which covered a small area where mussels experienced acute mortality. Consequently, we used only the REA to estimate gains from restoration for the LMPI case and compared this estimate to gains from the Certus case. Results showed that gains from restoration for the Certus, Inc. NRDAR case exceeded the losses from injury under both the Full and Reduced Scenarios (gain-to-loss ratio of 4.6 and 1.2, respectively). However, gains estimated under the Reduced Scenario were much lower than under the Full Scenario. Further research is needed to determine whether this discrepancy is due to higher than-expected mortality versus other causes such as mussel emigration from the restoration site and excystment of juvenile's mussels outside the monitoring area. Our study has clear implications for future NRDAR case development. In the case of emigration, gains should be credited towards off-setting losses, as those mussels are still alive and providing services both inside and outside of the monitoring/restoration area. However, gains should not be credited in the case of high mortality. Gains from restoration in the LMPI case were much lower than gains in the Certus case (1,442,480 DMYs vs.

6,842,634 DMYs, respectively). While we were not able to estimate injury for the LMPI case, given the large area involved (65 miles of stream), the chronic nature of the exposure, and the likely high number of mussels affected, even a small effect on services provided by mussels might result in a large injury over time. Further refinement of our REA is needed to complete analysis when injury is not a single, episodic and acute 100% kill. Regardless, the REA developed in this study should prove useful for future NRDAR cases involving freshwater mussels.

Introduction

Two Natural Resource Damage Assessment and Restoration (NRDAR) cases in the upper Tennessee River basin in Virginia (VA) and Tennessee (TN) are among the first and largest cases in the United States involving injury to freshwater mussels due to release of hazardous substances. The Certus, Inc. chemical spill occurred in 1998 in the Clinch River, VA, and the Lone Mountain Processing, Inc. (LMPI) occurred in 1996 in the Powell River of southwestern Virginia and northeastern Tennessee. Both rivers are tributaries to the Tennessee River and harbor some of the largest and most diverse freshwater mussel faunas in the United States.

The Certus, Inc. chemical spill released 1,350 gallons of Octocure-554 revised, a rubber accelerant, into a tributary of the Clinch River when a tanker truck overturned on U.S. Route 460 in Tazewell County, VA, on August 27, 1998 (U.S. Fish and Wildlife Service 2004). This spill resulted in the complete kill of all mussels in an approximately seven-mile impact zone, from Cedar Bluff, VA downstream to Richlands, VA in the Clinch River (Figure 1.2). A total of 6,207 dead mussels of thirteen species was collected from the surface of the substrate immediately following the spill, including 250 individuals of three federally listed endangered species (Table 3.1). However, detection of mussels is imperfect, as a substantial proportion of the population is buried in the substrate at any given time (Schwalb and Pusch 2007), especially outside of their breeding season. Consequently, a multiplier of three was used to account for buried mussels that died in place in the substrate and were undetected. This resulted in a final injury estimate of 18,621 mussels of thirteen species, which was used as the baseline condition and ultimate restoration target for the Certus, Inc. NRDAR case.

The LMPI spill was the result of the failure of a coal slurry impoundment at a coal processing plant in Lee County, VA, on October 24, 1996 (U.S. Fish and Wildlife Service 2003). Approximately 6,000,000 gallons of coal slurry were released into a series of tributaries of the Powell River. The resulting blackwater (a mixture of water, coal fines, and clay) affected a large stretch of the Powell River, and was ultimately deposited in Norris Reservoir, about 65 miles downstream from the release point. The deposition of coal fines and sediment throughout the affected area had a number of potential effects. Acute mortality of fishes in tributaries at the time of the spill was the most immediate impact. However, resuspension of coal fines during high-flow events likely continued to occur well after the initial spill. Because of resuspension of coal fines weeks to months after the spill, chronic toxicity to mussels was a concern. In addition to direct toxicity from coal fines, sedimentation likely interfered with oxygen exchange and mussel feeding, as coal fines were observed in the guts of mussels collected from the river following the spill. Sedimentation also may have directly suffocated mussels in the affected area. Finally, there was possible indirect loss of mussels due to loss of host fish and degradation of mussel habitat. While it is difficult to quantify the effects of this spill, it was assumed to have contributed to decline of mussel species in the Powell River.

While these two cases were among the largest NRDAR cases involving injury to endangered freshwater mussels, a number of other cases have arisen. In Virginia, for example, release of mercury from the DuPont-Waynesboro Facility from 1929 to 1950 likely has resulted in impacts to mussel populations in the South River downstream of Waynesboro (U.S. Fish and Wildlife Service 2017). This case resulted in a settlement that included $4 million for mussel restoration in the South River and South Fork Shenandoah River. A mussel kill in the Ohio River from the release of hazardous substances from a ferro-alloy manufacturing facility in 1999 resulted in over 990,000 mussels killed over a 20-mile section of river (U.S. Fish and Wildlife Service 2007), and a coal ash spill in the Dan River in 2014 likely affected the federally endangered James Spinymussel (*Pleurobema collina*) and other mussel species (Dan River Natural Resource Trustee Council 2015). The responsible party for the coal ash spill in the Dan River has either completed or is under court order to complete several restoration activities benefiting freshwater mussels, including the removal of the Pigg River Power Dam and the financing and transfer of up to 683 acres to North Carolina and Virginia State Parks (Dan River Natural Resource Trustee Council 2020). Together, these cases demonstrate that injury to freshwater mussel populations is an ongoing concern and future NRDAR cases would benefit from identifying appropriate scientific protocols for assessing injury and determining damages.

Resource Equivalency Analysis (REA) Background

Resource Equivalency Analysis (REA) is a resource-to-resource approach to injury quantification that assumes that services lost and restored are comparable, an approach similar to habitat equivalency analysis (HEA) (National Oceanic and Atmospheric Administration (NOAA) 2006). REA generally refers to a stepwise replacement model for killed or injured species, which first was used in the North Cape NRDAR case (Sperduto, Hebert, et al. 1999; Sperduto, Powers, and Donlan 2003). This approach is consistent with both the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA) and the Oil Pollution Act (OPA) NRDAR regulations, and is explicitly identified in the revised CERCLA regulations (2008). REA calculations using the stepwise replacement model involve basic population modeling, including elements of a Leslie matrix model (Leslie 1945) and associated life tables (see, e.g., Renshaw 1993; Singh and Uyenoyama 2004; Simpfendorfer 2005), with appropriate economic discounting to express the final results in terms of present value. This approach documents how individuals (faunal organisms) are lost per age class over time in a stepwise fashion based on survival rates and longevity, and seeks to measure how much it costs to replace the mussels (including their associated services) that the public lost as a result of the injury. While the common biological unit of measure is the number of individual animals killed (including forgone reproduction into perpetuity), REA also accommodates lethal and sublethal quantities of biomass lost.

Mathematically, a REA for a specific species, *S*, is calculated as:

$$DSYs_s = \sum_{t=0} \frac{N_{s,t}^B - N_{s,t}^I}{(1+r)^t}$$

where $N_{s,t}^B$ and $N_{s,t}^I$ represent the number of individuals in the population at time *t* under baseline and injured conditions, respectively. The *S* can be substituted for the specific species type (e.g., discounted bird-years (DBYs) for birds, DFYs for fish) (see, e.g., Sperduto, Hebert, et al. 1999; Zafonte and Hampton 2005). Here in this chapter, we adopt DMYs for discounted mussel-years. When population-level estimates are unavailable, the numerator can be simplified to account for the direct and indirect mortality as a result of the injury. The *r* represents the discount rate, which is the rate at which society as a whole is willing to trade off present for future benefits. Generally, people would prefer to have services provided by a resource or habitat now rather than at some time in the future. They also expect to be compensated for having lost services that should have been available in the past. The net benefits from short-term and longer-term restoration options can be compared only when converted to the same timeframe – present value (i.e., by discounting). REA can be adjusted to represent any time interval (e.g., years, months, or days) (Hampton and Zafonte 2006). The standard 3% annual discount rate is used in this analysis.

Case teams for NRDAR typically decide to use the REA model because of its: (1) appropriate focus on individuals killed and their replacement, (2) relatively reliable results that are transparent and reproducible, and (3) cost-effectiveness. More specifically, the current state-of-the-art REA has:

1) *Appropriate Focus.* As noted across the REA literature, the number of individuals killed in an incident can be counted or estimated. Although lost individual-years (e.g., mussel-years, bird-years, or fish-years) can be difficult to observe, simulations and arguments in the literature suggest that removing even a small number of individuals from a population can produce persistent impacts (e.g., Sperduto, Hebert, et al. 1999; Zafonte and Hampton 2005). Thus, it seems reasonable for the natural resource trustees responsible for assessing damages to focus on individuals killed using REA in developing a claim for damages.
2) *Relatively Reliable Results.* The public's valuation of a resource is not necessarily equal to the total replacement cost identified in a REA in the case of unique and scarce resources. Zafonte and Hampton (2007) conducted experiments to explore the degree to which violations of REA assumptions can result in either under-compensation or over-compensation to the public. Specifically, they examined whether the results of compensatory restoration (a term used in OPA; the CERCLA parallel is "the cost of projects that compensate for services lost pending restoration" (i.e., interim losses)[1]) diverged from those of monetized settlements. They found that the results of a traditional REA are consistent with that of a monetized approach except in cases where the demand for resources is inelastic (i.e., no substitutes) and the impact to local resources is severe (public values are likely affected). Zafonte and Hampton (2007) asserted that their results suggest that "the welfare biases intrinsic to a traditional REA methodology are probably minor for many NRDA cases". In sum, REA applies basic ecological concepts within a standard economic framework to provide reliable estimates of restoration projects that compensate for interim losses for many NRDAR cases.
3) *Cost-Effective Assessment.* Like HEA, a standard REA can be run and reviewed by all stakeholders, often using existing literature. Certain species require more local study, so even HEAs and REAs can become more expensive in those situations. "However, because it is easier and less costly to measure the total replacement cost than the total public value, REA has an advantage over other methods, especially for small to medium-sized incidents with minimal impact on rare species" (Kure Trustee Council 2008).

Despite its common use in NRDAR cases today, there are few published case studies that use REA/HEA for damage assessment in the peer-reviewed literature (Fonseca, Julius, and Kenworthy 2000; Penn and Tomasi 2002). One notable example is the 1997 Texaco Pipeline case in Lake Barre, Louisiana, which was one of the earliest uses of HEA in a NRDAR case (Penn and Tomasi 2002). In this spill, 6,561 barrels of crude oil were discharged into Lake Barre and affected about 1,750 hectares (ha) of marsh in total. Using a HEA approach, it was determined that planting 7.5 ha of additional marsh would compensate for the injured marshland. The low amount of restoration required relative to the injury was because only a small section of the 1750 ha was heavily oiled, while the majority of the affected area was only lightly

[1] https://www.govinfo.gov/content/pkg/FR-2008-10-02/html/E8-23225.htm

affected and expected to recover within 4 months. Further, Trustees expected that an additional 15.9 ha of marsh would become established due to vegetation spreading from the planted 7.5 ha. Since 1997, habitat and resource equivalency analyses have become common methods of estimating damages due to oil and chemical spills under NRDAR regulations (Fonseca, Julius, and Kenworthy 2000; Zafonte and Hampton 2007; Shaw and Wlodarz 2013). We believe more case studies are needed to further develop these methods, especially as regards NRDAR cases involving injury to freshwater mussel populations.

Objectives

Resource equivalency analysis has become a common method of calculating injury and damages in NRDAR cases (Zafonte and Hampton 2007) due to its relative simplicity compared to other valuation methods and its greater reliance on measurable endpoints from peer-reviewed literature (Thompson 1992; Jones and DiPinto 2018). However, the use of REA for injury and damages determination specific to freshwater mussels had not been developed at the time of the LMPI, Inc. NRDAR case and was underdeveloped during the Certus, Inc. NRDAR case. This background, along with the increasing number of NRDAR cases involving injury to freshwater mussels, warranted the development of a standardized REA for use specifically with freshwater mussel NRDAR cases. Therefore, the objectives of this chapter were to:

1) use a REA to retroactively analyze injury to mussel populations in the upper Clinch River, VA due to the Certus, Inc. chemical spill,
2) calculate the credit expected as a result of actual mussel restoration efforts from 2003 to 2019 (Full Scenario),
3) calculate the credit expected as a result of actual mussel restoration efforts from 2003 to 2019, but assuming that 75% of released mussels did not initially establish at release sites (i.e., only 25% of released mussels follow age-specific survival and recruitment rates in standard scenario) (Reduced Scenario), and
4) determine whether gains from mussel restoration in each scenario have equaled loss from injury.

We chose to focus our analysis on the Certus, Inc. NRDAR case due to its relative simplicity. The injury to mussels for Certus was easily quantifiable because it represented a single acute impact (e.g., 18,621 mussels killed). In contrast, the injury to mussels for the LMPI, Inc. NRDAR case likely was chronic and sublethal and was not quantified at the time of the spill. While we did not analyze the injury to mussel populations as a result of the LMPI chemical spill, we did conduct a REA to calculate the credit expected as a result of actual mussel restoration efforts in the Powell, River, VA and TN, and therefore we include herein a fifth objective:

5) calculate the credit expected as a result of actual mussel restoration efforts from 2003 to 2019 for the LMPI, Inc. coal slurry spill.

Methods

Resource Equivalency Analysis

We developed a REA model to estimate injury as total discounted mussel-years (DMYs) lost resulting from the kill of all mussels that occurred in the upper Clinch River during the Certus, Inc. chemical spill. We also used this REA to estimate gains from mussel restoration programs conducted by the Virginia Department of Wildlife Resources' Aquatic Wildlife Conservation Center (AWCC) and Virginia Tech's

Freshwater Mollusk Conservation Center (FMCC) for the Certus, Inc. and LMPI, Inc. NRDAR cases. For injury in the Certus case, we estimated the total expected DMYs lost by calculating the direct injury to the local mussel assemblage (i.e., populations of all species present at a site) present at the time of the incident and the indirect injury to the first and second generations of mussels that would have resulted from reproduction in lieu of the incident. The inclusion of one or more generations is at the discretion of Trustees based on their case-specific situation. For gains in the Certus, Inc. case, we performed two analyses. The first assumed that all released mussels successfully established at release sites and were subject to REA model assumptions, termed the full-establishment scenario (Full Scenario). The second analysis assumed that only 25% of released mussels successfully established and were subject to REA model assumptions, termed the reduced-establishment scenario (Reduced Scenario). For all analyses, the REA was conducted separately for each species injured or released.

Background and Assumptions

The REA uses a Leslie matrix population model to determine the expected number of mussels in each year had the incident not occurred. Age-specific survival rates were modified from those of Jones, Neves, and Hallerman (2012). Robust estimates of age-specific fecundity are not generally available for freshwater mussels. Consequently, we calculated the recruitment rate to the age-0 year class necessary to result in a stable age distribution, given the age-specific survival rates (see Figure 3.1 for the life-cycle diagram used in the Leslie matrix). For example, a recruitment rate of 0.1 and a breeding population with 1000 mussels would result in 100 recruits to the age-0 year class.

We assumed that mussels began breeding after entering the age-5 year class and that all age classes above 5 years contributed equally to recruitment. We also assumed that survival and recruitment were constant over time, resulting in a deterministic demographic model (i.e. we did not incorporate stochasticity). Finally, we used a discount rate of 3% for all analyses, a rate widely used in REA models and supported in the literature (National Oceanic and Atmospheric Administration (NOAA) 2006). This rate allows for injury and restoration at different time-periods to be compared in terms of present value (Desvousges et al. 2018)

Injury Calculation

The key inputs for injury determination are the estimated size of the total injured population, the number of species injured, the maximum age of each species, and the estimated number of mussels in each age class. Ideally, the total injured population should be estimated on site immediately after the incident using appropriate quantitative survey methods. Ideally, maximum age of species can be determined using shell thin-section data (Neves and Moyer 1988) of dead mussels from the injured population. If thin-section age data are unavailable, general life-history data available for a particular species (Rogers, Watson, and Neves 2001; Haag and Rypel 2011; Jones and Neves 2011) or expert opinion can be used instead. Ideally, the number of mussels in each age class should be estimated at the same time as the total injured population. However, if these data are unavailable or unreliable, a general age-class distribution for a given maximum age can be used. This general age-class structure is a result of a stable age-class distribution given the recruitment and age-specific survival rates used in the REA.

Other inputs include the start years for analysis, the first year in which reproduction was affected, and the base year used for discounting. The start year for analysis is the first year for which injury was estimated and typically is the same year as the incident. The start year for reproduction is the first year that reproduction was affected by an incident. This may be the same year as the incident or the next year, depending on whether reproduction has already occurred during the incident year. Finally, the base year for the discount rate is the year in which the discount rate will be one, which is the year that the analysis is conducted to ensure that all injury is quantified in present value.

Total injury is divided into direct loss (i.e., DMYs lost from mussels that were actually killed in a spill) and indirect loss (i.e., DMYs lost from mussels that would have been produced in the first and second generations). Direct loss is calculated by distributing the injured population into age classes based on either on-site estimates of the percentage of the population in each age class or a generalized age distribution of a stable population. Age-specific survival rates are used to determine the number of mussels in each age class for each subsequent year after the injury year, until no more individuals of the original injured population survive, which depends on the maximum age of the species. For each year, the total number of mussels is multiplied by the discount factor, which is one minus the discount rate (e.g., 3%) raised to the t power for that year (i.e., time t) to determine the total DMYs lost for that year in terms of present value. The discounting is shown in the equation presented above. The total direct loss is determined by summing the total DMYs lost for all years.

Indirect loss from the first generation is calculated by using the number of breeding-age mussels in each year (age 5 to maximum age) and the expected number of DMYs provided by a single recruit to the age-0 year class. The number of breeding-age mussels in each year is determined from the direct loss calculation. This number is multiplied by the recruitment rate to determine the expected number of mussel recruits in each year (age-0 year class). Next, the expected number of recruits each year is first multiplied by the expected DMYs provided by a single recruit produced in the first year that reproduction is affected over its lifetime, then multiplied by 0.97087, the discount rate per year. This is an expediting method to extrapolate into the future while limiting rounding error. Finally, the DMYs provided by the first generation in each year is summed for all years until no first generation mussels survive. Indirect loss from the second generation is calculated similarly to first generation loss. However, the number of breeding mussels in each year is based on the first-generation of recruits. This is determined by applying the age-specific survival rates to the first generation of recruits.

The expected DMYs per recruit is based on the discount rate and age-specific survival rates, and is determined by tracking a single hypothetical recruit that would have been produced in the first year an incident affected reproduction, if the incident had not occurred. For example, in the absence of the incident, a single recruit from the first year that reproduction was affected would be expected to produce 1 DMY if it survived until the end of its first year and if the base year for discounting was also the first year (Table 3.2). However, a mussel recruit has a survival rate of 0.3 to the age-1 year class, so the expected DMYs for that year is only 0.3. The expected DMYs are determined each year for the lifespan of the recruit (i.e., until maximum age) by multiplying the probability of the recruit surviving until that year by the discount factor of that year. To continue the example above, the expected discounted mussel-years in the second year would be calculated as follows:

$$\widehat{SY_2} = S_2 * D_2$$

where $\widehat{SY_2}$ is the expected DMYs provided in year 2, S_2 is the probability of surviving until year 2, and D_2 is the discount factor for year 2. Thus, $\widehat{SY_2}$ would be:

$$(0.3 * 0.95)(0.97) = 0.277.$$

The expected DMYs for each year are summed to determine the total DMYs expected from one recruit.

Gain Calculation

The inputs for calculation of gains from restoration are the expected number of mussels to be released each year of restoration, age upon release, the number of years restoration will occur, and the start year of restoration. Alternatively, one may allow for a variable number of releases in each year. All other inputs are identical to the injury calculation and include age-specific survival rates, base year for discounting, discount rate, and recruitment rate.

Total gains from restoration are calculated by applying the Leslie matrix projection (age-specific survival rate and recruitment) to the expected number of mussels released per year in each age class. The total number of mussels in each year is multiplied by the discount factor for that year to calculate DMYs in present value. The total DMYs for each year are summed through the 119th year from the base discount year and represent gains into perpetuity. Mathematically, a 3% discount rate leads to measurable gains over 119 years. Potential restoration programs can be tested until gains into perpetuity equal total losses.

Retrospective REA of Certus, Inc. NRDAR case

The REA was used to calculate the total injury for each species killed in the Certus chemical spill. Inputs for the total injured local population of each species came from the U.S. Fish and Wildlife Service (2004) estimate of mussels killed (see Chapter 1, Table 1.1). A general age class distribution was used to estimate the number of mussels in each age class. The start year for reproduction was 1999 because the spill occurred in late 1998 when most reproduction for 1998 had already occurred. The base year for discounting was 2020 to ensure that DMYs were in present value (PV) and to facilitate comparisons between the Certus and LMPI, Inc. NRDAR cases. The total injury was divided into direct and indirect losses and reported as discounted mussel-years (DMYs). Direct losses included all losses expected if the mussels (18,621) had not been killed. Indirect losses included losses from the expected first and second generations of mussels that would have been produced but for the kill. In addition to total injury across all years, injury was also calculated for each year in the analysis to determine over what time period the injury occurred.

For each injured species, mussel release data were used to determine the actual gains from mussel restoration activities conducted from 2003–2019. Gains were reported as DMYs and divided into four categories based on the site location of releases. Gains were partitioned into the following categories (see Figure 3.2):

1) sites in the immediate impact zone of the Certus, Inc. spill,
2) sites in Indian Creek, a Clinch River tributary directly adjacent to the impact zone,
3) a combination of the impact zone and Indian Creek sites, and
4) all other mussel release sites in the Clinch River located downstream in Russell County, VA.

Monitoring of release sites suggests that abundances of mussels are much lower than expected (~75% lower on average) based on the assumptions of our REA (see Chapter 2, Table 2.8). This low abundance may be due to high initial mortality upon release or downstream dispersal of mussels outside of the monitoring area. Consequently, we calculated gains under two scenarios. The full-establishment scenario (Full Scenario) assumed that all mussels released were subject to the assumptions of the REA (i.e., age-specific survival and recruitment rates). The reduced-establishment (Reduced Scenario) scenario assumed that only 25% of mussels released were subject to the REA model assumptions. That is, 75% of released individuals were removed from the virtual population at the beginning of the analysis to account for failure of most released mussels to establish at restoration sites.

The difference between total injury and total gain in mussel-years for each species was calculated and reported as a ratio of gain to injury for each scenario. Finally, injuries and gains in each year of the analysis were compared for each scenario, which included a total of 12 species that were not injured in the chemical spill, but were propagated and released from 2003 to 2019. Sites outside of the impact zone in the Clinch River in Russell County, VA, were chosen to reduce the risk of stocking mussels only at a single, relatively short, urban stream reach between Cedar Bluff and Richlands, VA. Species released at these downstream sites included species that occurred at those additional sites but were not injured in the impact zone of the Certus, Inc. chemical spill. Some of these species also acted as surrogate species for injured species that could not be propagated successfully at the time. For example, *Epioblasma brevidens*, *E. capsaeformis*, and *E. triquetra* served as surrogates for *E. aureola*. Gains from these species were calculated using the same methodology as for injured species and reported separately.

Retrospective REA of LMPI, Inc. NRDAR case

We used the REA to calculate the expected DMYs gained as a result of restoration in the Powell River, VA and TN (Figure 3.3). The spill occurred in late 1996; hence, the start year for reproduction was 1997, and 2020 was used as the base year for discounting (to facilitate comparisons between Certus and LMPI NRDAR cases). Mussel release data were used to determine actual gains from restoration conducted from 2004–2014. All gains were reported as DMYs. Injury was not estimated due to insufficient data to estimate injury to the affected mussel populations in the Powell River.

Results

Certus, Inc. REA

Total injury

Total injury into "perpetuity" (i.e., 119 years after base year for discounting) as a result of the Certus, Inc. chemical spill was estimated to be a loss of 714,025 DMYs (Table 3.3). Direct injury to the mussel assemblage was estimated as 290,900 DMYs, and indirect injury was estimated as 423,125 DMYs. *Villosa iris* was the most injured species, followed by *Lampsilis fasciola* and *Ptychobranchus subtentus*.

Total injury began in 1999 at 52,940 DMYs and decreased exponentially by year to less than 20 DMYs by 2078 (Figure 3.4). Direct injury as a percentage of total injury was highest in 1999 at 59%, decreased until 2028, increased until 2048, and finally decreased to 0 in 2078. The increase in the proportion of indirect to direct injury beginning in 2028 is a result of the two most injured species (*V. iris* and *L. fasciola*) both being modeled with maximum ages of 30 years. Consequently, 2028 was the last year with direct injury to these two species and subsequent years were comprised of a greater percentage of indirect injury.

Actual gains from species injured in Certus, Inc. chemical spill

Total actual gains from the mussel restoration program were estimated to be 3,026,780 DMYs for the Full Scenario and 783,770 for the Reduced Scenario (Table 3.4). This number includes gains only from species that were injured in the Certus, Inc. chemical spill (see Table 3.1). By species, the highest gains for both scenarios were from *Villosa vanuxemensis*, *Lampsilis fasciola*, and *V. iris*. Most of the gains (~73%) for both scenarios occurred at restoration sites either in the immediate impact zone of the Certus, Inc. chemical spill (1,978,887 and 485,229 DMYs for Full and Reduced Scenarios, respectively) or Indian Creek (358,748 and 89,964 DMYs for Full and Reduced Scenarios, respectively), a nearby tributary to the Clinch River unaffected by the spill. About 26% (786,584 and 196,651 DMYs for Full and Reduced Scenarios, respectively) of gains from restoration of species injured in the spill occurred at other sites in the Clinch River in Russell County, VA, 40 miles downstream of the impact zone, where mussels were released to reduce risk of restoring species to only one short, urban stream reach in Tazewell County.

Injury vs. gain

Overall, gains from restoration were much higher than losses due to injury from the Certus Inc. chemical spill for the Full Scenario and somewhat higher for the Reduced Scenario (Figure 3.5). For species that were successfully propagated and released, the ratio of gains to injury ranged from 0.5 for *Lasmigona costata* to 471.3 for *Villosa iris* (Table 3.5) for the Full Scenario. The ratio of gains to injury ranged from 0.1 for *Lasmigona costata* to 117.8 for *Villosa iris* for the Reduced Scenario. Of the 14 species injured during the Certus, Inc. chemical spill, three were not successfully propagated and released, including *Pleuronaia barnesiana*, *Pleurobema oviforme*, and *Quadrula strigillata*. *Epioblasma aureola* was successfully propagated and released only in 2017. The overall ratio of gains-to-loss for injured species was 4.2 under the Full Scenario and 1.1 under the Reduced Scenario. Under the Full Scenario, only *L. costata* had a ratio less than one for the released mussels. In contrast, *L. costata*, *Actinonaias pectorosa*, *V. iris*, and *Epioblasma aureola* had ratios less than one under the Reduced Scenario.

Gains from species not injured in Certus, Inc. chemical spill

Total actual gains from species not injured in the Certus, Inc. chemical spill were 3,815,854 DMYs under the Full Scenario and 987,275 DMYs under the Reduced Scenario, compared to the 3,026,780 DMYs gained from injured species. As such, these gains accounted for more than half (55.7%) of all gains from restoration efforts of the Certus, Inc. NRDAR case. The majority of these gains for the Full Scenario came from mussels released in Russell County, VA, primarily from *Epioblasma brevidens* and *E. capsaeformis* with 1,937,100 DMYs and 1,547,013 DMYs, respectively, for the Full Scenario (Table 3.6) and 484,275 DMYs and 386,753, respectively, for the Reduced Scenario.

LMPI, Inc. REA

Total gains from restoration

Total actual gains from the LMPI mussel restoration program at sites in the Powell River were estimated to be 1,442,480 DMYs (Table 3.7). By species, the highest gains were from *Epioblasma capsaeformis*, *E. brevidens*, and *Villosa iris*.

Discussion

Freshwater mussels are relatively sessile as adults, and even more so as juveniles, making them particularly susceptible to injury from release of a hazardous substance compared to other aquatic fauna. Further, 65% of the extant mussel species in North America are either endangered, threatened, or vulnerable (Haag and Williams 2014). A single chemical spill has the potential to kill the last population of some species. Indeed, the Certus, Inc. chemical spill destroyed one of only two remaining populations of *Epioblasma aureola*, and this species was successfully propagated only in 2017 and from but a few individuals. In several cases, injury to mussels has been chronic and/or covered a large area (U.S. Fish and Wildlife Service 2003; U.S. Fish and Wildlife Service 2007; Dan River Natural Resource Trustee Council

2015; U.S. Fish and Wildlife Service 2017). In such cases, injury to mussels (as DMYs lost) can be potentially quite large, and the restoration required to recover losses may either be infeasible or very challenging and would require substantial resources.

Our REA shows that DMYs gained from mussel restoration under both the Full and Reduced Scenarios exceeded DMYs lost; therefore, more effort was put into restoration activities than was needed to replace both direct and indirect losses due to the Certus chemical spill. However, monitoring at release sites has indicated that far fewer mussels have survived and established at restoration sites than expected based on the REA. This outcome can be partially accounted for by higher than expected mortality, emigration of released mussels from the monitoring area, as well as excystment of juvenile mussels outside of the monitoring area, although the extent of these factors cannot be quantified at this time. Another source of the discrepancy between REA predicted abundance and estimated abundance from monitoring may be lower survival rates of propagated mussels compared to wild mussels. This lower survival may occur for years after release, or it might be limited to a short period of higher mortality immediately after release. The degree to which the discrepancy can be attributed to these differing causes is highly relevant to future application of the REA to assessment of mussel injury. Gains from emigrating mussels or excystment of juveniles outside of the monitoring area should be accounted for. However, if higher mortality of propagated mussels compared to wild populations is the main factor, then future refinement of the REA should account for varying survival rates between propagated and wild mussels. Further study is needed to conduct a more in-depth examination of the factors affecting survival and establishment of propagated mussels at restoration sites. Regardless, even in the Reduced Scenario, which assumes very high initial mortality of released mussels and more accurately reflects monitoring data, the DMYs gained were still somewhat greater than DMYs lost due to injury.

Our REA analysis of the gains from the LMPI restoration program suggests that far fewer gains were realized as part of restoration efforts compared to the Certus, Inc. NRDAR case (1,442,480 DMYs vs. 6,842,634 DMYs). Injury for the LMPI case was chronic and sublethal. However, given the large area over which the injury occurred (~65 miles of stream reach) and the potential number of mussels affected, even a very small effect (e.g., 1%) on juvenile recruitment or adult mortality over time might result in a relatively large loss of DMYs. When compared to the injury over the relatively small area of the Certus case (~7 miles of stream), gains from restoration for the LMPI case may be less than needed to recover losses due to the release.

We focused our analysis on the Certus, Inc. chemical spill, because this case was characterized by an acute kill event of all mussels in a small section of river. This type of injury (i.e., acute effect from a short-term release) is among the most straightforward to quantify (Dunford, Ginn, and Desvousges 2004). The injury for this case was relatively easy to quantify with a census of fresh-dead mussels. In contrast, injury in the LMPI spill was characterized by chronic, sublethal effects that were not quantified at the time of the incident. However, given the spatial extent of river affected, the injury to mussels likely was large in terms of DMYs. In such cases, the necessary compensation needed for gains from restoration to equal losses from injury can be more difficult to determine. Unfortunately, such cases are not unheard of. In the Dupont NRDAR case for example, mercury was released into the South River from 1929 to 1950, but was not discovered until the 1970s (U.S. Fish and Wildlife Service 2017). In this case, quantification of injury was challenging because the initial releases occurred so long ago that baseline conditions, as well as injury before the release was recognized, were not well documented and because mercury still persisted in the system after multiple or high discharge events. Further, it was necessary to separate the effects of the mercury release from declines in mussel populations due to nearby urbanization and agriculture. REA can be adapted to allow increases in mortality and decreases in recruitment over time, rather than a simple 100% mortality rate at a single point in time. For example, one could estimate that a spill would cause a 5% increase in mortality over 20 years or a 1% decrease in recruitment for 10 years. To use a REA in a case where injury is long-term or potentially sub-lethal, a robust method of estimating how mortality/recruitment are likely to be affected by a spill and over what time frame is needed.

The discount rate in a REA allows for injury and restoration at different time periods to be compared in terms of present value (Desvousges et al. 2018). In the context of REA, a service provided (i.e., DMY) in the present is worth more than a service provided in the future. However, choice of discount rate can greatly affect the results of a REA and the consequent restoration needed. If all other inputs are the same, a higher discount rate will result in more restoration needed for gains to equal losses because the injury in the present is weighted more highly than restoration that might occur in the future. Conversely, a lower discount rate will weight future gains and losses more highly than a higher discount rate. In our analysis for example, the ratio of gains to injury for *Villosa iris* was 1.131. Using a discount rate of 4% instead of 3%, that ratio decreases to 0.82, suggesting that restoration was not sufficient. The National Oceanic and Atmospheric Administration recommends a discount rate of 3% for use with REA (NOAA 1999), and this is the rate most commonly used for NRDAR cases in the United States. However, discount rates in the European Union range from 3 to 6% (Shaw and Wlodarz 2013). Evans (2006) recommends a standard rate of 3 to 4% for the European Union, and the European Commission recommends a similar rate of 4% (European Commission 2005). World Bank projects commonly use even higher discount rates due to higher perceived risk of projects in developing countries (Lopez 2008; Shaw and Wlodarz 2013). Economists have long debated the appropriate discount rate when there are intergenerational (long-term) considerations (Cropper 2012; Office of Management and Budget 2021). Given mussels are long-lived species and the sensitive nature of mussel restoration outcomes (i.e., how successful they are on a long time-scale, such as >20 years), it might be appropriate to consider alternatives to the typical 3% discount rate.

Based on our REA using a 3% discount rate, it appears that mussel restoration for the Certus, Inc. NRDAR case was successful. Gains from restoration were greater than lost DMYs for most injured species. It should be noted that two facilities were needed to ensure that gains from restoration met lost DMYs for the Certus, Inc. NRDAR case. It is unlikely that one facility alone would have achieved the restoration goal for this case. In addition, a substantial number of DMYs were gained from augmentation of non-injured species at sites outside of the impact zone of the chemical spill. However, there is still uncertainty about survival of propagated mussels at restoration sites and how it compares to assumptions within the REA. Further work is needed to develop a more flexible REA for use when injury is chronic in nature and not a single, 100% kill that is more easily understood and quantified.

Acknowledgments

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Tables

Table 3.1. Mussel age data and kill estimates from the Certus, Inc. chemical spill that occurred in the Clinch River at Cedar Bluff, Tazewell County, VA, on August 27, 1998.[1]

Species	Min. Age	Max. Age	Mean Age	Collected	USFWS Kill Estimate
Actinonaias pectorosa	6	32	15.5	135	405
Epioblasma aureola	2	11	4.9	178	534
Lampsilis fasciola	8	33	18.5	962	2,886
Lampsilis ovata	5	38	14.2	62	186
Lasmigona costata	4	33	16.5	84	252
Medionidus conradicus	2	14	6.2	219	657
Pleuronaia barnesiana Pleurobema oviforme	4	51	18.8	610	1,830
Ptychobranchus fasciolaris	7	85	31.0	579	1,737
Ptychobranchus subtentus	9	55	21.9	35	105
Theliderma strigillata	11	63	44.5	20	60
Venustaconcha trabalis	4	29	11.3	52	156
Villosa iris	2	20	7.2	3,247	9,741
Villosa vanuxemensis	6	22	11.4	24	72
Total				6,207	18,621

[1] U.S. Fish and Wildlife Service (2004).

Table 3.2. Example calculation of expected discounted mussel-years (DMYs) from a single recruit (maximum age of 20) when base year for discounting is year 0 and the discount rate is 3%. The number of digits provided illustrate the calculations and do not represent actual precision of these estimates.

Year	Discount Factor	Probability of surviving to each year	DMYs present value
0	1.000	0.300	0.300
1	0.971	0.285	0.277
2	0.943	0.271	0.255
3	0.915	0.257	0.235
4	0.888	0.244	0.217
5	0.863	0.232	0.200
6	0.837	0.221	0.185
7	0.813	0.210	0.170
8	0.789	0.199	0.157
9	0.766	0.189	0.145
10	0.744	0.180	0.134
11	0.722	0.171	0.123
12	0.701	0.154	0.108
13	0.681	0.131	0.089
14	0.661	0.104	0.069
15	0.642	0.078	0.050
16	0.623	0.055	0.034
17	0.605	0.036	0.022
18	0.587	0.021	0.013
Total			2.783

Table 3.3. Total injury in present value of discounted mussel-years (DMYs) for each species directly affected by the Certus, Inc. chemical spill that occurred in the Clinch River at Cedar Bluff, Tazewell, VA, on August 27, 1998.

	DMYs Lost		
Species	Direct	Indirect	Total
Actinonaias pectorosa	4,457	7,986	12,443
Epioblasma aureola	3,390	7,660	11,050
Lampsilis fasciola	43,201	65,796	108,997
Lampsilis ovata	3,215	4,461	7,676
Lasmigona costata	3,772	5,745	9,517
Medionidus conradicus	5,897	10,598	16,495
Pleurobema oviforme	606	747	1,354
Pleuronaia barnesiana	36,992	45,576	82,568
Ptychobranchus fasciolaris	37,354	43,628	80,981
Ptychobranchus subtentus	2,009	2,636	4,645
Theliderma strigillata	1,213	1,494	2,707
Venustaconcha trabalis	2,335	3,557	5,892
Villosa iris	145,813	222,080	367,893
Villosa vanuxemensis	646	1,161	1,808
Total	290,900	423,125	714,025

Table 3.4. Total discounted mussel-years (DMYs) gained at sites in the Clinch River, VA, as a result of mussel restoration conducted from 2003–2019 for the Certus, Inc. NRDAR case using two restoration scenarios, Full and Reduced (see Methods). Estimates include only gains from species that were injured in the Certus, Inc. chemical spill that occurred at Cedar Bluff, VA, in 1998. Impact zone includes sites in the immediate vicinity of the Certus, Inc. chemical spill at Cedar Bluff (River Mile 324) downstream to Richlands, VA (River Mile 318). Indian Creek includes sites in this tributary located in Cedar Bluff. Other Sites includes sites located in the Clinch River, Russell County, VA, 40 miles downstream of the impact zone.

	Impact Zone		Indian Creek		Impact and Indian		Other		Total	
Species	Full	Reduced	Full	Reduced	Full	Reduced	Full	Reduced	Full	Reduced
Actinonaias pectorosa	14,136	3,534	31,144	7,786	45,280	11,320	619	155	45,899	11,475
Epioblasma aureola	22,524	5,631	16,481	4,120	39,006	9,751	0	0	39,006	9,751
Lampsilis fasciola	366,756	91,689	87,205	21,801	453,961	113,490	327,315	81,829	781,276	195,319
Lampsilis ovata	230,492	57,623	26,843	6,711	257,335	64,334	70,226	17,557	327,561	81,890
Lasmigona costata	3,987	997	163	41	4,150	1,038	512	128	4,662	1,166
Medionidus conradicus	210,932	52,733	27,127	6,782	238,059	59,515	18,069	4,517	256,128	64,032
Pleurobema oviforme	0	0	0	0	0	0	0	0	0	0
Pleuronaia barnesiana	0	0	0	0	0	0	0	0	0	0
Ptychobranchus fasciolaris	96,450	24,112	7,602	1,901	104,052	26,013	8,481	2,120	112,533	28,133
Ptychobranchus subtentus	87,514	21,879	0	0	87,514	21,879	4,211	1,053	91,725	22,931
Theliderma strigillata	0	0	0	0	0	0	0	0	0	0
Venustaconcha trabalis	16,796	3,978	11,662	2,916	28,459	7,115	67,402	15,631	95,861	23,965
Villosa iris	514,944	128,736	11,037	2,759	525,981	131,495	2,495	624	427,904	132,119
Villosa vanuxemensis	415,465	94,317	139,509	34,877	554,974	138,743	292,151	73,038	851,950	212,988
Total	1,979,997	485,229	358,774	89,694	2,338,772	584,693	791,481	196,651	3,034,506	783,770

Table 3.5. Difference between discounted mussel-years (DMYs) lost from the Certus, Inc. chemical spill and DMYs gained from restoration in the Clinch River, VA, from 2003–2019 for each species injured using two restoration scenarios, Full and Reduced (see Methods). Estimates include only gains from species that were injured in the Certus, Inc. chemical spill and restored at all sites in the Clinch River, VA, including species restored to sites located 40 miles downstream in Russell County, VA.

	Total DMYs Lost	Total DMYs Gained		Difference from DMYs Lost		Gain:Loss	
Species		Full	Reduced	Full	Reduced	Full	Reduced
Actinonaias pectorosa	12,443	45,899	11,475	33,456	-968	3.7	0.9
Epioblasma aureola	11,050	39,006	9,751	27,955	-1,299	3.5	0.9
Lampsilis fasciola	108,997	781,276	195,319	672,279	86,322	7.2	1.8
Lampsilis ovata	7,676	327,561	81,890	319,885	74,214	42.7	10.7
Lasmigona costata	9,517	4,662	1,166	-4,855	-8,352	0.5	0.1
Medionidus conradicus	16,495	256,128	64,032	239,634	47,538	15.5	3.9
Pleurobema oviforme	1,354	0	0	-1,354	-1,354	0.0	0.0
Pleuronaia barnesiana	82,568	0	0	-82,568	-82,568	0.0	0.0
Ptychobranchus fasciolaris	80,981	112,533	28,133	31,552	-52,848	1.4	0.3
Ptychobranchus subtentus	4,645	91,725	22,931	87,080	18,286	19.7	4.9
Theliderma strigillata	2,707	0	0	-2,707	-2,707	0.0	0.0
Venustaconcha trabalis	5,892	95,861	23,965	89,969	18,073	16.3	4.1
Villosa iris	367,893	427,904	132,119	60,011	-235,774	1.2	0.4
Villosa vanuxemensis	1,808	851,950	212,988	850,143	211,180	471.3	117.8
Total	714,025	3,034,506	783,770	2,320,481	69,745	4.2	1.1

Table 3.6. Total discounted mussel-years (DMYs) gained as a result of restoration of species not injured in the Certus chemical spill at sites in the Clinch River, VA, located 40 miles downstream in Russell County from 2003–2019 using two restoration scenarios, Full and Reduced (see Methods). *Released in Indian Creek, Tazewell County, VA.

Species	Total DMYs	
	Full	Reduced
Cyprogenia stegaria	2,126	532
Dromus dromas	455	114
Epioblasma brevidens	1,937,100	484,275
Epioblasma capsaeformis	1,547,013	386,753
Epioblasma triquetra	120,514	30,128
Eurynia dilatata	44,415	11,104
Fusconaia cor	7,826	1,957
Lasmigona holstonia*	64,456	16,114
Lemiox rimosus	18,265	4,566
Ligumia recta	69,092	17,273
Plethobasus cyphus	178	45
Pleuronaia dolabelloides	4,414	1,103
Total	3,815,854	953,964

Table 3.7. Total discounted mussel-years (DMYs) gained at sites in the Powell River, VA and TN, as a result of mussel restoration conducted from 2003–2014 for the LMPI NRDAR case.

Species	Total
Actinonaias pectorosa	72
Dromus dromas	53
Epioblasma brevidens	286,346
Epioblasma capsaeformis	717,253
Epioblasma triquetra	17,417
Lampsilis fasciola	85,236
Lampsilis ovata	105,474
Lemiox rimosus	4,312
Ligumia recta	16,733
Villosa iris	209,584
Total	1,442,480

Figures

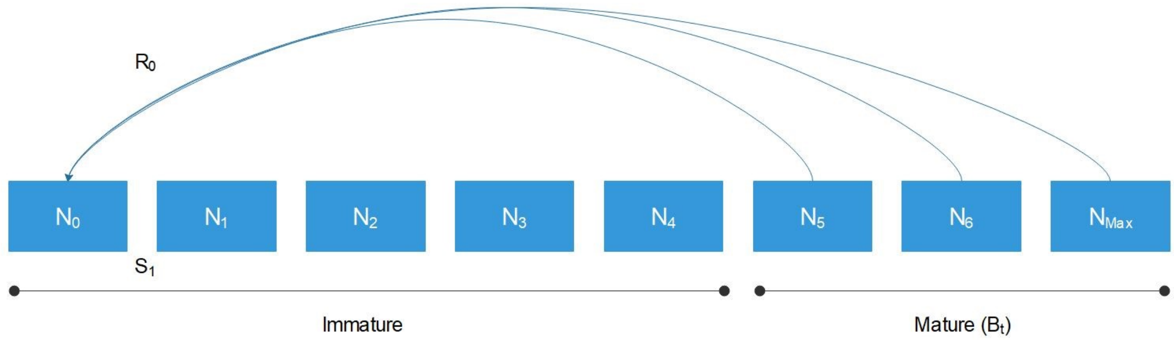

Figure 3.1. Generalized life-cycle diagram showing mussel age classes in years and recruitment used in a Leslie matrix for analysis of mussel injury and restoration for the Certus, Inc. NRDAR case. R_0 is recruitment into the age-0 year class. B_t is the number of individuals of breeding age. Diagram shows how mature adults recruit age-0 juveniles to the N_0 class.

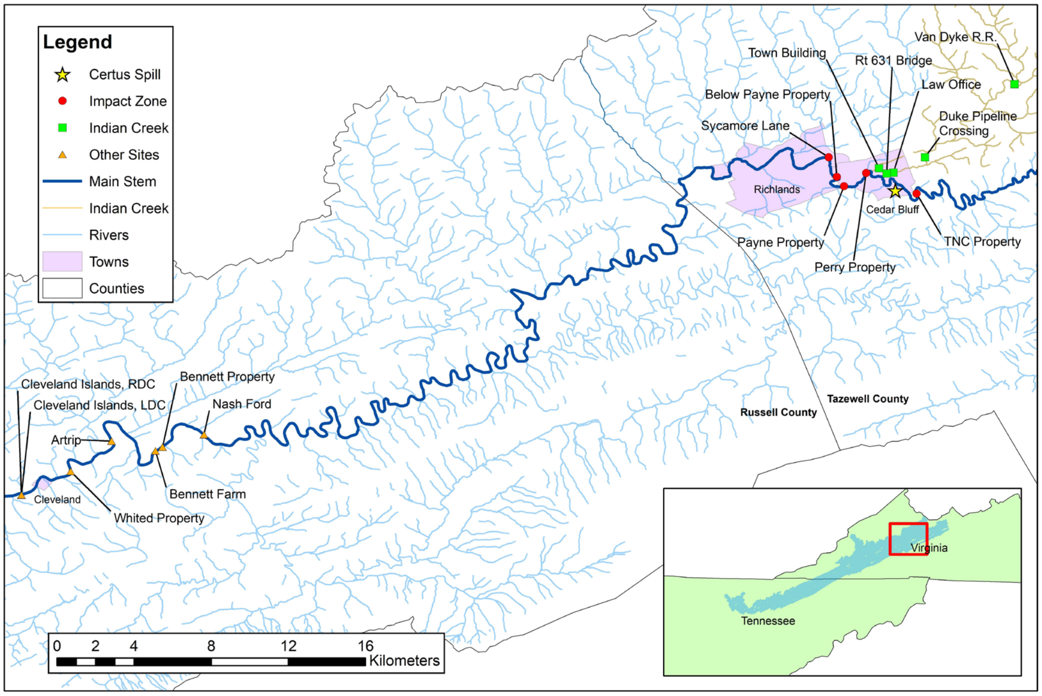

Figure 3.2. Location of restoration sites for the Certus, Inc. NRDAR case from 2003 to 2019. Impact zone sites were in the immediate impact zone of the chemical spill, Indian Creek sites were in a nearby tributary that was not affected by the spill, and all other sites were located on the main stem of the Clinch River, 40 miles downstream in Russell County, VA.

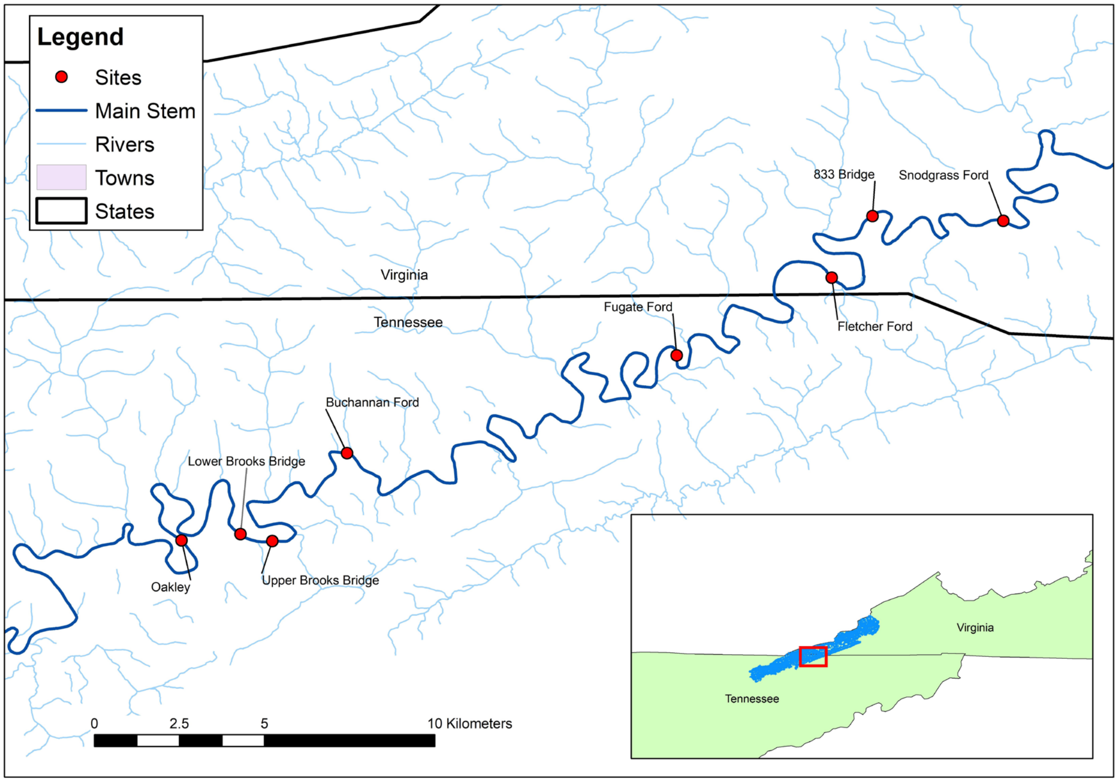

Figure 3.3. Location of restoration sites for the LMPI NRDAR mussel restoration project conducted from 2003 to 2014 in the Powell River, VA and TN.

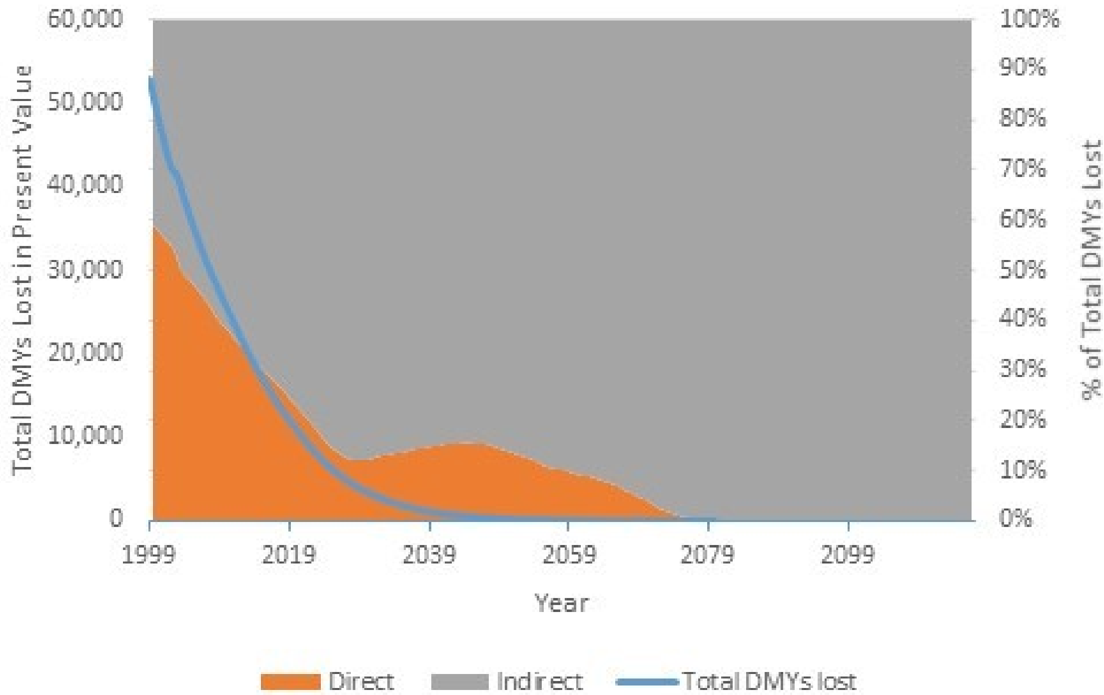

Figure 3.4. Total estimated injury as discounted mussel-years (DMYs) by year as a result of the Certus Inc. chemical spill that occurred in the Clinch River at Cedar Bluff, Tazewell County, VA, on August 27, 1998. Direct and indirect injury to mussels are presented as a percentage of total DMYs.

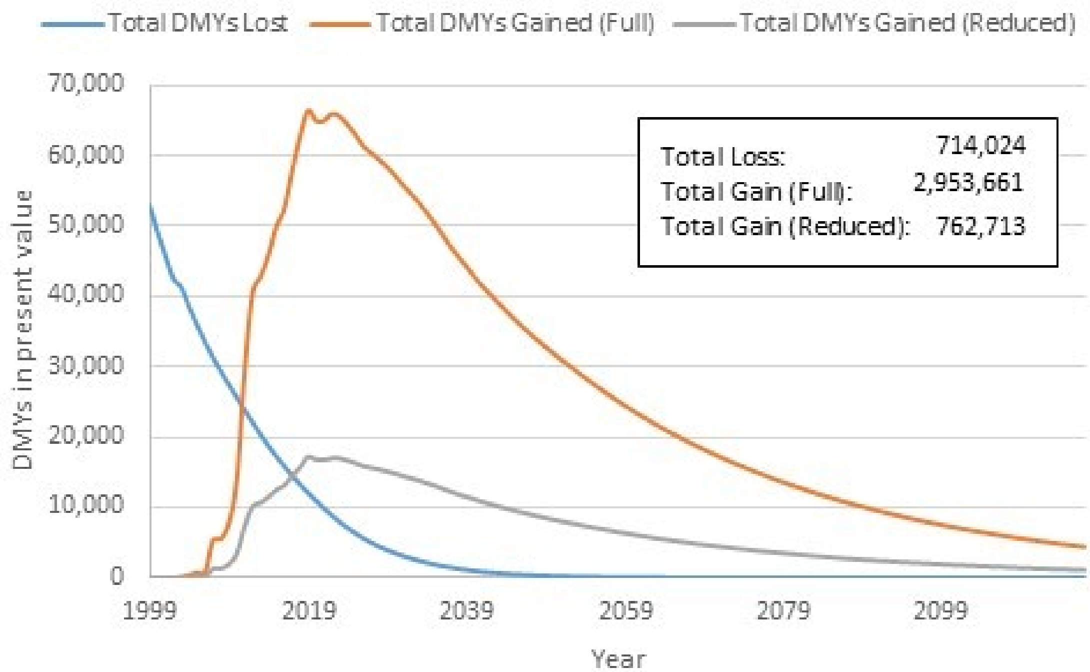

Figure 3.5. Total expected gain in discounted mussel-years (DMYs) over time from mussel restoration efforts at sites in the Clinch River, VA, conducted during the Certus Inc. NRDAR case compared to total expected injury as DMYs over time.

Chapter 4

Estimation of Costs to Produce Mussels at the Freshwater Mollusk Conservation Center and the Aquatic Wildlife Conservation Center for Population Restoration in the Clinch and Powell Rivers, Tennessee and Virginia

Abstract

The Certus, Inc. and Lone Mountain Processing, Inc. Natural Resource Damage and Assessment (NRDAR) cases are among the first and largest NRDAR cases in the United States involving injury to freshwater mussels. Restoration of mussel populations for these two cases was conducted from 2003 to 2019 by Virginia Tech's Freshwater Mollusk Conservation Center (FMCC) and the Virginia Department of Wildlife Resources' Aquatic Wildlife Conservation Center (AWCC) near Marion, VA. We estimated the annual costs of operating these facilities from 2003 to 2019 by examining and compiling available financial records and costs to produce mussels. All costs were converted to real costs (2020 $) using the Consumer Price Index for All Urban Consumers. We also determined the cost of propagating, raising, and successfully establishing a mussel at restoration sites under two different scenarios. The Full Restoration Scenario assumed that all released mussels initially survived and became established as part of the local breeding population. The Reduced Restoration Scenario assumed that only 25% of released mussels initially survived and became established as part of a local breeding population. The cost per established mussel was calculated under both restoration scenarios for each facility from 2010 to 2019. Mean annual real costs to operate FMCC were $111,061 per year while mean annual real costs to operate AWCC were $203,269 per year. However, the mean cost per established mussel at AWCC was $16.81 under the Full Restoration Scenario and $67.23 under the Reduced Restoration Scenario, and similarly, the mean cost per established mussel at FMCC was $14.75 under the Full Restoration Scenario and $59.02 under the Reduced Restoration Scenario. These data provide cost estimates for determining damages in future NRDAR cases involving injury to freshwater mussels, especially for the fauna of the upper Tennessee River basin. However, each NRDAR case will be unique and many factors will need to be considered to estimate mussel production costs, such as difficulty of working with certain species and the available production capacity at existing facilities.

Introduction

Two of the earliest and largest hazardous substance spills involving injury to mussel populations in the United States were the Certus, Inc. and the Lone Mountain Processing, Inc. (LMPI) Natural Resource Damage Assessment and Restoration (NRDAR) cases. The Certus, Inc. chemical spill occurred in the headwaters of the Clinch River in southwestern Virginia in 1998, killing an estimated 18,000 mussels of 13 species in an approximately seven-mile impact zone, including individuals of three federally endangered species. The LMPI spill occurred in Lee County, VA, in 1996 when 6,000,000 gallons of coal slurry were released into the Powell River, affecting mussel populations of more than 30 species over a 65-mile stretch of river from the spill site downstream to Norris Reservoir, Tennessee. Several other well-documented releases have affected mussel populations in the United States, including: (1) the DuPont Facility at Waynesboro, VA where mercury leaked from 1929 to 1950 into the South River, a headwater stream to the Shenandoah River (U.S. Fish and Wildlife Service 2017), (2) release of hazardous substances in the Ohio River from a ferro-alloy production facility in 1999 (U.S. Fish and Wildlife Service 2007), and (3) a coal ash spill in the Dan River in 2014 near Eden, North Carolina (Dan River Natural Resource Trustee Council 2015). The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) allows federal, state, and tribal governments to recover money from responsible parties to restore, replace, rehabilitate and/or acquire the equivalent of natural resources injured or services lost as a result of a release of a hazardous substance. Recovered monetary damages from NRDAR cases are used to restore natural resources (e.g., mussel populations) to their baseline level as well as to recover damages for service losses in the interim between the event and successful restoration. In the Certus, Inc. and LMPI NRDAR cases, recovered settlement funds mostly were used to propagate freshwater mussels at two facilities in southwest Virginia, the Freshwater Mollusk Conservation Center (FMCC) at Virginia Tech and the Virginia Department of Wildlife Resources (VDWR) Aquatic Wildlife Conservation Center (AWCC) near Marion, Virginia, and release those mussels at restoration and augmentation sites.

Mussel propagation is now a more common conservation strategy to replace mussels lost by spills and other harmful anthropogenic events. Mussel hatcheries collect gravid females from streams and use those mussels to propagate juvenile mussels and rear them to a stockable size, eventually releasing them at restoration and augmentation sites. However, the costs to produce and raise mussels have not been well documented. While the type of costs incurred for mussel restoration are reasonably well understood by hatchery personnel (Table 4.1), available data on the actual costs to operate a propagation facility are sparse. Thus, the costs to produce and cultivate mussels to stockable-size are not well documented. A better understanding of these costs will allow for a more effective determination of damages in NRDAR cases involving freshwater mussels. The purpose of this study was to estimate the cost to produce and culture a juvenile mussel to a stockable size at AWCC and FMCC for the Certus, Inc. and LMPI NRDAR cases from 2003 to 2019. While costs are specific to these two facilities, this information should still provide a framework for estimating damages in future NRDAR cases involving mussel populations in the Clinch and Powell rivers in Tennessee and Virginia, and other river systems throughout the United States.

Methods

Annual operational budgets for FMCC were compiled from 2003 to 2019 from cooperative agreements established between the United States Fish and Wildlife Service (USFWS), VDWR, and Virginia Tech and the corresponding budgets prepared by the Office of Sponsored Programs at Virginia Tech. Electronic data files for each respective budget and other financial data sources are archived at the

U.S. Fish and Wildlife Service Virginia Field Office, Gloucester, with titles and descriptions of each data file available in Appendix D. Expenditure of funds per year were broken down by cost category, e.g., salary, fringe benefits, equipment, materials and supplies, travel, and overhead (see Table 1.1 for cost category examples) as well as by project (e.g., Certus, Inc. and LMPI, Inc. NRDAR cases). Reported total costs included all years for FMCC, but mean costs were calculated by excluding the 2019 data because the costs in that year were low and only reflect minimal maintenance level grow-out costs for about 6 months during the final year of the project. Annual budgets for AWCC were compiled from 2004 to 2018 from financial records provided by the VDWR, Richmond Office. However, financial data for AWCC were only available by total expenditures per project per year and not by cost category. Reported total costs included all years for AWCC, but mean costs excluded the 2011 financial data because it was incomplete and likely did not reflect the actual costs for that year.

The annual cost to produce a mussel, raise it to a stockable size and release it at a site (i.e., the cost per mussel released) was calculated by dividing the total expenditures for each year by the number of mussels released in the following year, because mussels were usually grown at each facility to at least one year old before they were released. However, the cost per mussel released was only calculated beginning in 2010. In prior years from 2003 to 2009 mussels were commonly released shortly after their transformation on host fishes at much younger ages (2-4 weeks old) and at smaller sizes (<1 mm). Monitoring at release sites during this earlier restoration period (2003-2009) determined that establishment of juveniles at sites was more successful if they were grown to larger sizes (>20 mm long) before release. The mean cost per mussel from 2010 to 2019 was calculated by dividing the mean expenditures per year from 2009 to 2018 by the mean number of mussels released from 2010 to 2019.

The annual cost per mussel released was calculated under two restoration scenarios and applied to each facility. Under the Full Restoration scenario (see Chapter 3 for methodological details), all mussels released were assumed to have successfully established at the intended release site. In this scenario, "successfully established" means that mussels settled and burrowed into suitable habitat after their release and then experienced normal survival and reproductive rates over their lifetimes based on species specific longevities and survival rates (see Chapter 3; Jones, Neves, and Hallerman (2012)). That is, mussels released at a site successfully became part of a breeding population. However, it is possible that mortality of juveniles is higher than expected after release before stabilizing to more typical survival and reproductive rates, such as those in the Full Restoration scenario. Therefore, the Reduced Restoration scenario assumes that only 25% of released mussels successfully established at a site. This establishment rate was chosen based on monitoring of mussels at the Certus, Inc. and LMPI NRDAR release sites in the Clinch and Powell Rivers from 2015 to 2017, which showed that local populations were about 25% of their expected size based on number of mussels released and expected mortality and reproduction rates reported in Jones, Neves, and Hallerman (2012) (see Chapter 2 and Table 2.8). The remaining 75% of the released mussels are assumed to have died shortly after release in 1-7 days or moved downstream during high stream discharge events and either died or perhaps established themselves in other downstream areas. If for example, 1000 mussels were released at an annual operating cost of $1000, the cost per mussel released would be $1 under the Full Restoration scenario and $4 under the Reduced Restoration scenario. Examining both restoration scenarios helps biologists to explore a wider range of costs under different juvenile mussel survival regimes at release sites. For example, if habitat conditions were not optimal at certain restoration sites, then a Reduced Restoration scenario leading to higher cost per mussel might be more appropriate for planning purposes. All costs per mussel reported hereafter under the Full and Reduced Restoration scenarios can be more accurately defined as the cost per *established* mussel, regardless of the actual number of mussels released.

All costs to operate each facility were converted from actual (nominal) dollars to real dollars (adjusted for inflation) using the Consumer Price Index for All Urban Goods[1] (CPIAUCNS, available at: https://fred.stlouisfed.org/series/CPIAUCNS) and the following formula:

$$NC_t \times \frac{PI_{2020}}{PI_t} = C_{2020}$$

where NC_t is the nominal cost for year *t*, PI_{2020} is the price index for 2020, PI_t is the price index for year *t*, and C_{2020} is the real cost in 2020 dollars (adjusted for inflation). The nominal cost here would be the cost of operating each facility for a given year *t* as reported from historical financial data. All subsequent analyses (i.e., cost per mussel released) were reported using real dollars (2020).

Results

The sum of nominal costs[2] to operate FMCC over the entire lifespan of the project from 2003 to 2019 was $1,536,898, and adjusted to 2020 dollars, the sum of real costs over this same period was $1,802,288 (Table 4.2). The mean real cost per year was $111,061 (Figure 4.1), where on average 77% of costs were used for the Certus, Inc. NRDAR restoration case, 20% were used for the LMPI, Inc. NRDAR restoration case, and 3% for a project funded by The Nature Conservancy (TNC) (Figure 4.2). By cost category, 78% of costs at FMCC were used for salary, wages, and fringe benefits. Of the remaining 22%, most expenditures were for Virginia Tech overhead, equipment, travel, and materials/supplies (See Appendix E for a breakdown of FMCC costs per category). However, the sum of real costs to operate FMCC from 2010 to 2019 was $1,074,024, and the mean real cost per year (excluding 2019) was $115,668, which was used to determine the cost per mussel (Figure 4.1).

The sum of nominal costs to operate AWCC from 2004 to 2018 was $2,407,302 (Table 4.3). Adjusted to 2020 dollars, the sum of real costs to operate AWCC over this period was $2,742,433. The mean real cost per year was $203,269 for AWCC (Figure 4.1), where an average of 35% of costs were used for the Certus, Inc. NRDAR case, 26% were used for the LMPI NRDAR case, and 38% were used for other projects, such as the State Wildlife Grant program. However, the sum of real costs to operate AWCC from 2010 to 2018 was $1,799,663, and the mean real cost per year (excluding 2011) was $222,569, which was used to determine the cost per mussel (Figure 4.1).

The real cost to propagate, grow and release a mussel from 2010 to 2019 by FMCC under the Full Restoration scenario ranged from $4.36 per mussel in 2012 to $96.48 per mussel in 2010 with a mean cost of $14.75 per mussel, and under the Reduced Restoration scenario real costs ranged from $17.42 to $385.93 with a mean cost of $59.02 per mussel (Table 4.4 and Figure 4.3). The real cost to propagate,

[1] DOI economists recommend NRDAR case teams consult with their economists prior to using the mussel cost estimates contained in this report. They can assist with the conversions from actual (nominal) dollars to real dollars using DOI's best practices.

[2] Nominal and real costs are provided to provide the maximum flexibility for users. However, the grand totals are sums and are *not* converted to present value using the appropriate real or nominal discount rate.

grow and release a mussel from 2010 to 2019 for AWCC under the Full Restoration scenario ranged from $6.75 per mussel in 2013 to $40.96 per mussel in 2016 with a mean cost of $16.81 per mussel, and under the Reduced Restoration scenario real costs ranged from $27.01 to $163.82 per mussel with a mean of $67.23 per mussel (Table 4.4 and Figure 4.3).

Discussion

Resolution of a NRDAR case often involves the recovery of damages, i.e., financial restitution from a responsible party to fund restoration activities to restore or replace injured natural resources and/or their services. Therefore, a robust estimate of the cost to propagate, raise, and release mussels at restoration sites is vital for accurately determining damages in NRDAR cases involving injury to freshwater mussel populations. A survey of mussel propagation facilities in the United States by Southwick and Loftus (2017) showed that the average real cost (2020 $) of producing a mussel to taggable (i.e., >20 mm long) size ranged from $28.00 per individual for species in the genus *Actinonaias* to $229.12 for individuals of *Simpsonaias ambigua*. In comparison, average real costs for all species propagated at FMCC and AWCC ranged from $14.75 per mussel released under the full scenario to $67.23 per mussel established under the reduced scenario. Species-specific costs were not estimated in this study because data were not collected in a way to allow for tracking the costs separately for each species and cohort of mussels. For comparison, the costs of Southwick and Loftus (2017) did not include restocking costs (i.e., the cost of transporting mussels to restoration sites and releasing them). Southwick and Loftus (2017) also did not report the cost of producing mussels in any genera that only included threatened and endangered species (e.g., *Dromus*, *Epioblasma*), whereas the cost estimates for FMCC and AWCC include both restocking costs and costs to produce and grow endangered and threatened species, which in some cases can be more difficult and costly to propagate than non-endangered species. Although it should be noted that in some instances endangered and threatened species are not necessarily more costly to propagate. Both *Epioblasma capsaeformis* and *E. brevidens* are listed as endangered; however, both are relatively low cost to produce because (Table 4.5) these two species are moderately fecund, have readily available host fish (*Cottus* spp.) that are easy to collect and care for, and currently have large populations in the Clinch River where broodstock are easy to collect for propagation purposes. Together, these two species account for almost 25% of all mussels released by FMCC and AWCC, despite their status as federally endangered.

There was a wide range of annual costs per mussel produced and established under the Full or Reduced Scenarios at FMCC and AWCC, and costs per mussel were higher than usual at both facilities in 2015 and 2016 (Figure 4.3). There are a number of potential reasons for these variable annual production costs. Staff turnover and training of key personnel for example, may lead to a temporary loss of expertise and lead to a subsequent decrease in production efficiency. The suite of species being produced can greatly affect cost per mussel. Species that are difficult to collect in the field because they are rare, have low natural fecundity or utilize rare or difficult to obtain host fish, or host fish that are challenging to maintain in captivity, can lead to lower production efficiency and increase overall costs. For example, *Lampsilis fasciola* has high fecundity, it is relatively easy to collect gravid females of this species, and has a common fish host species (*Micropterus salmoides*) that is easy to obtain and care for in the lab (Table 4.5), and therefore this species is relatively lower cost to produce. In contrast, *Pleuronaia dolabelloides* has low fecundity, finding broodstock is difficult, and its host fishes are uncommon and difficult to care for, which makes it much more difficult and expensive to produce. Field conditions will also affect cost per mussel. If sampling conditions are unfavorable (e.g., river discharge too high or low, turbid water, etc.) collecting sufficient broodstock may be challenging or simply impossible, especially for species that are only gravid for short periods of time. Other factors that may drive up costs include diseases that can affect host fish and mussel survival in captivity and installation of new culture systems.

The goal of NRDAR is to restore the injured resource to baseline. Ideally, the restoration would establish a local population with a similar abundance and age/size class structure as the original population, and one that can reproduce and maintain itself over time. We examined costs under a Full Restoration scenario, in which all released mussels established at a restoration site and became part of a reproducing population, and a Reduced Restoration scenario, in which only 25% of mussels established at a site and became part of a reproducing population. This has important implications regarding determination of damages. The rate at which released mussels are expected to establish at a site needs to be considered during NRDAR case development and restoration planning. For example, to establish 1,000 mussels at $1.00 each under the Full Restoration Scenario would cost $1,000. However, to establish 1,000 mussels under the Reduced Restoration Scenario would cost $4,000 because 4,000 mussels would need to be released (i.e., only 25% of 4,000 would be expected to establish at a site). This cost may be reduced somewhat if producing larger cohorts of mussels can be made more efficient than smaller cohorts. Regardless, the cost per established mussel is almost certain to be higher (perhaps substantially) in cases where successful establishment is less than 100%. We recommend examining a range of scenarios with varying levels of mussel establishment as conducted here so that cost estimation for natural resource damages determination reflect a realistic level of successful establishment of released mussels.

While the mean annual cost of operating AWCC ($222,569) was higher than FMCC ($115,668) from 2009-2018, the mean cost per released mussel under the Full Restoration Scenario for example was similar ($16.81 at AWCC vs. $14.75 at FMCC). This is because the mean number of mussels released >6 months old from 2010 to 2019 was higher for AWCC (13,243) than for FMCC (7,840). Regardless, both facilities have been able to take advantage of various partnerships and resources in addition to NRDAR funds. For example, both facilities occasionally received funds from projects other than Certus and Lone Mountain Processing, Inc. NRDAR cases, which allowed each facility to make improvements to culture systems and increase production efficiency in ways that might not be possible with NRDAR funds alone.

The mussel production cost data from these two facilities provide a starting point to estimate damages in future cases involving propagation and restocking of mussels to restore injured freshwater mussels, especially in the Upper Tennessee River Basin where these facilities primarily operate. However, each new NRDAR case will be unique and many case-specific factors will have to be considered to estimate damages, such as the propagation and rearing difficulty of working with certain mussel species, whether host fish trials will be needed, whether current facilities will be able to handle new propagation work or will a new facility need to be established (i.e., startup costs), the leverage that can be obtained from new and existing partnerships, and the restocking (i.e., transport costs). Any increase in staffing requirements to meet case-related restoration goals can substantially increase costs, as labor represents a large proportion of the cost per mussel. Future studies examining the cost of propagating mussels should focus on species level costs, as it is difficult to examine this *post hoc* unless facility data are collected in such a way as to specifically examine this factor. It would also be beneficial to determine the cost of producing a cohort of mussels (e.g., mussels released from a single infestation of host fish), as much of the cost of production is incurred whether survival from transformation to release is high or low (i.e., a fixed cost).

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Tables

Table 4.1. Costs categories, types and descriptions associated with operating a mussel propagation facility that utilizes host fish to produce juvenile mussels.

Cost Categories	Description and Need	Yearly Cost
Personnel		
Full time staff	Manager, host species maintenance, handling and monitoring of broodstock and juvenile mussels, field work	Yes
Technicians	Supplement staff, especially during field season.	Yes
Grow-out Systems		
Tanks	Holding juvenile mussels and host fish	No
Pumps	Water recirculation	No
Chillers	Maintain ideal water temperature	No
Heaters	Maintain ideal water temperature	No
Pipes, fittings, etc.	Water delivery to tanks	No
Field-work Gear		
Waders	Used for collection of mussels	No
Wetsuit/drysuit	Used for collection of mussels	No
Collection bags	Holding collected mussels during sampling	No
Notebooks	Data recording	No
Coolers	Transport of mussels and fish to and from facility	No
Buckets	Holding of mussels in the field (for measuring, etc.)	No
Electrofisher	Capture of host fish	
Nets	Capture of host fish	
Mussel Care		
Food	Algae costs can vary based on source, e.g., whether producing on site or purchasing from a vendor	Yes
Reagents	For water quality testing and maintenance	Yes
Misc. equipment		
Petri dishes/Counting plates	Counting of juvenile mussels throughout time at facility	No
Microscopes	Counting and measuring of juvenile mussels	No
Buckets	Transport of mussels and fish to and from facility	No
Weighing scales		No
Calipers	Measuring of mussels/fish	No
Office Supplies		
Printer	Reports, datasheets, etc.	No
Printing supplies	Ink, paper, etc.	No
Computers	Data analysis, report preparation, etc.	No
Software licensing	Cost for proprietary software	No
Data storage	For databases and backups	No
Furniture	Desks, chairs, tables, bookshelves, etc.	No
Travel		
Vehicle	For conducting field work	Yes
Vehicle Maintenance	Oil changes, etc.	Yes
Mileage	Cost per mile driven	Yes
Lodging	Hotel cost	Yes
Per diem	Food and Miscellaneous expenses for field crew	Yes
Utilities		
Water supply	Cost of water if not from well or river	Yes
Telephone	Cost of telephone line	Yes
Internet	Cost of internet	Yes
Electricity	Cost of electricity	Yes
Heating (If separate from electricity)	Cost of heating facility	Yes
Rent or Lease	Cost of monthly rent or yearly lease if not covered by existing facility	Yes

Table 4.2. Actual (nominal) and real total costs (2020 $) from 2003 to 2019 to operate the Freshwater Mollusk Conservation Center at Virginia Tech, Blacksburg[1]. Funding was provided by the Certus, Inc. and Lone Mountain Processing, Inc. (LMPI) Natural Resource Damage Assessment and Restoration cases and a project sponsored by The Nature Conservancy (TNC) to restore freshwater mussels to the Clinch and Powell Rivers of southwestern Virginia and northeastern Tennessee. Nominal costs were adjusted to real costs in 2020 dollars using the Consumer Price Index for All Urban Consumers (https://fred.stlouisfed.org/series/CPIAUCNS) by multiplying the nominal costs by the ratio of the price index for 2020 to the price index for each year.

Project	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Grand Totals
Actual Costs																		
Certus	$0	$90,182	$30,458	$0	$80,781	$0	$89,494	$114,000	$74,072	$138,138	$110,185	$99,815	$100,000	$99,075	$75,532	$85,096	$25,001	$1,211,829
LMPI	$117,755	$0	$0	$73,987	$0	$78,928	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$270,670
TNC	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$54,399	$0	$0	$54,399
Annual Totals	$117,755	$90,182	$30,458	$73,987	$80,781	$78,928	$89,494	$114,000	$74,072	$138,138	$110,185	$99,815	$100,000	$99,075	$129,931	$85,096	$25,001	$1,536,898
Real Costs (2020 $)																		
Certus	$0	$123,569	$40,365	$0	$100,833	$0	$107,963	$135,308	$85,226	$155,717	$122,414	$109,122	$109,195	$106,837	$79,751	$87,707	$25,309	$1,389,316
LMPI	$165,670	$0	$0	$94,987	$0	$94,878	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$355,535
TNC	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$57,437	$0	$0	$57,437
Annual Totals	$165,670	$123,569	$40,365	$94,987	$100,833	$94,878	$107,963	$135,308	$85,226	$155,717	$122,414	$109,122	$109,195	$106,837	$137,188	$87,707	$25,309	$1,802,288

[1] Nominal and real costs are provided to provide the maximum flexibility for users. However, the grand totals are sums and are *not* converted to present value using the appropriate real or nominal discount rate.

Table 4.3. Nominal and real total costs from 2004 to 2019 to operate the Virginia Department of Wildlife Resources' Aquatic Wildlife Conservation Center near Marion, Virginia[1]. Funding was provided by the Certus, Inc. and Lone Mountain Processing, Inc. (LMPI) Natural Resource Damage Assessment and Restoration cases, the State Wildlife Grant (SWG) fund and projects sponsored by The Nature Conservancy (TNC) and the Town of Saint Paul (St. Paul), Virginia to restore freshwater mussels to the Clinch and Powell Rivers of southwestern Virginia and northeastern Tennessee. Nominal costs were adjusted to real costs in 2020 dollars using the Consumer Price Index for All Urban Consumers (https://fred.stlouisfed.org/series/CPIAUCNS) by multiplying the nominal costs by the ratio of the price index for 2020 to the price index for each year.

Project	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**Grand Totals**
Actual Costs																
Certus	$51,241	$29,672	$46,492	$54,436	$48,190	$69,278	$59,764	$15,678	$30,146	$56,767	$80,530	$82,125	$78,347	$81,189	$85,426	$869,282
LMPI	$11,447	$54,207	$44,925	$64,589	$132,860	$123,260	$61,203	$9,363	$73,159	$68,841	$0	$0	$0	$0	$0	$643,854
SWG	$0	$0	$15,000	$8,500	$0	$0	$0	$0	$24,029	$67,739	$149,555	$152,518	$145,501	$150,780	$158,649	$872,271
TNC	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$19,395	$0	$19,395
St. Paul	$0	$0	$0	$0	$0	$0	$0	$0	$2,500	$0	$0	$0	$0	$0	$0	$2,500
Annual Totals	$62,688	$83,879	$106,418	$127,525	$181,050	$192,538	$120,968	$25,041	$129,833	$193,347	$230,085	$234,644	$223,848	$251,364	$244,075	$2,407,302
Real Costs (2020 $)																
Certus	$70,211	$39,323	$59,689	$67,949	$57,928	$83,575	$70,935	$18,039	$33,982	$63,067	$88,039	$89,677	$84,485	$85,724	$88,047	$1,000,670
LMPI	$15,685	$71,838	$57,677	$80,622	$159,708	$148,697	$72,643	$10,773	$82,469	$76,481	$0	$0	$0	$0	$0	$776,593
SWG	$0	$0	$19,258	$10,610	$0	$0	$0	$0	$27,087	$75,257	$163,501	$166,543	$156,901	$159,202	$163,517	$941,874
TNC	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$20,478	$0	$20,478
St. Paul	$0	$0	$0	$0	$0	$0	$0	$0	$2,818	$0	$0	$0	$0	$0	$0	$2,818
Annual Totals	$85,896	$243,687	$136,623	$159,181	$217,637	$232,272	$143,577	$28,812	$146,355	$214,805	$251,540	$256,219	$241,386	$265,404	$251,564	$2,742,433

[1] Nominal and real costs are provided to provide the maximum flexibility for users. However, the grand totals are sums and are *not* converted to present value using the appropriate real or nominal discount rate.

Table 4.4. Annual real costs to operate the Freshwater Mollusk Conservation Center and the Aquatic Wildlife Conservation Center, as well as the number and cost of established mussels under the two restoration scenarios. The Full Restoration Scenario (FRS) assumed all released mussels successfully established at a site while the Reduced Restoration Scenario (RRS) assumed only 25% of released mussels successfully established at a site. The FRS and RRS cost per mussel was calculated using the mean real cost and mean number of established mussels under each scenario. Medians for cost per mussel were not calculated because the mean cost per mussel for each year is not weighted based on the number of mussels released (indicated by dash). Values other than real cost were not calculated for 2009 because mussels less than 6 months old were still being released (also indicated by dash). *Cost data were only available for 6 months in 2011. The mean real cost from 2009 to 2018 was used as the real cost for AWCC in 2011 and that number was used to calculate cost per mussel in 2012 for AWCC under both scenarios.

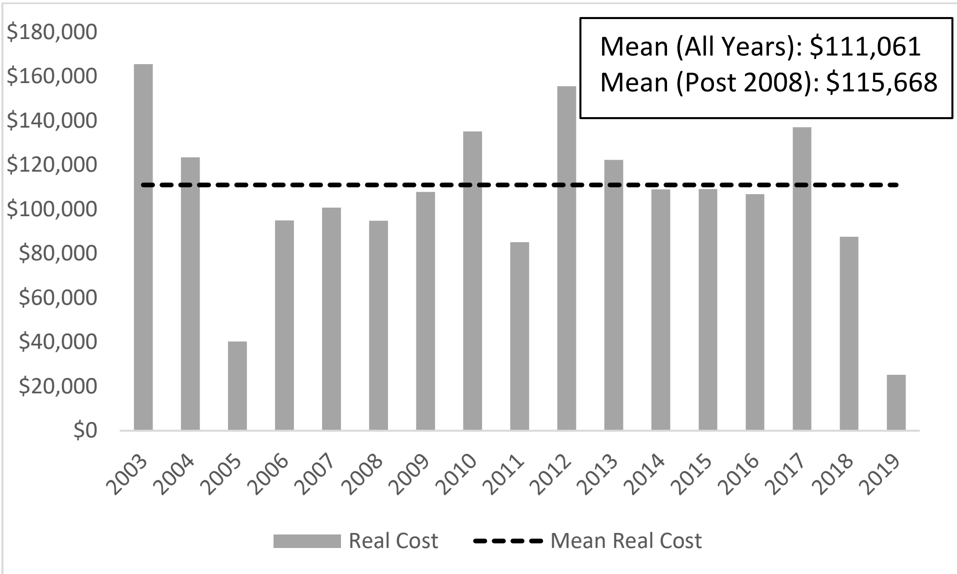
	Freshwater Mussel Conservation Center					Aquatic Wildlife Conservation Center				
Year	Real Cost (2020 $)	Number of Established Mussels (FRS)	Number of Established Mussels (RRS)	FRS Cost Per Mussel (2020 $)	RRS Cost Per Mussel (2020 $)	Real Cost (2020 $)	Number of Established Mussels (FRS)	Number of Established Mussels (RRS)	FRS Cost Per Mussel (2020 $)	RRS Cost Per Mussel (2020 $)
2009	$107,963	—	—	—	—	$232,272	—	—	—	—
2010	$135,308	1,119	280	$96.48	$385.93	$143,577	11,835	2,959	$19.63	$78.50
2011	$85,226	5,765	1,441	$23.47	$93.88	$222,569*	17,039	4,260	$8.43	$33.71
2012	$155,717	19,569	4,892	$4.36	$17.42	$146,355	13,742	3,436	$16.20*	$64.79*
2013	$122,414	10,664	2,666	$14.60	$58.41	$214,805	21,672	5,418	$6.75	$27.01
2014	$109,122	5,661	1,415	$21.62	$86.50	$251,540	16,485	4,121	$13.03	$52.12
2015	$109,195	2,142	536	$50.94	$203.78	$256,219	10,539	2,635	$23.87	$95.47
2016	$106,837	1,533	383	$71.23	$284.92	$241,386	6,256	1,564	$40.96	$163.82
2017	$137,188	6,163	1,541	$17.34	$69.34	$265,404	12,883	3,221	$18.74	$74.95
2018	$87,707	12,549	3,137	$10.93	$43.73	$251,564	10,004	2,501	$26.53	$106.12
2019	—	13,231	3,308	$6.63	$26.52	—	11,975	2,994	$21.01	$84.03
Mean	$115,668	7,840	1,960	$14.75	$59.02	$222,569	13,243	3,311	$16.81	$67.23
Median	$109,159	5,964	1,491	—	—	$241,386	12,429	3,107	—	—
Min.	$85,226	1,119	280	$4.36	$17.42	$143,577	6,256	1,564	$6.75	$27.01
Max.	$155,717	19,569	4,892	$96.48	$385.93	$265,404	21,672	5,418	$40.96	$163.82

Table 4.5. Number of mussels released of all ages from 2003 to 2019 by the Freshwater Mollusk Conservation Center and the Aquatic Wildlife Conservation Center, along with relative difficulty of propagating each species.

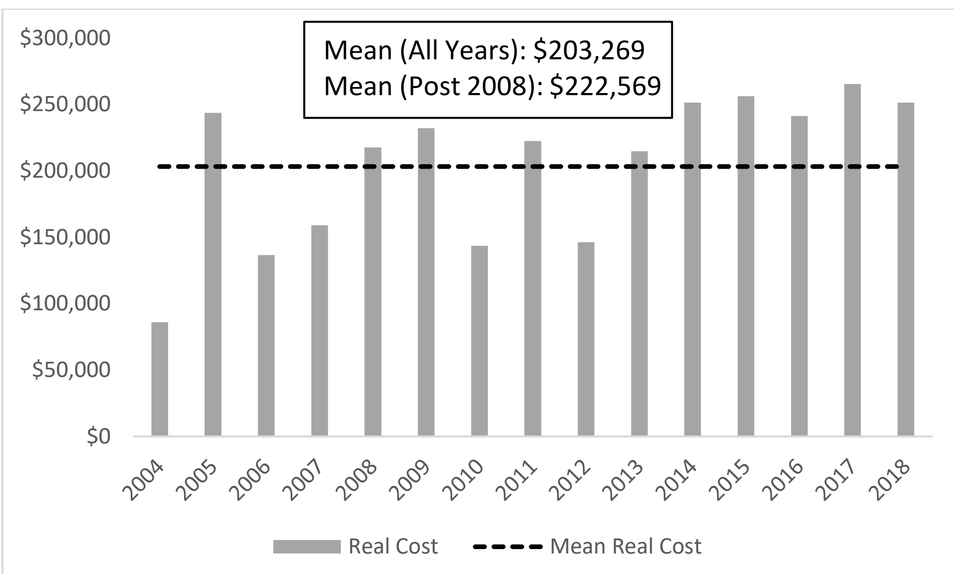
Species	Number released (all ages)	Percent of all releases	Overall difficulty	Fecundity	Short term vs long term brooder	Broodstock abundance	Primary fish host used	Fish host availability	Ease of keeping fish host
Lampsilis fasciola	195,616	19.28%	Easy	High	Long	Common	*Micropterus salmoides, M. dolomieu*	Common/Uncommon	Easy/Moderate/Difficult
Actinonaias pectorosa	180,331	17.78%	Easy	Very High	Long	Common	*Micropterus salmoides*	Common	Easy
Lampsilis ovata	132,383	13.05%	Easy	Very High	Long	Uncommon	*Micropterus salmoides, M. dolomieu*	Common/Uncommon	Easy/Moderate/Difficult
Epioblasma capsaeformis	131,623	12.98%	Easy	Moderate	Long	Common	*Cottus spp.*	Common	Easy/Moderate
Epioblasma brevidens	115,017	11.34%	Easy	Moderate	Long	Common	*Cottus spp., P. caprodes*	Common	Easy/Moderate
Villosa iris	68,314	6.73%	Easy	Medium/High	Long	Common	*Ambloplites rupestris*	Common/Uncommon	Easy
Actinonaias ligamentina	44,180	4.36%	Easy	Very High	Long	Common	*Micropterus salmoides*	Common	Easy
Villosa vanuxemensis	43,457	4.28%	Easy	Medium /High	Long	Common	*Cottus spp.*	Common	Easy/Moderate
Epioblasma aureola	27,155	2.68%	Moderate	Low	Long	Rare	*Cottus spp., Etheostoma flabellare*	Common/Uncommon	Easy/Moderate
Ligumia recta	21,170	2.09%	Moderate	Very High	Long	Rare	*Sander vitreus, S. canadensis, Micropterus salmoides*	Rare	Moderate/Difficult
Venustaconcha trabalis	13,279	1.31%	Moderate	Medium	Long	Rare	*Cottus spp., E. flabellare*	Common/Uncommon	Moderate/Difficult
Epioblasma triquetra	8,251	0.81%	Moderate	Medium	Long	Uncommon	*Cottus spp.*	Common	Easy/Moderate
Lampsilis abrupta	7,887	0.78%	Moderate	Very High	Long	Rare	*Micropterus salmoides*	Common	Easy
Medionidus conradicus	6,805	0.67%	Easy	Medium	Long	Common	*E. flabellare, E. rufilineatum*	Common/Uncommon	Easy/Moderate
Ptychobranchus subtentus	4,621	0.46%	Moderate	Very High	Long	Common	*E. caeruleum, E. rufilineatum, E. camurum*	Common/Uncommon	Easy/Moderate
Ptychobranchus fasciolaris	4,243	0.42%	Moderate	Medium	Long	Common	*E. caeruleum, E. rufilineatum, E. camurum*	Common/Uncommon	Easy/Moderate
Lasmigona holstonia	3,334	0.33%	Moderate	Low	Long	Uncommon	*C. bairdii, C. baileyi*	Common	Easy/Moderate
Dromus dromas	2,343	0.23%	Moderate	Very High	Long	Uncommon	*Percina evides, Etheostoma blennioides*	Uncommon/Rare	Moderate/Difficult
Lemiox rimosus	1,618	0.16%	Moderate	Low/Medium	Long	Rare	*E. blennioides, E. zonale*	Uncommon	Moderate
Eurynia dilatata	1,272	0.13%	Difficult	High	Short	Uncommon	*Cottus spp.*	Common	Easy/Moderate
Lasmigona costata	624	0.06%	Moderate	Medium	Long	Uncommon	*C. bairdii, C. baileyi*	Common	Easy/Moderate
Fusconaia cor	283	0.03%	Difficult	Low	Short	Uncommon	*Cyprinella galactura, Luxilus chrysocephalus*	Uncommon/Rare	Moderate/Difficult
Pleuronaia barnesiana	157	0.02%	Difficult	Low	Short	Rare	*Cyprinella galactura*	Uncommon/Rare	Moderate/Difficult
Cyprogenia stegaria	129	0.01%	Moderate	Medium	Long	Rare	*Percina caprodes, Cottus spp.*	Common	Easy/Moderate

Species	Number released (all ages)	Percent of all releases	Overall difficulty	Fecundity	Short term vs long term brooder	Broodstock abundance	Primary fish host used	Fish host availability	Ease of keeping fish host
Pleuronaia dolabelloides	100	0.01%	Difficult	Low	Short	Rare	*Cyprinella galactura, Luxilus chrysocephalus*	Uncommon/Rare	Moderate/Difficult
Alasmidonta viridis	82	0.01%	Moderate	Low	Long	Rare	*Cottus spp.*	Common	Easy/Moderate
Strophitus undulatus	39	0.00%	Moderate	Low	Long	Rare	*Cottus spp.*	Common	Easy/Moderate
Fusconaia cuneolus	29	0.00%	Difficult	Low/Medium	Short	Uncommon	*Micropterus salmoides*	Common	Easy
Epioblasma florentina walkeri	22	0.00%	Difficult	Medium	Long	Rare	*E. flabellare, Cottus spp.*	Common/Uncommon	Easy/Moderate
Plethobasus cyphyus	3	0.00%	Difficult	Low	Short	Rare	*Notemigonus crysoleucas*	Common	Easy/Moderate

Figures

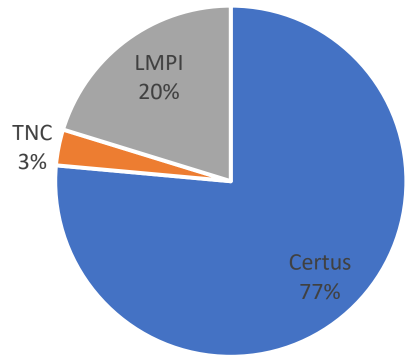


(A) Freshwater Mollusk Conservation Center

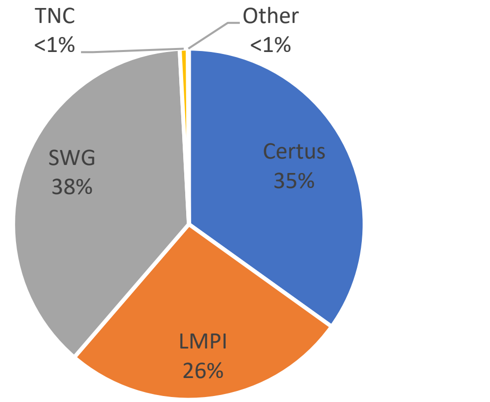


(B) Aquatic Wildlife Conservation Center

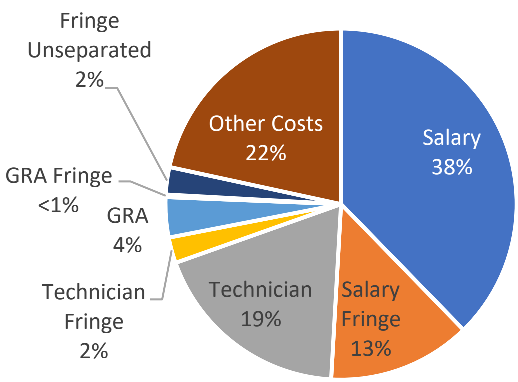
Figure 4.1. Total real costs (2020 $) per year to operate Virginia Tech's Freshwater Mollusk Conservation Center from 2003 to 2018 and the Virginia Department of Wildlife Resources' Aquatic Wildlife Conservation Center from 2004 to 2018. The 2019 data were excluded from the mean cost estimate for FMCC because the costs were minimal, and 2011 data was excluded from the mean cost estimate for AWCC as only six months of data were available for that year. Horizontal lines represent the mean cost over all years for both facilities; however, the annual operational costs for 2019 were not included in the mean estimation for Freshwater Mollusk Conservation Center.



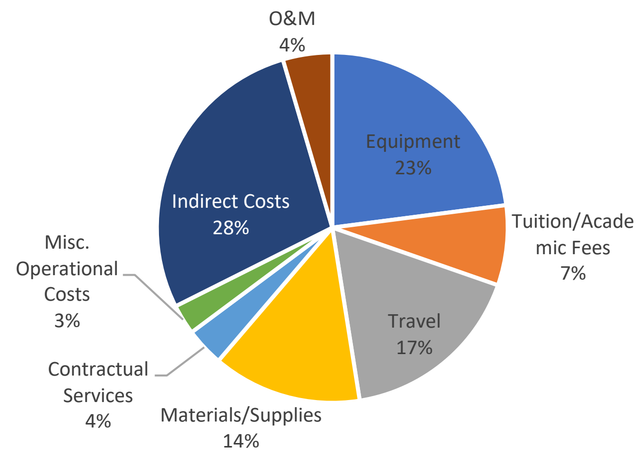
(A) Total cost by project for FMCC from 2003 to 2019



(B) Total cost by project for AWCC from 2004 to 2019

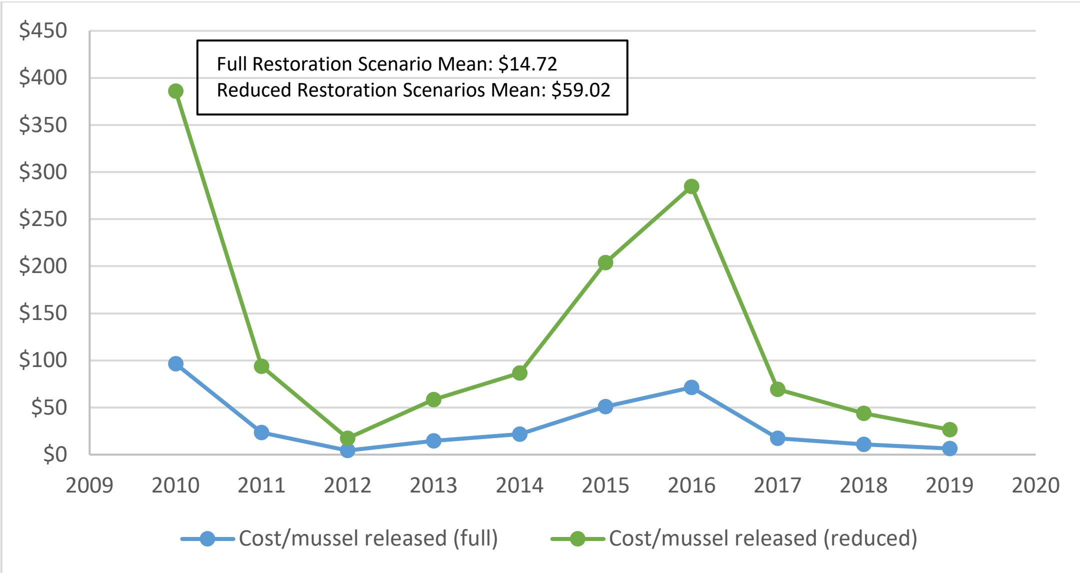


(C) Total cost by category for FMCC from 2003 to 2019

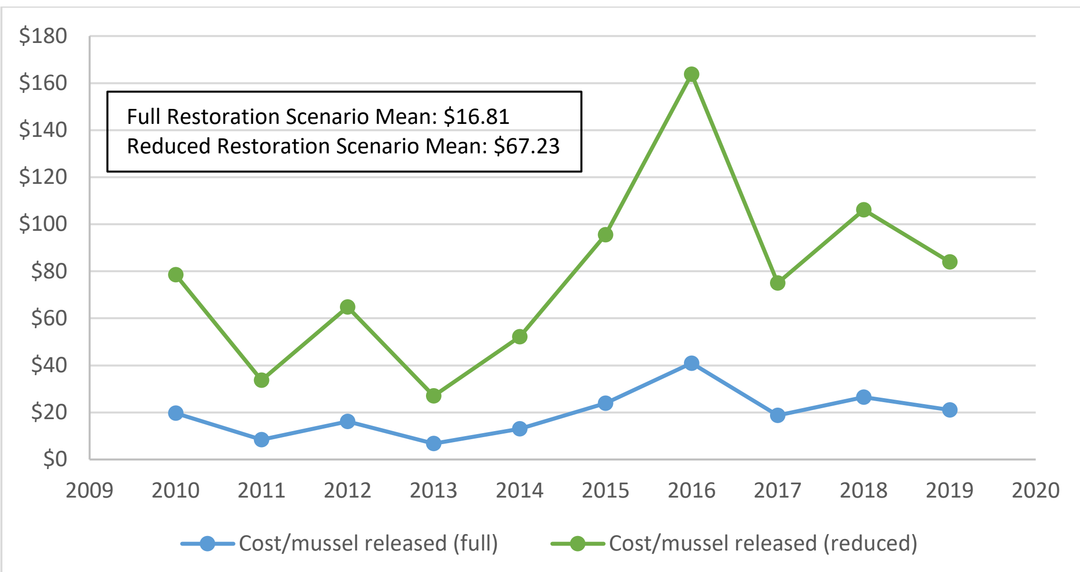


(D) Breakdown of other costs from subfigure (c). Miscellaneous operational costs include publication costs, departmental direct costs and and telephones

Figure 4.2. Real costs to operate Virginia Tech's Freshwater Mollusk Conservation Center (FMCC) from 2003 to 2019 and the Virginia Department of Wildlife Resources' Aquatic Wildlife Conservation Center (AWCC) from 2004 to 2018 itemized by project and cost category. Figures A and B include costs by project for FMCC and AWCC. Projects include the Certus, Inc. and Lone Mountain Processing, Inc. NRDAR cases, a project sponsored by The Nature Conservancy (TNC), and the State Wildlife Grant (SWG) fund. Figure C shows all salary, wage, and fringe benefit cost categories as well as total percent of all other costs, such as graduate research assistantship (GRA). Figure D shows the breakdown of Other Costs shown in Figure C.



(a). Freshwater Mollusk Conservation Center (FMCC)



(b). Aquatic Wildlife Conservation Center (AWCC)

Figure 4.3. Real cost per mussel released under two restoration scenarios for the Freshwater Mollusk Conservation Center (FMCC) and Aquatic Wildlife Conservation Center (AWCC). The Full Restoration Scenario assumed all released mussels successfully established at a restoration site, whereas the Reduced Restoration Scenario assumed that only 25% of released mussels established at a site.

Appendices

Appendix A

Mussels >6 months old released by AWCC from 2006–2019 for projects other than Certus, Inc. and LMPI NRDAR cases.

Species	Number Released	Year	Project	River	Release Site
Epioblasma capsaeformis	2	2006	SWG	Clinch	Clinchport
Lampsilis ovata	25	2006	SWG	Clinch	Clinchport
Ligumia recta	100	2006	SWG	Clinch	Clinchport
Ligumia recta	75	2006	TWRA Partnership	Clinch	Sneedville
2006 Total	202				
Ligumia recta	104	2007	SWG	Clinch	Clinchport
Ligumia recta	128	2007	SWG	Clinch	Slant
2007 Total	232				
Ligumia recta	50	2008	SWG	Clinch	Clinchport
Villosa iris	6	2008	SWG	Copper Creek	Dickensonville
Ligumia recta	150	2008	SWG	Clinch	Slant
2008 Total	206				
Ligumia recta	75	2009	SWG	Clinch	Clinchport
Villosa iris	1,866	2009	SWG	Copper Creek	Dickensonville
Lampsilis fasciola	258	2009	SWG	Clinch	Slant
Ligumia recta	100	2009	SWG	Clinch	Slant
2009 Total	2,299				
Ptychobranchus subtentus	562	2010	SWG	North Fork Holston	Clarke Property
Epioblasma brevidens	150	2010	TWRA Partnership	Duck	Lillards Mill
Villosa vanuxemensis	100	2010	SWG	Clinch	St. Paul
Epioblasma capsaeformis	350	2010	TWRA Partnership	Big South Fork Cumberland	Station Camp Creek Island
Epioblasma brevidens	655	2010	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Epioblasma capsaeformis	350	2010	TWRA Partnership	Nolichucky	TWRA Canoe Launch
2010 Total	2,167				
Venustaconcha trabalis	7	2011	SWG	Copper Creek	Dickensonville
Epioblasma brevidens	248	2011	TWRA Partnership	Duck	Lillards Mill
Epioblasma triquetra	330	2011	TWRA Partnership	Duck	Lillards Mill
Epioblasma brevidens	158	2011	SWG	Clinch	Slant

Species	Number Released	Year	Project	River	Release Site
Epioblasma capsaeformis	350	2011	SWG	Clinch	Slant
Lampsilis abrupta	86	2011	SWG	Clinch	Slant
Lampsilis fasciola	276	2011	SWG	Clinch	Slant
Lampsilis ovata	177	2011	SWG	Clinch	Slant
Villosa vanuxemensis	202	2011	SWG	Clinch	Slant
Epioblasma brevidens	50	2011	SWG	Clinch	Speers Ferry
Epioblasma capsaeformis	50	2011	SWG	Clinch	Speers Ferry
Lampsilis abrupta	88	2011	SWG	Clinch	Speers Ferry
Lampsilis fasciola	371	2011	SWG	Clinch	Speers Ferry
Lampsilis fasciola	500	2011	SWG	Clinch	St. Paul
Villosa vanuxemensis	150	2011	SWG	Clinch	St. Paul
Epioblasma brevidens	250	2011	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Epioblasma capsaeformis	999	2011	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Lampsilis fasciola	100	2011	TWRA Partnership	Pigeon	Wilton Springs
Villosa iris	100	2011	TWRA Partnership	Pigeon	Wilton Springs
2011 Total	4,492				
Lampsilis abrupta	433	2012	SWG	Clinch	Clinchport
Lampsilis fasciola	150	2012	SWG	Clinch	Clinchport
Lampsilis ovata	200	2012	TWRA Partnership	Pigeon	Denton
Lampsilis abrupta	300	2012	TWRA Partnership	Clinch	Kyles Ford
Epioblasma brevidens	250	2012	TWRA Partnership	Duck	Lillards Mill
Epioblasma triquetra	150	2012	TWRA Partnership	Duck	Lillards Mill
Epioblasma capsaeformis	500	2012	TWRA Partnership	Hiwassee	McClary Island
Epioblasma brevidens	235	2012	SWG	Clinch	Slant
Epioblasma triquetra	225	2012	SWG	Clinch	Slant
Lampsilis abrupta	632	2012	SWG	Clinch	Slant
Lampsilis fasciola	246	2012	SWG	Clinch	Slant
Lampsilis ovata	181	2012	SWG	Clinch	Slant
Dromus dromas	13	2012	SWG	Clinch	Speers Ferry
Epioblasma brevidens	250	2012	SWG	Clinch	Speers Ferry
Epioblasma capsaeformis	250	2012	SWG	Clinch	Speers Ferry
Epioblasma triquetra	232	2012	SWG	Clinch	Speers Ferry
Lampsilis abrupta	459	2012	SWG	Clinch	Speers Ferry
Lampsilis fasciola	228	2012	SWG	Clinch	Speers Ferry
Lampsilis ovata	130	2012	SWG	Clinch	Speers Ferry
Lemiox rimosus	73	2012	SWG	Clinch	Speers Ferry
Lampsilis fasciola	150	2012	SWG	Clinch	St. Paul
Villosa vanuxemensis	150	2012	SWG	Clinch	St. Paul
Epioblasma brevidens	250	2012	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Epioblasma capsaeformis	500	2012	TWRA Partnership	Nolichucky	TWRA Canoe Launch

Species	Number Released	Year	Project	River	Release Site
2012 Total	6,187				
Epioblasma brevidens	1,722	2013	SWG	Clinch	Clinchport
Epioblasma capsaeformis	785	2013	SWG	Clinch	Clinchport
Lampsilis abrupta	1,981	2013	SWG	Clinch	Clinchport
Lampsilis fasciola	597	2013	SWG	Clinch	Clinchport
Lampsilis ovata	80	2013	SWG	Clinch	Clinchport
Lampsilis abrupta	200	2013	TWRA Partnership	Elk	Harms Mill
Lampsilis abrupta	100	2013	TWRA Partnership	Clinch	Kyles Ford
Lampsilis abrupta	300	2013	TWRA Partnership	Duck	Lillards Mill
Epioblasma brevidens	395	2013	TWRA Partnership	Duck	Lillards Mill
Lampsilis abrupta	121	2013	TWRA Partnership	Duck	Littlelot Hwy 230
Epioblasma capsaeformis	200	2013	TWRA Partnership	Emory	Oakdale Bridge
Epioblasma brevidens	700	2013	SWG	Clinch	Slant
Epioblasma capsaeformis	1,007	2013	SWG	Clinch	Slant
Epioblasma brevidens	1,002	2013	SWG	Clinch	Speers Ferry
Epioblasma capsaeformis	776	2013	SWG	Clinch	Speers Ferry
Epioblasma triquetra	23	2013	SWG	Clinch	Speers Ferry
Lampsilis abrupta	2,296	2013	SWG	Clinch	Speers Ferry
Lampsilis fasciola	393	2013	SWG	Clinch	Speers Ferry
Lampsilis ovata	3	2013	SWG	Clinch	Speers Ferry
Lemiox rimosus	63	2013	SWG	Clinch	Speers Ferry
Ligumia recta	100	2013	SWG	Clinch	Speers Ferry
Epioblasma brevidens	300	2013	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Epioblasma capsaeformis	400	2013	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Lampsilis abrupta	130	2013	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Lampsilis fasciola	238	2013	TWRA Partnership	Pigeon	Wilton Springs
2013 Total	13,912				
Epioblasma brevidens	2,087	2014	SWG	Clinch	Clinchport
Epioblasma capsaeformis	565	2014	SWG	Clinch	Clinchport
Fusconaia cor	138	2014	SWG	Clinch	Clinchport
Lampsilis abrupta	149	2014	SWG	Clinch	Clinchport
Medionidus conradicus	133	2014	SWG	Clinch	Clinchport
Lampsilis abrupta	118	2014	TWRA Partnership	Clinch	Kyles Ford
Epioblasma brevidens	435	2014	TWRA Partnership	Duck	Lillards Mill
Epioblasma capsaeformis	154	2014	TWRA Partnership	Emory	Oakdale Bridge
Epioblasma brevidens	1,918	2014	SWG	Clinch	Speers Ferry
Epioblasma capsaeformis	634	2014	SWG	Clinch	Speers Ferry
Lampsilis abrupta	161	2014	SWG	Clinch	Speers Ferry
Lemiox rimosus	22	2014	SWG	Clinch	Speers Ferry

Species	Number Released	Year	Project	River	Release Site
Ligumia recta	17	2014	SWG	Clinch	Speers Ferry
Medionidus conradicus	151	2014	SWG	Clinch	Speers Ferry
Lampsilis fasciola	106	2014	SWG	Clinch	St. Paul
Lampsilis ovata	22	2014	SWG	Clinch	St. Paul
Villosa iris	554	2014	SWG	Clinch	St. Paul
Epioblasma brevidens	550	2014	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Lasmigona holstonia	30	2014	Baptist Valley Sewer	Johnson Branch	White House
2014 Total	7,944				
Epioblasma brevidens	877	2015	SWG	Clinch	Clinchport
Epioblasma capsaeformis	348	2015	SWG	Clinch	Clinchport
Lampsilis fasciola	144	2015	SWG	Clinch	Clinchport
Lampsilis ovata	1	2015	SWG	Clinch	Clinchport
Ligumia recta	189	2015	SWG	Clinch	Clinchport
Medionidus conradicus	197	2015	SWG	Clinch	Clinchport
Epioblasma brevidens	913	2015	SWG	Powell	Fletcher Ford
Epioblasma capsaeformis	309	2015	SWG	Powell	Fletcher Ford
Ligumia recta	221	2015	SWG	Powell	Fletcher Ford
Epioblasma brevidens	510	2015	TWRA Partnership	Duck	Lillards Mill
Epioblasma brevidens	786	2015	SWG	Clinch	Slant
Epioblasma capsaeformis	324	2015	SWG	Clinch	Slant
Ligumia recta	185	2015	SWG	Clinch	Slant
Medionidus conradicus	207	2015	SWG	Clinch	Slant
Villosa iris	198	2015	SWG	Clinch	Slant
Epioblasma brevidens	857	2015	SWG	Clinch	Speers Ferry
Epioblasma capsaeformis	325	2015	SWG	Clinch	Speers Ferry
Fusconaia cuneolus	29	2015	SWG	Clinch	Speers Ferry
Ligumia recta	191	2015	SWG	Clinch	Speers Ferry
Epioblasma brevidens	503	2015	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Epioblasma capsaeformis	306	2015	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Lemiox rimosus	86	2015	TWRA Partnership	Nolichucky	TWRA Canoe Launch
2015 Total	7,706				
Epioblasma brevidens	197	2016	TVA/TNC	Powell	Brooks Bridge/Oakley Property
Villosa iris	150	2016	TVA/TNC	Powell	Brooks Bridge/Oakley Property
Lasmigona holstonia	414	2016	Baptist Valley Sewer	Cavitts Creek	Dollar General
Lasmigona holstonia	455	2016	Baptist Valley Sewer	South Fork Clinch	Dunford Park
Lasmigona holstonia	389	2016	Baptist Valley Sewer	North Fork Clinch	GOD Trailer
Lampsilis abrupta	100	2016	TWRA Partnership	Clinch	Kyles Ford
Epioblasma brevidens	250	2016	TWRA Partnership	Duck	Lillards Mill
Cyprogenia stegaria	91	2016	SWG	Clinch	Speers Ferry

Species	Number Released	Year	Project	River	Release Site
Villosa iris	361	2016	SWG	Clinch	St. Paul
Epioblasma brevidens	250	2016	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Epioblasma capsaeformis	100	2016	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Lampsilis abrupta	100	2016	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Lasmigona holstonia	126	2016	Baptist Valley Sewer	Johnson Branch	White House
Lasmigona holstonia	867	2016	Baptist Valley Sewer	North Fork Clinch	Your Grate Escape Restaurant
2016 Total	3,850				
Epioblasma brevidens	950	2017	SWG	Powell	Fletcher Ford
Epioblasma capsaeformis	220	2017	SWG	Powell	Fletcher Ford
Epioblasma brevidens	250	2017	TVA/TNC	Powell	Lower Brooks Bridge
Epioblasma capsaeformis	250	2017	TVA/TNC	Powell	Lower Brooks Bridge
Lampsilis fasciola	300	2017	TVA/TNC	Powell	Lower Brooks Bridge
Villosa iris	20	2017	TVA/TNC	Powell	Lower Brooks Bridge
Villosa vanuxemensis	500	2017	TVA/TNC	Powell	Lower Brooks Bridge
Epioblasma brevidens	60	2017	TVA/TNC	Powell	Oakley Property/Upper Brooks Bridge
Epioblasma capsaeformis	60	2017	TVA/TNC	Powell	Oakley Property/Upper Brooks Bridge
Villosa iris	60	2017	TVA/TNC	Powell	Oakley Property/Upper Brooks Bridge
Epioblasma brevidens	1,050	2017	SWG	Powell	Rt. 833 Bridge
Epioblasma capsaeformis	219	2017	SWG	Powell	Rt. 833 Bridge
Lampsilis fasciola	25	2017	SWG	Clinch	St. Paul
Villosa iris	159	2017	SWG	Clinch	St. Paul
Villosa vanuxemensis	50	2017	SWG	Clinch	St. Paul
Epioblasma brevidens	800	2017	TWRA Partnership	Duck	Venable Springs
Epioblasma brevidens	955	2017	TWRA Partnership	Elk	Veto Fish Trap
Epioblasma capsaeformis	500	2017	TWRA Partnership	Elk	Veto Fish Trap
Lampsilis abrupta	133	2017	TWRA Partnership	Elk	Veto Fish Trap
2017 Total	6,561				
Medionidus conradicus	366	2018	SWG	Copper Creek	Above 619 Bridge
Venustaconcha trabalis	200	2018	SWG	Copper Creek	Above 619 Bridge
Villosa vanuxemensis	200	2018	SWG	Copper Creek	Above 619 Bridge
Epioblasma brevidens	500	2018	TWRA Partnership		Released to Don Hubbs (Partnership with TWRA)
Epioblasma capsaeformis	250	2018	TWRA Partnership		Released to Don Hubbs (Partnership with TWRA)
Ptychobranchus subtentus	100	2018	TWRA Partnership		Released to Don Hubbs (Partnership with TWRA)
Venustaconcha trabalis	250	2018	TWRA Partnership		Released to Don Hubbs (Partnership with TWRA)
Epioblasma brevidens	550	2018	SWG	Powell	Rt. 833 Bridge
Epioblasma capsaeformis	457	2018	SWG	Powell	Rt. 833 Bridge
Venustaconcha trabalis	45	2018	SWG	Clinch	Speers Ferry

Species	Number Released	Year	Project	River	Release Site
Epioblasma brevidens	80	2018	SWG	Clinch	St. Paul Boat Launch
Lampsilis fasciola	25	2018	SWG	Clinch	St. Paul Boat Launch
Medionidus conradicus	80	2018	SWG	Clinch	St. Paul Boat Launch
Villosa vanuxemensis	131	2018	SWG	Clinch	St. Paul Boat Launch
Venustaconcha trabalis	100	2018	SWG	Copper Creek	Dickensonville Site 2
2018 Total	3,334				
Villosa vanuxemensis	500	2019	SWG	Copper Creek	Above 619 Bridge
Epioblasma brevidens	182	2019	SWG	Clinch	Clinchport Boatramp
Epioblasma capsaeformis	170	2019	SWG	Clinch	Clinchport Boatramp
Lemiox rimosus	50	2019	SWG	Clinch	Clinchport Boatramp
Venustaconcha trabalis	50	2019	SWG	Clinch	Clinchport Boatramp
Ligumia recta	50	2019	SWG	Clinch	Crafts Mill
Villosa vanuxemensis	375	2019	SWG	Clinch	Crafts Mill
Epioblasma capsaeformis	200	2019	Alabama Partnership	Paint Rock	
Venustaconcha trabalis	100	2019	Alabama Partnership	Paint Rock	
Venustaconcha trabalis	300	2019	Alabama Partnership	Paint Rock	
Lampsilis fasciola	799	2019	North Carolina Partnership	Little Tennessee	
Lampsilis ovata	300	2019	North Carolina Partnership	Little Tennessee	
Strophitus undulatus	39	2019	North Carolina Partnership	Little Tennessee	
Epioblasma brevidens	304	2019	LMU Partnership	Powell River	Rt. 833 Bridge
Epioblasma capsaeformis	100	2019	LMU Partnership	Powell River	Rt. 833 Bridge
Ligumia recta	50	2019	LMU Partnership	Powell River	Rt. 833 Bridge
Villosa vanuxemensis	350	2019	LMU Partnership	Powell River	Rt. 833 Bridge
Lemiox rimosus	50	2019	SWG	Clinch	Speers Ferry
Venustaconcha trabalis	50	2019	SWG	Clinch	Speers Ferry
Villosa vanuxemensis	125	2019	SWG	Clinch	St. Paul Boat Launch
Alasmidonta viridis	82	2019	SWG	Plum Creek	Crab Orchard Bridge
Epioblasma brevidens	500	2019	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Lemiox rimosus	750	2019	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Venustaconcha trabalis	750	2019	TWRA Partnership	Nolichucky	TWRA Canoe Launch
2019 Total	6,226				
Grand Total	65,318				

Appendix B

Mussels >6 months old released by FMCC from 2007–2019 for projects other than Certus, Inc. and LMPI NRDAR cases.

Species	Number Released	Year	Project	River	Release Site
Villosa iris	2,060	2007	TWRA Partnership	Clinch	Horton Ford, Hancock County, TN
2007 Total	2,060				
Lampsilis ovata	299	2008	TWRA Partnership	Clinch	Horton Ford, Hancock County, TN
2008 Total	299				
Epioblasma capsaeformis	32	2009	TWRA Partnership	Clinch	Frost Ford, Hancock County, TN
Epioblasma capsaeformis	32	2009	TWRA Partnership	Clinch	Horton Ford, Hancock County, TN
Lampsilis ovata	200	2009	TWRA Partnership	Clinch	Horton Ford, Hancock County, TN
2009 Total	264				
Epioblasma capsaeformis	2,000	2012	TWRA Partnership	Multiple TWRA Sites	
Epioblasma brevidens	18	2012	TWRA Partnership	Nolichucky	Evans Island
Epioblasma brevidens	18	2012	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Epioblasma capsaeformis	36	2012	TWRA Partnership	Nolichucky	TWRA Canoe Launch
Epioblasma capsaeformis	36	2012	TWRA Partnership	Nolichucky	Evans Island
Epioblasma capsaeformis	1,007	2012	TWRA Partnership	Paint Rock	Jackson County, AL, RM 33
Epioblasma capsaeformis	36	2012	TWRA Partnership	Nolichucky	Upper Hales
Epioblasma brevidens	18	2012	TWRA Partnership	Nolichucky	Upper Hales
2012 Total	3,169				
Epioblasma capsaeformis	1,056	2013	TWRA Partnership	Multiple TWRA Sites	
Epioblasma brevidens	200	2013	TWRA Partnership	Multiple TWRA Sites	
2013 Total	1,256				
Epioblasma capsaeformis	500	2014	TWRA Partnership		
2014 Total	500				
Epioblasma triquetra	20	2016	TVA/TNC	Powell	Oakley Property
Villosa iris	50	2016	TVA/TNC	Powell	Oakley Property
Epioblasma capsaeformis	50	2016	TVA/TNC	Powell	Oakley Property
Epioblasma capsaeformis	352	2016	TVA/TNC	Powell	Lower Brooks Bridge
Epioblasma triquetra	80	2016	TVA/TNC	Powell	Lower Brooks Bridge
2016 Total	552				

Species	Number Released	Year	Project	River	Release Site
Venustaconcha trabalis	20	2017	TWRA Partnership	Holston	Beech Creek
Villosa iris	266	2017	TVA/TNC	Powell	Lower Brooks Bridge
Lampsilis fasciola	791	2017	TVA/TNC	Powell	Lower Brooks Bridge
Epioblasma brevidens	500	2017	TVA/TNC	Powell	Lower Brooks Bridge
Epioblasma capsaeformis	100	2017	TVA/TNC	Powell	Lower Brooks Bridge
2017 Total	1,677				
Venustaconcha trabalis	210	2018	TWRA Partnership	Holston	Beech Creek
2018 Total	210				
Epioblasma capsaeformis	500	2019	Alabama Department of Natural Resources and Conservation	Paint Rock	River Mile 50
Epioblasma brevidens	203	2019	TWRA Partnership	Nolichucky	TWRA Canoe Launch
2019 Total	703				
Grand Total	10,690				

Appendix C

Google Earth Photographs of Mussel Release and Monitoring Sites

Figure C.1: Google Earth photographic image of the Payne Property site (RM 322.1) on the Clinch River, Tazewell County, VA. Photograph taken on March 28, 2012. White lines delineate the surveyed area and yellow pin indicates the survey start location.

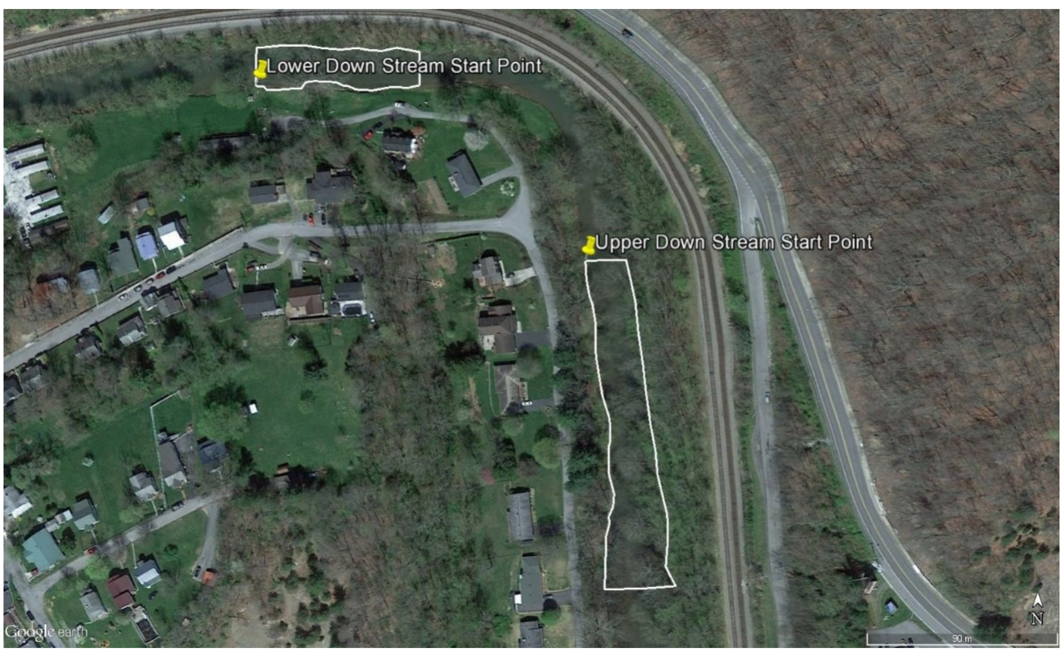


Figure C.2: Google Earth photographic image of the Sycamore Lane site (RM 320) on the Clinch River, Tazewell County, VA. Photograph taken on March 28, 2012. White lines delineate the surveyed area and yellow pin indicates the survey start location.

Figure C.3: Google Earth photographic image of the Bennett Property site (RM 277.5) on the Clinch River, Russell County, VA. Photograph taken on March 28, 2012. White lines delineate the surveyed area and yellow pin indicates the survey start location.

Figure C.4: Google Earth photographic image of the Artrip site (RM 274.5) on the Clinch River, Russell County, VA. Photograph taken on March 28, 2012. White lines delineate the surveyed area and yellow pin indicates the survey start location.

Figure C.5: Google Earth photographic image of the Whited Property site (RM 272.7) on the Clinch River, Russell County, VA. Photograph taken on March 28, 2012. White lines delineate the surveyed area and yellow pin indicates the survey start location.

Figure C.6: Google Earth photographic image of the Cleveland Islands, Right Descending Channel, site (RM 270) on the Clinch River, Russell County, VA. Photograph taken on March 28, 2012. White lines delineate the surveyed area and yellow pin indicates the survey start location.

Figure C.7: Google Earth photographic image of the Upper Brooks Bridge site (RM 95.3) on the Powell River, Claiborne County, TN. Photograph taken on October 21, 2015. White lines delineate the surveyed area and yellow pin indicates the survey start location.

Figure C.8: Google Earth photographic image of the Lower Brooks Bridge site (RM 94.7) on the Powell River, Claiborne County, TN. Photograph taken on October 21, 2015. White lines delineate the surveyed area and yellow pin indicates the survey start location.

Figure C.9: Google Earth photographic image of the Oakley Property site (RM 89.7) on the Powell River, Claiborne County, TN. Photograph taken on October 21, 2015. White lines delineate the surveyed area and yellow pin indicates the survey start location.

Appendix D

Financial data sources used to determine annual mussel propagation and restoration expenses of the Aquatic Wildlife Conservation Center and the Freshwater Mollusk Conservation Center from 2003 to 2019.

Table D.1. Description of financial data sources obtained from U.S. Fish and Wildlife Service (USFWS) Virginia Field Office (VAFO), Virginia Department of Wildlife Resources (VDWR), and Virginia Tech Office of Sponsored Programs (OSP) used to determine annual expenses at the VDWR's Aquatic Wildlife Conservation Center (AWCC) and at Virginia Tech's Freshwater Mollusk Conservation Center (FMCC) to propagate mussels for the Certus, Inc. and Lone Mountain Processing, Inc (LMPI) NRDAR cases and other projects from 2003 to 2019.

File name	File type	Source	Description
AWCC			
2003 AWCC LMPI Acquisition Request	wpd	USFWS VAFO	Acquisition request (AR) to cooperative agreement between United States Fish and Wildlife Service (USFWS) and the Virginia Department of Wildlife Resources (VDWR)
2003 AWCC LMPI Agreement	wpd	USFWS VAFO	Supplemental agreement to cooperative agreement between USFWS and VDWR outlining purpose, objectives, funding, etc.
2008 AWCC Funds Request	doc	USFWS VAFO	Memo requesting release of funds from the LMPI, Inc. NRDAR fund
2010 AWCC LMPI Agreement	pdf	USFWS VAFO	Cooperative agreement between USFWS and VDWR
AWCC Expenses 10-1-15 to 9-30-18	xlsx	VDWR file from Fred Leckie	Summary of expenses of AWCC from October 2015 to September 2018
Certus and SWG Summary 2012-01 to 2018-09	xlsx	VDWR file from Fred Leckie	Summary of funds used for Certus NRDAR project and State Wildlife Grant (SWG) projects. Some expenses for Certus are given in more detail in following files. Only source for SWG expenses
Certus Detail 2012-01 to 2012-09	xlsx	VDWR file from Fred Leckie	Detailed breakdown of Certus expenses from January 2012 to September 2012
Certus Detail 2012-10 to 2013-09	xlsx	VDWR file from Fred Leckie	Detailed breakdown of Certus expenses from October 2012 to September 2013
Certus Detail 2013-10 to 2014-09	xlsx	VDWR file from Fred Leckie	Detailed breakdown of Certus expenses from October 2013 to September 2014
Certus Detail 2014-10 to 2015-09	xlsx	VDWR file from Fred Leckie	Detailed breakdown of Certus expenses from October 2014 to September 2015
LM and CE Expenditures Jan. 2004 - July 2011	xlsx	VDWR file from Fred Leckie	Summary of Lone Mountain and Certus expenses from 2005 to 2012. Years in file are assumed to be off by one year (e.g., expenses for 2005 are actually expenses for 2004) based on timing of expenses from more detailed sources. File is only source for expenses for most years between 2004 and 2011.
LMPI Detail 2012-01 to 2012-04	xlsx	VDWR file from Fred Leckie	Detailed breakdown of LMPI expenses from January 2012 to April 2012
LMPI Detail 2012-05 to 2012-09	xlsx	VDWR file from Fred Leckie	Detailed breakdown of LMPI expenses from May 2012 to September 2012
LMPI Detail 2012-10 to 2013-03	xlsx	VDWR file from Fred Leckie	Detailed breakdown of LMPI expenses from October 2012 to March 2013
LMPI Detail 2013-04 to 2013-08	xlsx	VDWR file from Fred Leckie	Detailed breakdown of LMPI expenses from April 2013 to August 2013
LMPI Summary 2012-01 to 2013-09	xlsx	VDWR file from Fred Leckie	Summary of expenses from January 2012 to September 2013. Used to confirm more detailed expenses.

File name	File type	Source	Description
Fred Leckie Email	pdf	VDWR file from Fred Leckie	Copy of email from Fred Leckie that clarifies how funds were spent among years.
FMCC			
2003 VT LMPI Agreement	wpd	USFWS VAFO	Supplemental agreement to cooperative agreement between USFWS and Virginia Tech for LMPI NRDAR case
2004 VT CERTUS Agreement	doc	USFWS VAFO	Supplemental agreement to cooperative agreement between USFWS and Virginia Tech for Certus NRDAR case
2005 VT CERTUS Agreement	doc	USFWS VAFO	Supplemental agreement to cooperative agreement between USFWS and Virginia Tech for Certus NRDAR case
2006 VT LMPI Agreement	doc	USFWS VAFO	Supplemental agreement to cooperative agreement between USFWS and Virginia Tech for LMPI NRDAR case
2007 VT CERTUS Agreement	doc	USFWS VAFO	Supplemental agreement to cooperative agreement between USFWS and Virginia Tech for Certus NRDAR case
2008 VT LMPI Agreement	pdf	USFWS VAFO	Supplemental agreement to cooperative agreement between USFWS and Virginia Tech for LMPI NRDAR case
2009 VT CERTUS Agreement	pdf	USFWS VAFO	Supplemental agreement to cooperative agreement between USFWS and Virginia Tech for Certus NRDAR case
2009 VT CERTUS OSP Budget	xlsx	OSP	Budget on file with Virginia Tech's Office of Sponsored Programs
2010 VT CERTUS Agreement	pdf	USFWS VAFO	Supplemental agreement to cooperative agreement between USFWS and Virginia Tech for Certus NRDAR case
2010 VT CERTUS OSP Budget	xlsx	OSP	Budget on file with Virginia Tech's Office of Sponsored Programs
2011 VT CERTUS OSP Budget	xlsx	OSP	Budget on file with Virginia Tech’s Office of Sponsored Programs
2012 VT CERTUS OSP Budget	xlsx	OSP	Budget on file with Virginia Tech’s Office of Sponsored Programs
2013 and 2014 VT CERTUS OSP Budget	xlsx	OSP	Budget on file with Virginia Tech’s Office of Sponsored Programs
2015 and 2016 VT CERTUS OSP Budget	xlsx	OSP	Budget on file with Virginia Tech’s Office of Sponsored Programs
2017 and 2019 VT CERTUS OSP Budget	xlsx	OSP	Budget on file with Virginia Tech’s Office of Sponsored Programs
Misc.			
CERTUS Budgets Compiled	xlsx		Compiled budgets for Certus funds at FMCC and AWCC. Used for confirmation or if no other data was available. Other sources took priority if there was a discrepancy.
LMPI Budgets Compiled	xlsx		Compiled budgets for LMPI funds at FMCC and AWCC. Used for confirmation or if no other data was available. Other sources took priority if there was a discrepancy.

Appendix E

Detailed costs by category to operate the Freshwater Mollusk Conservation Center from 2003 to 2019.

Table E.1. Nominal cost of operating the Freshwater Mollusk Conservation Center from 2003 to 2019. Costs include all projects.

Category	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Grand Total
Salary, Wages, and Benefits[1]																		
Salary	$37,000	$26,780	$6,735	$27,437	$31,304	$32,448	$48,898	$58,243	$35,044	$52,574	$53,250	$55,380	$21,163	$18,972	$40,064	$27,779	$13,201	$586,272
Salary Fringe	$12,950	$8,034	$2,239	$0	$14,008	$0	$23,960	$27,520	$0	$14,983	$20,701	$22,152	$9,973	$8,880	$19,104	$14,306	$6,799	$205,609
Technician	$26,000	$12,500	$0	$16,983	$12,522	$14,739	$0	$0	$12,000	$24,000	$12,000	$13,720	$47,360	$47,360	$31,000	$18,000	$0	$288,184
Technician Fringe	$9,100	$3,750	$0	$0	$5,604	$0	$0	$0	$0	$1,800	$975	$1,063	$3,670	$3,670	$2,403	$1,395	$0	$33,430
GRA	$0	$0	$11,496	$0	$0	$0	$0	$0	$9,366	$10,316	$7,610	$0	$2,000	$2,000	$11,260	$5,277	$0	$59,325
GRA Fringe	$0	$0	$719	$0	$0	$0	$0	$0	$0	$722	$618	$0	$113	$135	$902	$528	$0	$3,737
Fringe Unseparated[2]	$0	$0	$0	$17,341	$0	$20,566	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$37,907
Indirect Costs[3]	$10,705	$8,198	$2,769	$6,726	$7,343	$7,175	$8,136	$10,061	$9,662	$18,018	$0	$0	$0	$0	$0	$0	$0	$88,793
Equipment[4]	$18,000	$15,920	$0	$1,000	$3,000	$3,000	$3,000	$3,326	$2,500	$3,000	$2,000	$0	$3,000	$3,000	$2,587	$3,000	$2,001	$68,334
Travel[5]	$4,000	$2,500	$6,000	$2,000	$2,000	$1,000	$3,000	$5,000	$3,000	$3,500	$3,500	$3,500	$3,500	$3,500	$6,000	$3,500	$1,000	$56,500
Materials/Supplies[6]	$0	$0	$500	$0	$0	$0	$2,500	$9,850	$2,500	$4,000	$4,000	$4,000	$4,683	$7,000	$4,400	$4,000	$1,000	$48,433
Tuition/Academic Fees[7]	$0	$0	$0	$0	$0	$0	$0	$0	$0	$5,225	$5,531	$0	$1,038	$1,058	$8,711	$5,311	$0	$26,874
Contractual Services[8]	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$3,500	$3,500	$3,500	$2,000	$0	$12,500
O&M	$0	$10,000	$0	$2,500	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$12,500
Misc. Operational Costs[9]	$0	$2,500	$0	$0	$5,000	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$1,000	$8,500
Grand Total	$117,755	$90,182	$30,458	$73,987	$80,781	$78,928	$89,494	$114,000	$74,072	$138,138	$110,185	$99,815	$100,000	$99,075	$129,931	$85,096	$25,001	$1,536,898

[1] Includes all costs related to personnel compensation
[2] Fringe for these two years was not separated in supplemental agreement
[3] Negotiated rate for indirect cost 10% paid to Virginia Tech by USFWS
[4] Items above $2,500 (e.g., chillers)
[5] Gas, mileage, hotels for collecting fish and mussels
[6] Items below $2,500 (e.g., food for fish/mussels, water quality reagents, etc.)
[7] Fees for graduate research assistant support only
[8] Water quality analysis at Virginia Tech laboratory facilities
[9] Includes publication costs, departmental direct costs (for administrative support within the Department of Fish and Wildlife Conservation), and telephones and other miscellaneous operations

Table E.2. Real costs in 2020 dollars of operating the Freshwater Mollusk Conservation Center from 2003 to 2019. Costs include all projects. Nominal costs were adjusted to real costs in 2020 dollars using the Consumer Price Index for All Urban Consumers (https://fred.stlouisfed.org/series/CPIAUCNS) by multiplying the nominal costs by the ratio of the price index for 2020 to the price index for each year.

Category	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Grand Total
Salary, Wages, and Benefits																		
Salary	$52,055	$36,694	$8,926	$35,225	$39,075	$39,005	$58,989	$69,129	$40,321	$59,264	$59,160	$60,544	$23,109	$20,458	$42,301	$28,631	$13,364	$686,251
Salary Fringe	$18,219	$11,008	$2,967	$0	$17,485	$0	$28,905	$32,664	$0	$16,890	$22,998	$24,218	$10,890	$9,576	$20,171	$14,745	$6,883	$237,619
Technician	$36,579	$17,128	$0	$21,803	$15,630	$17,717	$0	$0	$13,807	$27,054	$13,332	$14,999	$51,715	$51,071	$32,732	$18,552	$0	$332,120
Technician Fringe	$12,803	$5,138	$0	$0	$6,995	$0	$0	$0	$0	$2,029	$1,083	$1,162	$4,007	$3,958	$2,537	$1,438	$0	$41,151
GRA	$0	$0	$15,235	$0	$0	$0	$0	$0	$10,776	$11,629	$8,455	$0	$2,184	$2,157	$11,889	$5,439	$0	$67,763
GRA Fringe	$0	$0	$953	$0	$0	$0	$0	$0	$0	$814	$687	$0	$123	$146	$953	$544	$0	$4,219
Fringe Unseparated	$0	$0	$0	$22,263	$0	$24,722	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$46,985
Indirect Costs	$15,061	$11,233	$3,670	$8,635	$9,166	$8,625	$9,815	$11,942	$11,117	$20,311	$0	$0	$0	$0	$0	$0	$0	$109,574
Equipment	$25,324	$21,814	$0	$1,284	$3,745	$3,606	$3,619	$3,948	$2,876	$3,382	$2,222	$0	$3,276	$3,235	$2,732	$3,092	$2,026	$86,180
Travel	$5,628	$3,426	$7,952	$2,568	$2,496	$1,202	$3,619	$5,935	$3,452	$3,945	$3,888	$3,826	$3,822	$3,774	$6,335	$3,607	$1,012	$66,487
Materials/Supplies	$0	$0	$663	$0	$0	$0	$3,016	$11,691	$2,876	$4,509	$4,444	$4,373	$5,114	$7,548	$4,646	$4,123	$1,012	$54,015
Tuition/Academic Fees	$0	$0	$0	$0	$0	$0	$0	$0	$0	$5,890	$6,145	$0	$1,133	$1,141	$9,198	$5,474	$0	$28,981
O&M	$0	$13,702	$0	$3,210	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$16,912
Contractual Services	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$3,822	$3,774	$3,695	$2,061	$0	$13,353
Misc. Operational Costs	$0	$3,426	$0	$0	$6,241	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$0	$1,012	$10,679
Grand Total	$165,670	$123,569	$40,365	$94,987	$100,833	$94,878	$107,963	$135,308	$85,226	$155,717	$122,414	$109,122	$109,195	$106,837	$137,188	$87,707	$25,309	$1,802,288