



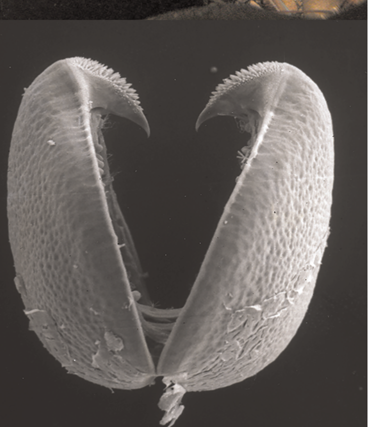

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REGULAR ARTICLE

A REVISED LIST OF THE NORTH AMERICAN SPHAERIIDAE: COMMON NAMES, SCIENTIFIC NAMES, AND DISTRIBUTIONS

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ABSTRACT

The Sphaeriidae of North America, including species found in Canada, Mexico, and the United States, is reviewed, and the taxonomy is summarized and updated to modern standards. This family of small freshwater bivalves is to be added to the Freshwater Mollusk Conservation Society List of Common and Scientific Names of Freshwater Bivalves of North America. We have included a revised taxonomy and the known distributions for Canada, Mexico, and the United States. The total sphaeriid fauna of North America consists of 49 native and introduced species.

KEY WORDS: Sphaeriidae, Fingernail Clam, Pillclam, Peaclam, Canada, Mexico, United States, freshwater, bivalve

INTRODUCTION

During the Freshwater Mollusk Conservation Society (FMCS) Bivalve Common and Scientific Names Subcommittee Meeting on April 10, 2023, in Portland, Oregon, we discussed expanding the FMCS List of Common and Scientific Names of Freshwater Bivalves of North America (hereafter FMCS List) to include the family Sphaeriidae. Known as fingernail clams, pillclams, or peaclams, they have a Recent global distribution, excluding Antarctica. This family was included in the Common and Scientific Names of Aquatic Invertebrates from the United States and Canada (Turgeon et al. 1988, 1998); however, it was not included in Williams et al. (2017) and has not been featured in the FMCS List (FMCS 2019, 2021, 2023, 2025). At the 2025 FMCS meeting in Ann Arbor, Michigan, the subcommittee discussed this addition and noted the current limited active systematic and taxonomic research on this family in Canada, Mexico, and the United States. The subcommittee agreed to add Sphaeriidae to the FMCS List, but noted that this would first require the development, peer review, and publication of an updated and revised list of this family throughout North America. The aim of our work is to produce this list based on a review of the literature. Because the taxonomy reported from Canada and the United States has not been updated in the past 16 yr, we constructed Table 1 (comparison of classifications) and Table 2 (revised list of sphaeriid species) by using Turgeon et al. (1998), Mackie (2007), Bogan and Naranjo-Garcia (2024), Graf and Cummings (2025),and MolluscaBase (2025).

LITERATURE REVIEW

Sphaeriidae is the second largest freshwater bivalve family, currently containing 260 recognized modern species in two subfamilies and eight genera (MolluscaBase 2025) or 272 recognized species in nine genera (Graf and Cummings

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Table 1. Comparison of the various classifications for the Sphaeriidae of Canada and the United States, with an updated generic placement of the species.

Turgeon et al. (1998)	Mackie (2007)	MolluscaBase (2025)	Graf and Cummings (2025)	Updated Canada and US list
Eupera cubensis (Prime, 1865)	*Eupera cubensis*	*Eupera cubensis*	*Eupera cubensis*	*Eupera cubensis* (Prime, 1865)
Musculium lacustre (Müller, 1774)	*Musculium lacustre*	*Sphaerium lacustre*	*Sphaerium lacustre*	*Sphaerium lacustre* (Müller, 1774)
Musculium partumeium (Say, 1822)	*Musculium partumeium*	*Sphaerium partumeium*	*Sphaerium partumeium*	*Sphaerium partumeium* (Say, 1822)
Musculium securis (Prime, 1852)	*Musculium securis*	*Sphaerium securis*	*Sphaerium securis*	*Sphaerium securis* (Prime, 1852)
Musculium transversum (Say, 1829)	*Musculium transversum*	*Sphaerium transversum*	*Sphaerium transversum*	*Sphaerium transversum* (Say, 1829)
Pisidium adamsi Stimpson, 1851*	*Pisidium adamsi**	*Euglesa adamsii*	*Euglesa adamsii*	*Euglesa adamsii* (Stimpson, 1851)
Pisidium amnicum (Müller, 1774)	*Pisidium amnicum*	*Pisidium amnicum*	*Pisidium amnicum*	*Pisidium amnicum* (Müller, 1774)
Pisidium casertanum (Poli, 1791)	*Pisidium casertanum*	*Euglesea casertana*	*Euglesa casertana*	*Euglesa casertana* (Poli, 1791)
Pisidium compressum Prime, 1852	*Pisidium compressum*	*Euglesa compressa*	*Euglesa compressa*	*Euglesa compressa* (Prime, 1852)
Pisidium conventus Clessin, 1877	*Pisidium conventus*	*Conventus conventus*	*Conventus conventus*	*Conventus conventus* (Clessin, 1877)
Pisidium cruciatum Sterki, 1895	*Pisidium cruciatum*	*Pisidium cruciatum*	*Pisidium cruciatum*	*Pisidium cruciatum* Sterki, 1895
Pisidium dubium (Say, 1817)	*Pisidium dubium*	*Pisidium dubium*	*Pisidium dubium*	*Pisidium dubium* (Say, 1817)
Pisidium equilaterale Prime, 1852	*Pisidium equilaterale*	*Euglesa equilateralis*	*Euglesa equilateralis*	*Euglesa equilateralis* (Prime, 1852)
Pisidium fallax Sterki, 1896	*Pisidium fallax*	*Euglesa fallax*	*Euglesa fallax*	*Euglesa fallax* (Sterki, 1896)
Pisidium ferrugineum Prime, 1852	*Pisidium ferrugineum*	*Euglesa ferruginea*	*Euglesa ferruginea*	*Euglesa ferruginea* (Prime, 1852)
Pisidium henslowanum (Sheppard, 1825)	*Pisidium henslowanum*	*Euglesa henslowana*	*Euglesa henslowana*	*Euglesa henslowana* (Sheppard, 1825)
Pisidium idahoense Roper, 1890	*Pisidium idahoense*	*Pisidium idahoense*	*Pisidium idahoense*	*Pisidium idahoense* Roper, 1891
Pisidium insigne Gabb, 1868	*Pisidium insigne*	*Conventus insigne*	*Conventus insigne*	*Conventus insigne* (Gabb, 1868)
Pisidium lilljeborgi (Clessin in Esmark and Hoyer, 1886)	*Pisidium lilljeborgi*	*Euglesa lilljeborgii*	*Euglesa lilljeborgii*	*Euglesa lilljeborgii* (Clessin in Esmark & Hoyer, 1886)
Pisidium milium Held, 1836	*Pisidium milium*	*Euglesa milium*	*Euglesa milium*	*Euglesa milium* (Held, 1836)
	Pisidium moitessierianum Paladilhe, 1866	*Odhneripisidium moitessierianum*	*Odhneripisidium moitessierianum*	*Odhneripisidium moitessierianum* (Paladilhe, 1866)
Pisidium nitidum Jenyns, 1832	*Pisidium nitidum*	*Euglesa nitida*	*Euglesa nitida*	*Euglesa nitida* (Jenyns, 1832)
Pisidium punctatum Sterki, 1895	*Pisidium punctatum*	*Pisidium punctatum*	*Pisidium punctatum*	*Pisidium punctatum* Sterki, 1896
Pisidium punctiferum (Guppy, 1867)	*Pisidium punctiferum*	*Pisidium punctiferum*	*Pisidium punctiferum*	*Pisidium punctiferum* (Guppy, 1867)
Pisidium rotundatum Prime, 1852	*Pisidium rotundatum*	*Euglesa rotundata*	*Euglesa rotundata*	*Euglesa rotundata* (Prime, 1852)
Pisidium sanguinichristi Taylor, 1987		*Pisidium sanguinichristi*	*Pisidium sanguinichristi*	*Euglesa variabilis/* or *Euglesa casertana*
Pisidium subtruncatum Malm, 1855	*Pisidium subtruncatum*	*Euglesa subtruncata*	*Euglesa subtruncata*	*Euglesa subtruncata* (Malm, 1855)
Pisidium supinum Schmidt, 1850	*Pisidium supinum*	*Euglesa supina*	*Euglesa supina*	*Euglesa supina* (Schmidt, 1851)
Pisidium ultramontanum Prime, 1865	*Pisidium ultramontanum*	*Euglesa ultramontana*	*Euglesa ultramontana*	*Euglesa ultramontana* (Prime, 1865)
Pisidium variabile Prime, 1852	*Pisidium variabile*	*Euglesa variabilis*	*Euglesa variabilis*	*Euglesa variabilis* (Prime, 1852)
Pisidium ventricosum Prime, 1851	*Pisidium ventricosum*	*Euglesa ventricosa*	*Euglesa ventricosa*	*Euglesa ventricosa* (Prime, 1851)
Pisidium walkeri Sterki, 1895	*Pisidium walkeri*	*Euglesa walkeri*	*Euglesa walkeri*	*Euglesa walkeri* (Sterki, 1895)
Sphaerium corneum (Linnaeus, 1758)	*Sphaerium corneum*	*Sphaerium corneum*	*Sphaerium corneum*	*Sphaerium corneum* (Linnaeus, 1758)
Sphaerium fabale (Prime, 1852)	*Sphaerium fabale*	*Sphaerium fabale*	*Sphaerium fabale*	*Sphaerium fabale* (Prime, 1852)
Sphaerium nitidum Clessin in Westerlund, 1876	*Sphaerium nitidum*	*Sphaerium nitidum*	*Sphaerium nitidum*	*Sphaerium nitidum* Clessin in Westerlund, 1876
Sphaerium occidentale (Lewis, 1856)	*Sphaerium occidentale*	*Sphaerium occidentale*	*Sphaerium occidentale*	*Sphaerium occidentale* (Lewis, 1856)
Sphaerium patella (Gould, 1850)	*Sphaerium patella*	*Sphaerium patella*	*Sphaerium patella*	*Sphaerium patella* (Gould, 1850)
Sphaerium rhomboideum (Say, 1822)	*Sphaerium rhomboideum*	*Sphaerium rhomboideum*	*Sphaerium rhomboideum*	*Sphaerium rhomboideum* (Say, 1822)
Sphaerium simile (Say, 1817)	*Sphaerium simile*	*Sphaerium simile*	*Sphaerium simile*	*Sphaerium simile* (Say, 1817)
Sphaerium striatinum (Lamarck, 1818)	*Sphaerium striatinum*	*Sphaerium striatinum*	*Sphaerium striatinum*	*Sphaerium striatinum* (Lamarck, 1818)

* Misspelled, originally described as *P. adamsii*.

2025). Bogan (2008) reported 196 species, whereas Graf (2013), Lee (2019), and Graf and Cummings (2021) listed the current worldwide Sphaeriidae diversity at 227 species. The most recent subfamily Sphaeriinae phylogeny was presented by Bespalaya et al. (2023).

The Sphaeriidae of Mexico was reviewed, and the taxonomy was updated by Bogan and Naranjo-Garcia (2024); it is included in Table 2. The Sphaeriidae of North America (Canada and the United States only) was revised by Herrington (1962). An illustrated key to the North American species was provided by Burch (1972, 1975). Sphaeriidae was included in the two editions of the American Fisheries Society list of Common and Scientific Names of Aquatic Invertebrates from the United States and Canada (Turgeon et al. 1988, 1998). The most recent work covering the taxonomy, distribution, and biology of this family in North America was produced by Mackie (2007). He overlooked *Pisidium sanguinichristi* (Taylor 1987) and recognized *Pisidium moitessierianum* (Paladilhe 1866), now *Odhneripisidium moitessierianum,* as introduced into the Great Lakes. The common name Pygmy Peaclam was used for *P. moitessierianum* by Kipp et al. (2025d) and the introduced distribution confirmed (see Grigorovich et al. 2000; Korniushin et al. 2001; Kipp et al. 2025c). Hein et al. (2025) presented analyses of two genes that showed *Pisidium sanguinichristi* was not an endemic species but rather a local population of a widespread species.

Table 2. Revised list of the Sphaeriidae reported from North America: Canada (Can), Mexico (Mex), and the United States (US). Mexican taxa, common names, and distribution from Bogan and Naranjo-Garcia (2024). North American distributions are presented as postal codes for U.S. states, Canada post abbreviations for Canadian provinces, and abbreviated Mexican states.

New North American Sphaeriidae List	English Common Name	Spanish Common Name	North American Distribution
Conventus conventus (Clessin, 1877)	Alpine Peaclam		**Can:** AB, BC, MB, NT, ON, QC, SK, YT. **US:** AK, AL, CO, MI, NY, WA
Conventus insigne (Gabb, 1868)	Tiny Peaclam		**Can:** BC, ON, PI. **US:** AZ, CA, COL, ID, ME, MI, MT, NM, NV, NY, OR, PA, WA, WY. **Mex:** VER
Euglesa adamsii (Stimpson, 1851)	Adams Peaclam[a]		**Can:** NB, NF, NS, ON, QT, SK. **US:** AL, CO, DE, FL, GA, IA, IL, IN, KS, MA, ME, MI, MT, NH, NJ, NY, OH, PA, TN, TX, VA, WI
Euglesa casertana (Poli, 1791)	Ubiquitous Peaclam		**Can:** AB, BC, MB, NB, NF, NT, NS, ON, PI, QC, SK, YT. **US:** AL, AK, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IA, IL, IN, KS, KY, LA, MA, MD, M, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VT, VA, WA, WV, WI, WY. **Mex:** EDOMEX, VER
Euglesa compressa (Prime, 1852)	Ridgebeak Peaclam	Almeja Comprimida	**Can:** AL, BC, MB, NT, ON, PI, QC, SK. **US:** AL, AK, AZ, AR, CO, GA, IA, ID, IL, IN, KS, MA, ME, MI, MN, MO, MT, NC, ND, NJ, NM, NV, NY, OH, OR, PA, SD, TN, TX, UT, VT, WI, WY. **Mex:** COAH, NL, SLP
Euglesa equilateralis (Prime, 1852)	Round Peaclam		**Can:** NB, ON, QC. **US:** MA, ME, NJ, NY, PA, RI, VA
Euglesa fallax (Sterki, 1896)	River Peaclam		**Can:** AB, MB, NT, ON, SK, QC. **US:** AL, IA, IL, ME, MI, MN, NJ, NY, OH, WA
Euglesa ferruginea (Prime, 1852)	Rusty Peaclam		**Can:** AB, BC, MB, NB, NT, ON, QC, SK, YT. **US:** CA, IL, IN, MA, ME, MI, MT, NJ, NY, OH, OR, UT, WA, WY
Euglea globularis (Clessin in Westerlund, 1873)	Rotund Peaclam		**US:** CO
Euglesa henslowana (Sheppard, 1825)[*]	Henslow Peaclam		**Can:** ON. **US:** MI, NY
Euglesa lilljeborgii (Clessin in Esmark & Hoyer, 1886)	Lilljeborg Peaclam		**Can:** AB, BC, MB, NT, ON, QC, SK, YT. **US:** CA, CO, IN, MA, MI, MT, NY, UT, VT, WA, WI
Euglesa milium (Held, 1836)	Quadrangular Pillclam		**Can:** BC, MB, NF, ON, SK. **US:** CO, ME, MI, MN, MT, NY, OR, UT
Euglesa nitida (Jenyns, 1832)	Shiny Peaclam		**Can:** AB, BC, MB, NB, NF, NT, ON, PI, QC, SK, YT. **US:** CA, CO, IA, ID, IL, IN, KS, MA, ME, MI, MN, MT, ND, NJ, NM, NY, NV, OH, PA, RI, TX, UT, VT, VA, WA, WI. **Mex:** COAH
Euglesa rotundata (Prime, 1852)	Fat Peaclam		**Can:** AB, MB, NF, NT, ON, QC, SK. **US:** FL, IL, MA, ME, MI, MT, NJ, NY, UT, VT, WA
Euglesa subtruncata (Malm, 1855)	Shortended Peaclam		**Can:** AB, BC, MB, NT, ON, QC, SK, YT. **US:** CA, MI, MT, ND, NY, SD, WI, WY
Euglesa supina (Schmidt, 1851)[*]	Humpbacked Peaclam		**Can:** ON. **US:** MA, MI, NY, OR
Euglesa ultramontana (Prime, 1865)	Montane Peaclam		**US:** CA, OR
Euglesa variabilis (Prime, 1852)	Triangular Peaclam		**Can:** AB, BC, MB, NB, NF, NS, NT, ON, PI, QC, SK. **US:** AL, CA, CO, CT, IA, ID, IL, IN, KS, MA, ME, MI, MN, MT, NJ, NM?, NY, OH, OR, PA, RI, SD, TN, UT, VT, WA, VA, WY?
Euglesa ventricosa (Prime, 1851)	Globular Peaclam		**Can:** AB, MB, NF, NT, ON, QC, SK. **US:** CA, CO, IL, MA, ME, MI, MN, MT, ND, NJ, NY, OH, SD, UT, VT, WA, WI. **Mex:**?
Euglesa walkeri (Sterki, 1895)	Walker Peaclam		**Can:** AB, MB, NT, ON, QC, SK. **US:** AK, IA, IL, MA, ME, MI, MN, MO, MT, NY, OH, PA, RI, SD, VA
Eupera cubensis (Prime, 1865)[**]	Mottled Fingernailclam		**US:** AL, FL, GA, IL, LA, ME, MI, MS, NC, SC, TX, VA.[b] **Mex:** COAH
Eupera insignis Pilsbry, 1926		Almeja distinguida	**Mex:** VER
Eupera singleyi (Pilsbry, 1889)		Almeja de singley	**Mex:** YUC
Odhneripisidium moitessierianum (Paladilhe, 1866)[*c,d]	Perforated Peaclam		**US:** MI, MN, OH
Pisidium amnicum (Müller, 1774)[*]	Greater European Peaclam		**Can:** ON. **US:** MI, NJ, NY, OH, PA
Pisidium cruciatum Sterki, 1895	Ornamented Peaclam		**Can:** ON. **US:** AL, AR?, IL, MI, MS, MT, OH, WI
Pisidium dubium (Say, 1817)	Greater Eastern Peaclam		**Can:** ON, QC, YT? **US:** AL, AK, FL, GA, MA, MI, MN, NJ, NY, PA, SC, TN, VT, VA
Pisidium idahoense Roper, 1891	Giant Northern Peaclam		**Can:** AB, MB, NB, NT, ON, PI, SK, YT. **US:** CA, ID, IN, MI, MT, WA, WI
Pisidium punctatum Sterki, 1896	Perforated Peaclam		**Can:** BC, MB, ON, SK. **US:** AL, IL, IN, MA, MI, OH, VA, WI

Table 2, continued.

New North American Sphaeriidae List	English Common Name	Spanish Common Name	North American Distribution
Pisidium punctiferum (Guppy, 1867)	Striate Peaclam		**US:** AL, AZ, FL, MS, NM, TX. **Mex:** SIN
Pisidium vegae Pilsbry, 1926		Almeja de vega	**Mex:** QR
Sphaerium corneum (Linnaeus, 1758)*[e,f]	European Fingernailclam		**Can:** ON. **US:** FL, IL, GA, MI, NY, TN, VT
Sphaerium fabale (Prime, 1852)	River Fingernailclam		**Can:** ON. **US:** AL, GA, ID, MD, MI, MT, NJ, NY, OH, PA, TN, VA, WA, WV
Sphaerium lacustre (Müller, 1774)	Lake Fingernailclam		**Can:** AB, BC, MB, NF, NT, ON, PI, QC, SK, YT. **US:** AL, CA, CO, DE, FL, GA, HI, IA, ID, IL, IN, KS, LA, MA, ME, MI, MN, MT, NE, NH, NJ, NY, OH, OR, PA, RI, SD, TN, VA, WA, WI, WY
Sphaerium martensi Pilsbry, 1899		Almeja de martens	**Mex:** MICH
Sphaerium mexicanum Dall, 1905		Almeja mexicana	**Mex:** SLP
Sphaerium nitidum Clessin in Westerlund, 1876	Arctic Fingernailclam		**Can:** AB, BC, MB, NL, NT, ON, QC, SK, YT. **US:** AK, IA, ID, MA, ME, MI, MN, MT, NY, OR, UT, WA
Sphaerium novoleonis Pilsbry, 1904		Almeja de Nuevo León	**Mex:** NL
Sphaerium occidentale (Lewis, 1856)	Herrington Fingernailclam		**Can:** MB, ON, QC. **US:** AL, CA, CO, CT, DE, GA, IA, ID, IL, IN, KS, MA, ME, MI, MN, MT, NJ, NY, OH, OR, PA, SC, TN, UT, VA, VT, WA, WI
Sphaerium partumeium (Say, 1822)	Swamp Fingernailclam		**Can:** MB, NB, NF, ON, QC, SK **US:** AL, CA, CT, DE, FL, GA, IA, IL, IN, KS, LA, MA, Me, MI, MN, MO, MS, MT, NC, ND, NE, NJ, NV, NY, OH, OK, PA, RI, SC, TN, TX, VT, VA, WI. **Mex:** TAB
Sphaerium patella (Gould, 1850)	Rocky Mountain Fingernailclam		**US:** CA, CO, GA, ID
Sphaerium queretaronis Pilsbry, 1926		Almeja de Querétaro	**Mex:** QRO, VER
Sphaerium rhomboideum (Say, 1822)	Rhomboid Fingernailclam		**Can:** BC, MB, NB, NFL, ON. **US:** CT, ID, NY, OH, PA, RI, VT, WI
Sphaerium securis (Prime, 1852)	Pond Fingernailclam		**Can:** AL, BC, MB, NB, NF, NS, ON, QC, SK. **US:** AL, AR, CT, FL, IA, IL, IN, KS, LA, MA, ME, MD, MI, MN, MS, MT, NC, ND, NE, NJ, NY, OH, PA, RI, SC, TN, TX, VA, WA, WI
Sphaerium simile (Say, 1817)	Grooved Fingernailclam		**Can:** AL, MB, ON, QC, SK. **US:** AL, DE, IA, ID, IL, IN, KS, MA, ME, MI, MN, MT, NJ, NY, OH, PA, SD, VA, WY
Sphaerium striatinum (Lamarck, 1818)	Striated Fingernailclam		**Can:** AB, BC, MB, NB, NT, ON, QC, SK, YT. **US:** AL, AZ, AR, CA, CO, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, ME, MD, MI, MN, MO, MS, MT, NC, ND, NE, NJ, NM, NY, NV, OH, OR, PA, RI, SD, TN, TX, UT, VA, WA, WI, WY. **Mex:** JAL, QRO, VER
Sphaerium subtransversum Prime, 1860		Almeja subtransversa	**Mex:** EDOMEX, MICH, TAB, YUC
Sphaerium transversum (Say, 1829)	Long Fingernailclam		**Can:** AL, BC, MB, NT, ON, QC, SK. **US:** AL, AR, CO, FL, GA, IA, ID, IL, IN, KS, KY, LA, ME, MI, MN, MO, MT, NC, ND, NE, NJ, NY, OH, OK, PA, TN, TX, VA, WV, WI. **Mex:** QRO, TLAX
Sphaerium triangulare (Say, 1829)		Almeja triangular	**Mex:** EDOMEX, GTO, JAL, MICH

? Species record for this state is in the literature but needs to be confirmed. * Introduced.
** Native and introduced.
[a] B. Watson (23 October 2025, personal communication).
[b] Changed to Adams Peaclam to match species name.
[c] Kipp et al. (2025c).
[d] Kipp et al. (2025d).
[e] Kipp et al. (2025a).
[f] Kipp et al. (2025b).

They identified it as either *Euglesa variabilis* or *Euglesa casertana.*

Bogan and Naranjo-Garcia (2024) listed *Pisidium obtusale* Pfeiffer, 1821 *non* Lamarck,1818 from Mexico, but in error changed it to the primary senior homonym *Pisidium obtusale* Lamarck, 1818. *Pisidum obtusale* Pfeiffer, 1821 *non* Lamarck, 1818 is considered a junior synonym of *Euglesa ventricosa* (Prime 1851) (Clarke 1973; Burch 1975).

Euglesa globularis (Clessin in Westerlund 1873) was considered a junior synonym of *Euglesa casertanum* (Poli 1791), but it was subsequently recognized as a separate and valid species (Glöer and Meier-Brook 2003). Guralnick (2005) explored *Pisidium* (*Cyclocalyx* Dall, 1903) by using 16S rRNA to test relationships of western North American sphaeriids. He found *P. casertanum* was a species complex with a clade separate from *P. casertanum* from Europe, but it was not named. Glöer and Zettler (2005) recognized *P. casertanum* and *P. globularis* as separate species. Bespalaya et al. (2020) examined *E. globularis* in northeast Asia by using 16S RNA data, including Guralnick (2005) sequences from two highland lakes of Colorado, sequences from northern Russia, and sequences from western Europe. These analyses confirmed that the specimens from Colorado are *E. globularis*. Bespalaya et al. (2023) examined the phylogeny of the subfamily Sphaeriinae and confirmed the *E. globularis* species group as containing three species and placed them in the subgenus *Amureuglesa* Korniushin 1996. They noted that *E. globularis* distribution included Europe, Asia, and North America. This species has been added to Table 2. We could not find a common name for *Euglesa globularis*, so we have coined the new common name Globular Peaclam.

SUMMARY

The Sphaeriidae taxonomy for Canada and the United States is updated in Table 1, including comparisons of various classifications and updated generic placement. The authors and dates for the columns, Turgeon et al. (1998) and Updated Canada and US list, are included to clarify which taxa were moved from their original genus of description with the addition of parentheses around the author and date in the revised column. For those species that have been moved, Table 2 summarizes the North American fauna, including common names (both English and Spanish where available) for Canada, Mexico, and the United States. The distribution data for Canada and the United States is from Mackie (2007) and may not include all the states or provinces where the species occurs. Confirming the distributions and delineation of species of Sphaeriidae in North America is an area for future research, which the authors strongly encourage (e.g., Frankiewicz 2024). Currently, this fauna comprises 34 species known from Canada, 19 from Mexico, and 40 from the United States. In total, there are 49 North American sphaeriid fauna. Halabowski et al. (2024) provided an overview of the European Sphaeriidae, including a functional flow diagram of the systematics, zoogeography and conservation needs, that may be used as a starting point for the revision of the North American Sphaeriidae.

We welcome constructive comments and suggestions regarding this revised and updated list.

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REGULAR ARTICLE

RESTORING FRESHWATER MUSSELS TO THE CLINCH AND POWELL RIVERS: MONITORING AND EVALUATION OF THE CERTUS INC. AND LONE MOUNTAIN PROCESSING INC. NATURAL RESOURCE DAMAGE ASSESSMENT CASES IN VIRGINIA AND TENNESSEE, USA

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ABSTRACT

Freshwater mussels are particularly susceptible to injury from releases of hazardous substances. Natural recolonization of injured mussel populations can take decades because of their complex life history. Hence, hatchery propagation and stocking of mussels is commonly used for recovering injured populations. In recent decades, several Natural Resource Damage Assessment and Restoration (NRDAR) cases have involved freshwater mussels, but none have analyzed whether restoration was successful. Our study represents the first evaluation of restoration success of freshwater mussels in an NRDAR context. Its purpose was to determine whether mussel restoration was successful for two large-scale, multiyear (>10 years) NRDAR cases in the Clinch and Powell rivers of Virginia and Tennessee. We used mussel release data compiled from 2004–2017 and a Leslie matrix model to estimate the expected abundance of mussels at nine restoration and monitoring sites. We then compared expected abundances to abundance values estimated from quadrat surveys conducted from 2015–2021 at these same nine sites. Estimated abundances were 57–85% lower than expected. We conducted mark-recapture surveys at two sites and the data from this independent method supported our quadrat survey results; i.e., abundance estimates were much lower than the expected abundance values. However, we observed evidence of successful restoration, such as released mussels reaching breeding ages and presence of mussels at low- to medium densities (0.02–0.48 m^{-2}) at restoration sites, and we confirmed limited recruitment of two species. Nonetheless, lower-than-expected abundance suggests that either mussels are settling or recruiting outside of restoration sites and/or that survival and recruitment of released mussels are lower than expected. Further study is needed to determine to what extent each of these factors explain lower-than-expected abundance to better estimate the scope of restoration required in future NRDAR cases. Finally, we developed a set of metrics to determine whether restoration was successful in this study and for application to future cases involving freshwater mussels.

KEY WORDS: freshwater mussels, NRDAR, quadrat-sampling, restoration, monitoring

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INTRODUCTION

Freshwater mussels provide many ecosystem services, including regulating services (biofiltration of water), supporting services (nutrient cycling and storage, habitat modification, and environmental monitoring), provisioning services (food and products made from shell), and cultural services (providing cultural and existence values) (Vaughn 2018). Unfortunately, freshwater mussels (Unionida) are among the most imperiled groups of freshwater organisms in North America (Vaughn and Taylor 1999; Lopes-Lima et al. 2018). 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). Water pollution and water quality degradation are among the most frequently cited causes of mussel decline (Downing et al. 2010). Due to their sessile nature, mussels are highly susceptible to injury from releases of hazardous substances into aquatic ecosystems. Releases of contaminants into rivers can drastically reduce the diversity and abundance of local mussel populations (Sheehan et al. 1989). Further, the limited dispersal capabilities of mussels and their complex life history (which involves fish hosts for dispersal) make natural recolonization difficult and unlikely in the short term (~10–20 years) (Patterson et al. 2018; Irmscher and Vaughn 2018).

Natural Resource Damage Assessment and Restoration (NRDAR) regulations allow the federal government to assess injury to natural resources resulting from the release of a hazardous substance and to recover damages from responsible parties (43 CFR § 11). There have been numerous NRDAR cases involving injury to freshwater mussels in recent decades. Two are particularly relevant to our study. In 1996, a release of coal slurry from the Lone Mountain Processing Inc. (LMPI) facility near St. Charles, Virginia, impacted a large section of the Powell River, affecting 15 species of federally listed endangered mussels present in the river (U.S. Fish and Wildlife Service 2003). Two years later, a tanker truck operated by Certus Inc. overturned in Cedar Bluff, Virginia (U.S. Fish and Wildlife Service 2004); the resulting spill of a hazardous chemical killed an estimated 18,621 mussels of 14 species, three of which were listed as federally endangered at the time and one other that was listed as endangered after the spill (Hyde and Jones 2021). Additional examples include a decades-long mercury release from the DuPont-Waynesboro facility that affected mussel populations in the South River from 1929 to 1950, resulting in a $4 million settlement for mussel restoration in the South River and South Fork Shenandoah River (U.S. Fish and Wildlife Service 2017); the 1999 release of hazardous substances from a ferro-alloy manufacturing facility that killed over 990,000 mussels in the Ohio River, including individuals of two federally listed endangered species (U.S. Fish and Wildlife Service 2007); and a 2014 coal ash spill in the Dan River (Dan River Natural Resource Trustee Council 2020) that adversely affected the federally endangered James Spinymussel (*Parvaspina collina*) and other mussel species. Together, these cases show that injury to freshwater mussel populations is an ongoing concern.

In these cases, injury assessment and restoration goals varied considerably. For the DuPont-Waynesboro case, an injury of 650,000 mussels was estimated by using reference sites, historical species composition, and impacted habitat to determine expected density and applying the estimated density to the area injured (U.S. Fish and Wildlife Service 2017). For the Ohio River NRDAR case (U.S. Fish and Wildlife 2007), injury was established by documenting the presence of dead mussels immediately downstream from the discharge area of the facility. Surveys at one downstream site found mussel mortality to be 100 percent, and additional surveys estimated 990,000 mussels killed. The selected restoration alternative included translocation of adult mussels, release of infested host fishes, and propagation and release of juvenile mussels. The goal was to restore density in the affected areas to one mussel per square meter, requiring long-term survival of 195,000 individuals to age five. In the Dan River NRDAR case, a Habitat Equivalency Analysis (HEA) was used to determine the level of restoration needed for gains in services to equal the loss of services from the injury. In this case, mussel propagation was not among the selected restoration activities, although three species (Yellow Lampmussel (*Lampsilis cariosa*), Green Floater (*Lasmigona subviridis*), and Notched Rainbow (*Venustaconcha constricta*)) were successfully propagated by the Virginia Fisheries and Aquatic Wildlife Center as part of the pre-NRDAR restoration process (Brian Watson, Virginia Department of Wildlife Resources, personal communication). Rather, several habitat restoration and conservation alternatives were used, including the transfer of 618 acres of land to North Carolina and Virginia state parks and the removal of the Pigg River Power Dam (Dan River Natural Resource Trustee Council 2020). Injury to mussel populations for the Certus Inc. NRDAR case was quantified by counting the number of fresh-dead mussels in the affected area and multiplying by three to account for mussels buried in the substrate (U.S. Fish and Wildlife Service 2001). The primary restoration goal for the Certus Inc. case was to restore the mussel assemblage to approximate baseline conditions, i.e., "the condition of the natural resources and services that would have existed had the incident not occurred," which was accomplished by propagating and releasing most species injured in the spill (U.S. Fish and Wildlife Service 2004). Injury for the LMPI NRDAR case was largely sublethal and defined, but not quantified, as acute (time of spill) and chronic (resuspension over time) toxicity from exposure to hazardous substances, indirect mortality of glochidia due to loss of host fishes, and indirect losses due to habitat degradation from silt and sedimentation (U.S. Fish and Wildlife Service 2003). The primary goal was to restore mussel assemblages to approximate baseline conditions, which was accomplished by propagating and releasing mussels from a targeted suite of injured species in the mussel assemblage. Compared to more recent cases, baseline conditions for the Certus Inc. and LMPI

NRDAR cases were less explicitly defined, and it was difficult to measure success of restoration based on the resulting restoration goals to restore to pre-injury baseline. These examples clearly demonstrate that the methods for determining the extent of injury and subsequent required restoration vary widely from case to case.

Natural recolonization of injured mussel assemblages may take many years, during which time the services they provide would be lost. Given this lag time, release of propagated mussels to restoration sites is a common action in NRDAR cases. Stocking mussels satisfies the "restoration or rehabilitation of injured natural resources to a condition where they can provide the level of services available at baseline" criteria of NRDAR regulations (43 CFR § 11). Further, NRDAR regulations also allow for "the replacement and/or acquisition of equivalent natural resources capable of providing such services. . ." in lieu of, or in addition to, restoration/rehabilitation (43 CFR § 11). In cases involving injury to freshwater mussels, replacement of equivalent services equates to releasing mussels outside of the impacted area or the use of species capable of providing services similar to those afforded by the injured species (replacement/acquisition). It also may be necessary to correct for differences in services provided by juvenile vs. adult mussels because most propagation involves releases of mussels <5 years old, which might not provide the full suite of services afforded by older individuals (Patterson et al. 2018). The extent to which mussels successfully establish at a site poses implications for the number of mussels that need to be released for successful restoration. A lower rate of establishment and survival at a site would require releasing a higher number of mussels over a longer period compared to a site with higher rates of mussel establishment and survival. Further, if mussels are experiencing higher mortality after release than what they would experience naturally, there would be a corresponding decrease in the expected services provided over their lifetime.

Few published studies have analyzed the success of mussel restoration, particularly in a NRDAR context. Although long-term monitoring is conducted in some cases (e.g., restored populations of *P. collina* have been monitored for over 10 years), results of such monitoring are seldom published in the primary literature (Lavictoire and West 2024). Thus, the purpose of our study was to monitor restored mussel populations in the Clinch and Powell rivers for the Certus Inc. and LMPI NRDAR cases to determine whether, and to what extent, restoration efforts for these cases were successful. The successful restoration of injured resources assumes that released mussels are establishing and reproducing at restoration sites. We developed the following metrics for assessing successful mussel restoration: (1) settling into suitable habitat and surviving after release, (2) surviving at rates high enough to reach breeding age, (3) being fertilized, resulting in gravid females, (4) producing recruits that successfully establish, and finally (5) that released mussels and their recruits continue successfully breeding to the point that the mussel assemblage is self-sustaining and stable in the long term (Table 1). These criteria are predicated on the successful establishment of released mussels in sufficient numbers. We focused only on abundance, density, and growth for the Certus Inc. and LMPI cases, because these metrics were developed after the design and implementation of the monitoring program for each case. Future monitoring designs should attempt to measure as many of these metrics as feasible. The objectives of this study were to (1) estimate the expected number of mussels surviving at restoration sites, (2) estimate abundance and density of mussel species at restoration sites, (3) determine whether estimated abundance and density differed from expected abundance and density at restoration sites, (4) determine the shell length growth rate of released mussels, and (5) determine whether, and to what extent, restoration goals were achieved for the Certus Inc. and LMPI NRDAR cases.

Detailed Case Background

The Certus Inc. and LMPI Natural Resource Damage Assessment and Restoration (NRDAR) cases in the upper Tennessee River basin of Virginia are among the first and largest cases in the United States involving injury to freshwater mussels due to release of hazardous substances (Hyde and Jones 2021). The Certus Inc. chemical spill released 5,110 liters of Octocure-554 revised, a rubber accelerant, into a tributary of the Clinch River when a tanker truck overturned in Tazewell County, Virginia, on August 27, 1998. An estimated 18,621 mussels, including 750 individuals of three endangered species (Golden Riffleshell (*Epioblasma aureola*), Purple Bean (*Venustaconcha trabalis*), and Rough Rabbitsfoot (*Theliderma strigillata*)), were killed along an 11-kilometer section of stream (U.S. Fish and Wildlife Service 2004). Further, since the spill, both Fluted Kidneyshell (*Ptychobranchus subtentus*) and Slabside Pearlymussel (*Pleuronaia dolabelloides*) have been listed as endangered. The loss of their local Clinch River populations likely contributed to the listing of these species.

The LMPI coal slurry spill occurred when a holding pond failed at a processing plant in Lee County, Virginia, on October 24, 1996. The spill released 22.7 million liters 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, Tennessee, 105 kilometers downstream from the release. Although 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 were killed, some of which are host fishes for the 15 federally endangered mussel species found in the impacted river reach (U.S. Fish and Wildlife Service 2003). Coal fines and sediment also were deposited in the substrate throughout the affected length of the Powell River and likely continued to impose chronic, sub-lethal impacts due to

Table 1. Monitoring criteria and evidence required for documenting successful establishment and reproductive success of restoring freshwater mussel populations.

Criteria	Evidence	Documentation Difficulty	Strength of Evidence
Settlement into habitat post release	Observation of mussels burrowing into substrate	*Easy*: healthy mussels will typically begin burrowing into habitat minutes after release and can be confirmed visually.	*Weak*: settlement does not guarantee continued survival
Mussels survive until breeding age	Recovery of stocked juvenile mussels >3–5 yrs. post release as mature adults	*Easy*: requires one or more monitoring events at the appropriate interval post release.	*Weak to Medium*: Survival does not guarantee future recruitment
Mussel abundance or density in affected area reaches a target or baseline level	Quantitative survey to estimate abundance or density	*Moderately Difficult*: multiple quantitative monitoring events 5–10 yrs. or more post release required, especially if natural recruitment is necessary to reach baseline.	*Medium*
Mussel growth and condition	Shell length and body weight growth rates are normal relative to individuals in a healthy population	*Easy*: requires measuring length and weight over time and comparing to a baseline population(s).	*Medium*
Reproductive behaviors and successful fertilization resulting in gravid females	Visual confirmation of gravid females with developed glochidia, females displaying mantle lures or releasing conglutinates	*Easy*: short-term brooders often have narrow windows of gravidity, requiring either precise timing of monitoring or multiple monitoring events in a single year. Gravidity of long-term brooders is easier to document.	*Medium*
Production of recruits from released mussels	Visual confirmation of recruits (i.e., individuals of only 0–2 years old); estimate of the number of recruits (quantitative)	*Moderate*: juveniles are small and buried in the substrate, making them difficult to detect even when present. Typically requires multiple years of systematic excavation of substrate to detect their presence.	*Strong*
Recruits successfully establish and breed in sufficient numbers to sustain a stable population	Long-term data showing an initial increase in population size from releases and associated recruitment, which eventually plateaus and stabilizes	*Difficult*: long-lived species (20–80 years) require periodic monitoring decades into the future to determine population stability. Shorter-lived species (<20 years) are easier to document but still require monitoring for 20–30 years.	*Very strong*

resuspension during high flow events in 1996 and 1997 (U.S. Fish and Wildlife Service 2003). 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 (Michalak et al. 2017).

The principal 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 (Hyde and Jones 2021). Consequently, many mussels released as part of restoration were at sites in the immediate impact zone of the Clinch River between Cedar Bluff, Virginia (River Mile (RM) 324) and Richlands, Virginia (RM 318). However, mussels also were released downstream at other restoration sites in the Clinch River in Russell County, Virginia (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 areas of Cedar Bluff and Richlands. Further, the ability to propagate some affected species was limited. Notably, Oyster Mussel (*Epioblasma capsaeformis*) and Cumberlandian Combshell (*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 and non-listed mussel species affected by the spill in the Powell River. Not all federally listed mussel species impacted by the spill could be propagated and restored due to technological limitations (e.g., undeveloped propagation techniques including 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.

METHODS

Study Area

Mussels were sampled at six release and monitoring sites in the Clinch River (Fig. 1) and three of six release and monitoring sites in the Powell River (Fig. 1). These nine sites were sampled from 2015 to 2017 and again in either 2020 or 2021 (Table 2). This period was chosen because, by 2015, most of the restoration had been conducted for the Certus and LMPI NRDAR cases. However, monitoring at the Oakley Property in the Powell River, Tennessee, only began in 2016 and therefore it was not sampled in 2015.

Local mussel populations at two of the sites in the Clinch River, the Sycamore Lane site near Richlands, Virginia (RM 320), and the Payne Property site near Cedar Bluff, Virginia (RM 322.1), in Tazewell County, Virginia, were impacted directly in 1998 by the Certus Inc. chemical spill. These sites were selected for restoration because they represented some of the best and largest available mussel habitat patches in the impact zone. Four additional sites located further downstream in Russell County—Bennett Property (RM 277.5), Artrip (RM 274.5), Whited Property (RM 272.7), and Cleveland Island (RM 270)—were not directly impacted by the spill. However, they were chosen as additional restoration sites for the Certus Inc. NRDAR project to reduce risk for potential future impacts to the two sites located in the impact zone between Cedar Bluff and Richlands, Virginia, as well as to replace/acquire natural resources and services equivalent to those lost during the spill. Additionally, these four sites had suitable habitat for mussels, a viable mussel assemblage, and suitable host fishes. All three Powell River monitoring sites were within the area affected by the LMPI coal slurry spill and are in Claiborne County, Tennessee—upper Brooks Bridge (RM 95.3), lower Brooks Bridge (RM 94.7), and Oakley Property (RM 89.7).

In the Clinch River, the Bennett Property had the highest number of released mussels >6 months old (28,538), most of which were *E. capsaeformis* and *E. brevidens* (see Table 1.18 in Hyde and Jones 2021). The Sycamore Lane (21,417) and Payne Property (15,314) sites in Tazewell County had the next highest numbers of released mussels, followed by Artrip (11,066), Cleveland Islands (7,344), and Whited (1,297).

In the Powell River, most of the mussels released were at the Lower (4,583) and Upper Brooks Bridge (4,211) sites, although an additional 1,301 mussels were released at the Oakley Property. Many of the released mussels at the Powell River sites were either *E. capsaeformis* or *E. brevidens*. The number and timing of mussel releases varied widely based upon the species released at restoration sites, how difficult those species were to propagate (e.g., how available gravid females were from year to year), and year-to-year differences in culture success.

Quadrat Sampling

We used a systematic quadrat sampling design at all nine restoration sites (Strayer and Smith 2003). We used multiple random starts for the location of the first quadrat of each systematic sample and all subsequent. We used a quadrat size of 0.25 m^2 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 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 et al. 2012). Survival was assumed to be high because mussels are generally long-lived species (Hart et al. 2001; Villella et al. 2004; Hua 2015). Recruitment from released mussels in the wild was assumed to be zero because released mussels were sub-adults. Assuming no recruitment and high survival also ensured that sufficient quadrats were sampled the first year because the density estimate was lower than if we had assumed recruitment, i.e., lower densities require more quadrats. We used a power analysis (Strayer et al. 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., a 15% target level of precision was used in this study. We calculated

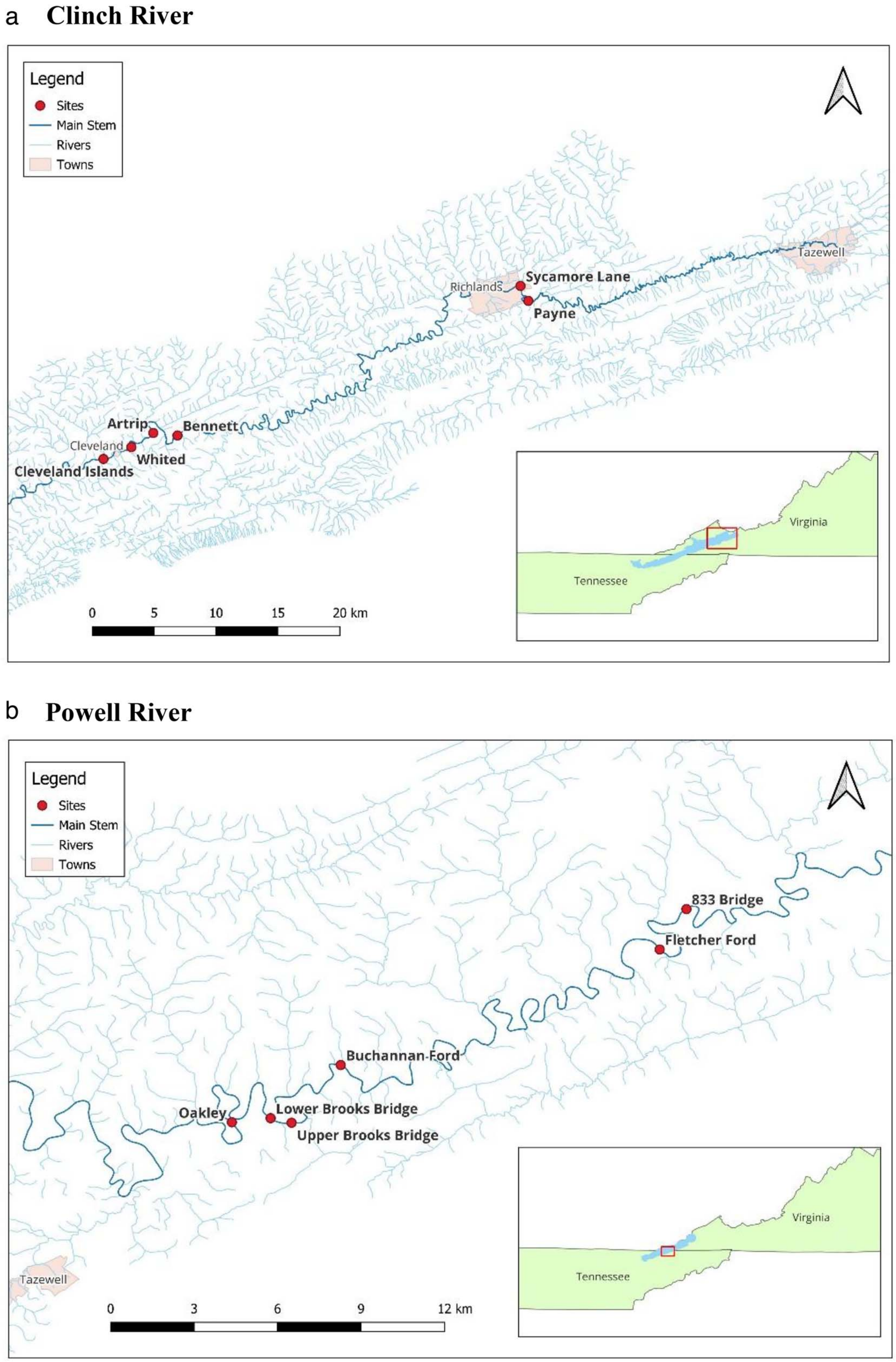

Figure 1. Locations of mussel population restoration and monitoring sites (red dots) in the upper Clinch River, Russell and Tazewell counties, Virginia, for the Certus Inc. NRDAR case and the Powell River, Claiborne County, Tennessee, and Lee County, Virginia, for the Lone Mountain Processing Inc. NRDAR case. Only monitoring data from lower three sites in the Powell were assessed and included in his study. Blue area in inset is the Clinch (a) and Powell (b) river watersheds. Mussel releases occurred from 2003–2019.

Table 2. Nine sites quantitatively sampled for the Certus Inc. and Lone Mountain Processing Inc. NRDAR mussel-restoration cases in the Clinch and Powell rivers, Tennessee and Virginia. Site length, mean width, and area are all rounded to nearest whole number (area is calculated using unrounded length and width). Sample sizes for quadrat surveys are number of quadrats sampled and sample sizes for mark-recapture surveys are number of individuals sampled. Dash (-) indicates the site was not sampled that year. We used river mile because it corresponds to the unit used in USGS topographic maps.

Site	River	River Mile	Length (m)	Mean Width (m)	Area (m^2)	Latitude/Longitude	Survey Method	Sample Size (N) 2015	2016	2017	2020	2021
Payne Property, VA	Clinch	322.1	151	23	3,531	37.081642°, −81.778816°	Quadrat	306	337	260	200	—
							Mark-recapture	137	147	141		
Sycamore Lane, VA	Clinch	320	245	20	4,756	37.095162°, −81.785898°	Quadrat	299	347	255	229	—
							Mark-recapture	194	418	644		
Bennett Property, VA	Clinch	277.5	235	36	8,407	36.959511°, −82.097550°	Quadrat	196	201	210	258	—
Artrip, VA	Clinch	274.5	210	21	4,380	36.961647°, −82.119429°	Quadrat	364	313	321	—	344
Whited Property, VA	Clinch	272.7	146	38	5,577	36.948771°, −82.139325°	Quadrat	341	316	307	—	243
Cleveland Islands, VA	Clinch	270	240	18	4,440	36.938084°, −82.164613°	Quadrat	202	264	254	—	335
Upper Brooks Bridge, TN	Powell	95.3	150	34	5,038	36.534982°, −83.442999°	Quadrat	317	312	306	—	224
Lower Brooks Bridge, TN	Powell	94.7	184	38	7,063	36.536824°, −83.451406°	Quadrat	236	322	359	—	216
Oakley Property, TN	Powell	89.7	58	20	1,150	36.535212°, −83.467035°	Quadrat	—	142	220	—	165

n starting with the most common species at each site and added fewer 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 the 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 × 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 location of suitable habitat. River width was measured at 10-m 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 Hyde and Jones 2021, Appendix C for Google Earth images of sites). We also used these measurements to calculate 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. For mussels found in each quadrat, we identified them to species, sexed them as male/female (for dimorphic species), and measured them to the nearest tenth millimeter using dial calipers (length only). We recorded any mussels visible on the surface as "surface," whereas those not visible were recorded as "subsurface." For mussels previously tagged at AWCC and FMCC prior to release, we also recorded the tag color and number.

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 in the area surveyed. Density was determined by dividing abundance by the area of the site sampled. We calculated 95% confidence intervals for abundance using the formula

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

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

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

where M is the number of possible systematic samples, m is the number of random starts, $\overline{x}$ is the mean number of mussels per systematic sample, and x_i is the number of mussels in random start (Smith et al. 2001). Variance for density can be calculated by dividing $var(\hat{N})$ by the squared area. We performed the same calculations on the subset of mussels 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 mark-recapture sampling to independently estimate abundance and density at those two sites. We used a robust design, mark-recapture framework (Pollock 1982) to sample these Clinch River sites 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. 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, we used view-scopes or slowly walked through transect areas and visually inspected for mussels. Substrate was not excavated during sampling. Each individual mussel was identified to species, sexed for dimorphic species, and measured for length to the nearest tenth millimeter using dial calipers. 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 two 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 equal 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 for 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, whereas 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 et al. 2004; Meador et al. 2011). Likelihood estimates were based on Akaike's Information Criterion (AIC) (Akaike 1973) modified for small sample sizes ($AICc$) (Sugiura 1978):

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

where $L(\hat{\theta})$ 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

Table 3. Mussel species that were assessed for expected abundance and density from 2015–2017 and again in either 2020 or 2021 at sites outside of the impact zone of the Certus Inc. chemical spill in the Clinch River, Virginia, and Powell River, Tennessee. These six species did not occur at restoration and monitoring sites before being released or occurred at very low densities. X indicates species was released at and assessed for expected abundance at that site.

	Clinch River, VA				Powell River, TN		
Species (6)	Bennett	Artrip	Whited Property	Cleveland Islands	UpperBrooks Bridge	LowerBrooks 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						

$<$2.0. To estimate the abundance of both the total mussel assemblage and the population of Rainbow Mussel (*Cambarunio iris*) at the Payne Property, we used the top model in each case.

Due to low recapture rates, we could not use the robust design model to estimate abundance at Sycamore Lane, although it was used to estimate abundance for both *C. 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). Abundance estimates from the mark-recapture estimators (Lincoln-Petersen and the robust design model) were compared to estimates of mussels from quadrats, i.e., the combined surface and subsurface mussels and the surface-only mussels.

Expected vs. Estimated Mussel Abundance

We used a Leslie matrix model 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, and in either 2020 or 2021. 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. Maximum age and breeding age were chosen to represent a typical mussel species. The recruitment rate was set so that the population growth rate would be stable over the long-term. Survival rates were based on Jones et al. (2012), who based their survival rates on an empirical study of dead shells and a catch-curve analysis of shell-length at age and unpublished survival rates from field studies by the Virginia Department of Wildlife Resources, and survival rates reported for other long-lived mussel species (Musick 1999; Akçakaya et al. 2004). We assumed all mussels died after reaching 40 years of age.

We used the mussel release data compiled in Hyde and Jones (2021) 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- and 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 3.). Hence, the natural mussel assemblage at sites in the Clinch River in Russell County, Virginia, and in the Powell River, Tennessee, 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, 2017, and either 2020 or 2021 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

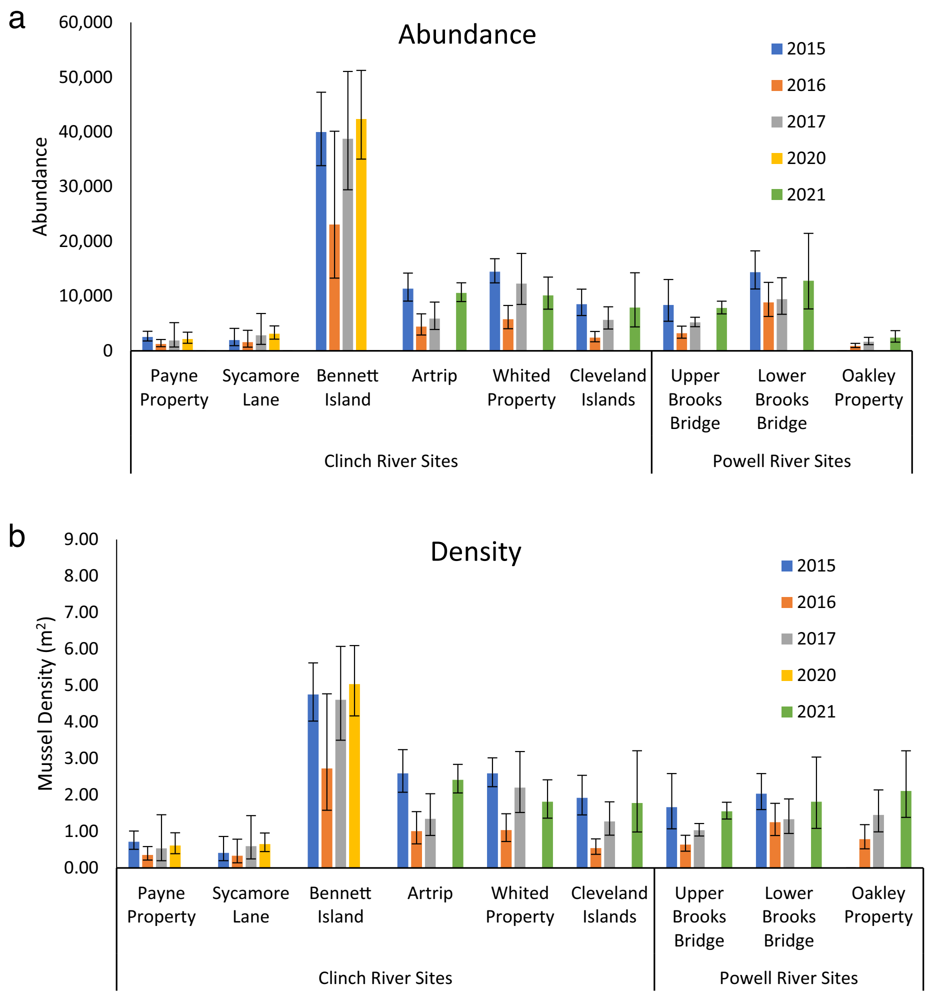

Figure 2. Estimated abundances (a) and densities (b) of freshwater mussels at population restoration and monitoring sites in the Clinch and Powell rivers, Virginia and Tennessee, based on quadrat sampling conducted from 2015–2017, and again in 2020 and 2021. Sites are ordered from upstream to downstream within each river, and error bars represent 95% confidence intervals.

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, as this was likely due to measurement error (since shell length, unlike mass, typically cannot decrease), 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 Wavyrayed lampmussel (*Lampsilis fasciola*), Kidneyshell (*Ptychobranchus fasciolaris*), and Mountain Creekshell (*Leaunio 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 *C. iris* initially tagged during 2015 mark-recapture sampling and tracked the mean lengths of this cohort in 2016 and 2017. Finally, we compared mean lengths of *P. fasciolaris* among all sites using 95% confidence intervals.

RESULTS

Quadrat Monitoring Data

Across all nine monitoring sites, mussel abundances and densities were generally higher in 2017 compared to 2016, but lower than the first year of monitoring in 2015 (Fig. 2). Sampling in 2020 and 2021 found similar estimates as prior years. In the Clinch River, the Bennett Property had the highest abundances and densities of all sites in all four monitoring years. In the Powell River, Lower Brooks Bridge generally had the highest abundances across all sites and years and the highest densities in 2015 (2.03 m^2) and 2016 (1.25/m^2), but the highest density observed during the study was at the Oakley Property in 2021 (2.10/m^2).

Clinch River.—Total mussel assemblage abundance and density at the Payne Property ranged from 1,257 individuals (0.36/m^2) in 2016 to 2,537 individuals (0.72/m^2) in 2015 (Tables 4 and 5; Fig. 2). *Cambarunio iris* was the most abundant species, followed by *Lampsilis fasciola* and *Ptychobranchus fasciolaris*. All three of these species were released at

Table 4. Estimated abundances of freshwater mussels at population restoration and monitoring sites based on quadrat sampling in the Clinch River, Virginia from 2015–2017, and again in either 2020 or 2021. Site at Cleveland Islands was located in the lower-half of the right-descending channel.

	Payne Property				Sycamore Lane				Bennett				Artrip				Whited Property				Cleveland Islands			
Species (27)	2015	2016	2017	2020	2015	2016	2017	2020	2015	2016	2017	2020	2015	2016	2017	2020	2015	2016	2017	2020	2015	2016	2017	2020
Ortmanniana ligamentina	0	0	0	0	0	0	0	0	0	0	0	130	0	0	0	0	0	0	0	0	0	0	0	0
Actinonaias pectorosa	92	0	0	210	0	0	0	0	16,985	14,053	18,094	16,032	3,369	1,847	1,692	3,051	6,934	2,965	8,138	6,364	2,638	875	1,468	1,537
Amblema plicata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67	0	0
Cambarunio iris	1,292	838	1,141	909	827	877	1,418	1,247	858	669	1,281	1,825	1,540	504	655	1,033	589	141	145	616	352	67	70	477
Cyclonaias tuberculata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67	70	0
Cyprogenia stegaria	0	0	0	0	0	0	0	0	0	0	0	130	0	0	0	0	0	0	0	0	0	0	0	0
Epioblasma brevidens	0	0	0	0	0	0	0	0	3,603	1,338	3,203	7,429	915	224	819	2,313	0	0	0	68	0	0	140	318
Epioblasma capsaeformis	0	0	0	0	0	0	0	0	3,774	1,506	3,683	4,041	578	112	491	295	327	0	0	0	1,495	202	559	265
Epioblasma triquetra	0	0	0	0	0	0	0	0	0	0	0	652	0	0	0	0	0	0	0	0	0	0	0	0
Eurynia dilatata	0	0	0	70	0	0	0	0	686	0	0	261	1,059	392	710	1,033	1,897	1,059	1,599	1,300	1,670	269	1,259	2,280
Fusconaia cor	0	0	0	0	0	0	0	0	686	0	160	391	144	0	55	0	65	71	145	68	176	135	350	371
Fusconaia cuneolus	0	0	0	0	0	0	0	0	0	0	0	130	0	0	0	0	0	0	0	0	0	0	0	106
Fusconaia subrotunda	0	0	0	0	0	0	0	0	0	0	0	0	48	0	0	0	65	0	0	0	0	67	0	106
Lampsilis fasciola	692	293	380	559	255	219	224	125	858	167	480	521	385	168	164	295	654	71	218	68	88	135	70	53
Lampsilis ovata	0	0	0	0	0	55	0	125	172	0	0	130	0	0	0	0	65	0	0	0	0	0	0	53
Lasmigona costata	0	0	0	0	64	55	0	0	172	0	160	261	144	0	0	49	65	0	0	0	0	0	0	0
Leaunio vanuxemensis	46	0	109	140	64	55	0	187	343	0	160	0	0	56	164	148	0	0	0	0	0	0	0	53
Lemiox rimosus	0	0	0	0	0	0	0	0	0	0	160	130	0	0	0	49	0	0	73	0	0	0	0	0
Ligumia recta	0	0	0	0	0	0	0	0	0	0	160	130	0	0	0	49	0	0	0	0	0	0	0	0
Medionidus conradicus	0	0	0	0	255	55	75	125	7,892	2,677	6,565	6,647	1,444	448	273	787	1,439	494	581	547	967	135	210	212
Pleuronaia barnesiana	0	0	54	0	0	0	0	0	0	0	0	0	0	0	0	0	65	0	0	0	0	0	0	0
Pleuronaia dolabelloides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	55	0	0	0	0	0	0	0	70	53
Pleuronaia spp.	0	0	0	70	0	0	0	0	858	335	1,601	652	578	504	327	886	1,308	494	799	411	879	202	979	1,272
Ptychobranchus fasciolaris	369	126	217	210	255	164	746	748	2,059	1,506	2,882	1,825	1,059	112	491	443	981	494	581	684	264	202	420	742
Ptychobranchus subtentus	46	0	0	0	255	110	373	561	1,029	502	160	782	0	56	0	0	0	0	0	0	0	0	0	0
Theliderma cylindrica	0	0	0	0	0	0	0	0	0	167	0	0	96	0	0	0	0	0	0	0	0	0	0	0
Venustaconcha trabalis	0	0	0	0	0	0	0	0	0	0	0	261	0	0	0	98	0	0	0	0	0	0	0	0
Grand Total	**2,537**	**1,257**	**1,901**	**2,168**	**1,975**	**1,590**	**2,836**	**3,118**	**39,975**	**22,920**	**38,749**	**42,360**	**11,359**	**4,423**	**5,896**	**10,529**	**14,454**	**5,789**	**12,279**	**10,126**	**8,529**	**2,423**	**5,665**	**7,898**

Table 5. Estimated densities of freshwater mussels at population restoration and monitoring sites based on quadrat sampling in the Clinch River, Virginia from 2015 to 2017. Density is reported as individuals per m^2. Site at Cleveland Islands was located in the lower-half of the right-descending channel.

	Payne Property				Sycamore Lane				Bennett				Artrip				Whited Property				Cleveland Islands			
Species (27)	2015	2016	2017	2020	2015	2016	2017	2020	2015	2016	2017	2020	2015	2016	2017	2021	2015	2016	2017	2021	2015	2016	2017	2021
Ortmanniana ligamentina	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Actinonaias pectorosa	0.03	0.00	0.00	0.06	0.00	0.00	0.00	0.00	2.02	1.67	2.15	1.91	0.77	0.42	0.39	0.70	1.24	0.53	1.46	1.14	0.59	0.20	0.33	0.35
Amblema plicata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
Cambarunio iris	0.37	0.24	0.32	0.26	0.17	0.18	0.30	0.26	0.10	0.08	0.15	0.22	0.35	0.12	0.15	0.24	0.11	0.03	0.03	0.11	0.08	0.02	0.02	0.11
Cyclonaias tuberculata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00
Cyprogenia stegaria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Epioblasma brevidens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.43	0.16	0.38	0.88	0.21	0.05	0.19	0.53	0.00	0.00	0.00	0.01	0.00	0.00	0.03	0.07
Epioblasma capsaeformis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.18	0.44	0.48	0.13	0.03	0.11	0.07	0.06	0.00	0.00	0.00	0.34	0.05	0.13	0.06
Epioblasma triquetra	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eurynia dilatata	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.03	0.24	0.09	0.16	0.24	0.34	0.19	0.29	0.23	0.38	0.06	0.28	0.51
Fusconaia cor	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.02	0.05	0.03	0.00	0.01	0.00	0.01	0.01	0.03	0.01	0.04	0.03	0.08	0.08
Fusconaia cuneolus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Fusconaia subrotunda	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.02
Lampsilis fasciola	0.20	0.08	0.11	0.16	0.05	0.05	0.05	0.03	0.10	0.02	0.06	0.06	0.09	0.04	0.04	0.07	0.12	0.01	0.04	0.01	0.02	0.03	0.02	0.01
Lampsilis ovata	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Lasmigona costata	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.02	0.03	0.03	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leaunio vanuxemensis	0.01	0.00	0.03	0.04	0.01	0.01	0.00	0.04	0.04	0.00	0.02	0.00	0.00	0.01	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Lemiox rimosus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Ligumia recta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Medionidus conradicus	0.00	0.00	0.00	0.00	0.05	0.01	0.02	0.03	0.94	0.32	0.78	0.79	0.33	0.10	0.06	0.18	0.26	0.09	0.10	0.10	0.22	0.03	0.05	0.05
Pleuronaia barnesiana	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pleuronaia dolabelloides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01
Pleuronaia spp.	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.10	0.04	0.19	0.08	0.13	0.12	0.07	0.20	0.23	0.09	0.14	0.07	0.20	0.05	0.22	0.29
Ptychobranchus fasciolaris	0.10	0.04	0.06	0.06	0.05	0.03	0.16	0.16	0.24	0.18	0.34	0.22	0.24	0.03	0.11	0.10	0.18	0.09	0.10	0.12	0.06	0.05	0.09	0.17
Ptychobranchus subtentus	0.01	0.00	0.00	0.00	0.05	0.02	0.08	0.12	0.12	0.06	0.02	0.09	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Theliderma cylindrica	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Venustaconcha trabalis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Grand Total	**0.72**	**0.36**	**0.54**	**0.62**	**0.39**	**0.32**	**0.61**	**0.67**	**4.74**	**2.73**	**4.61**	**5.07**	**2.58**	**1.02**	**1.34**	**2.41**	**2.59**	**1.04**	**2.20**	**1.80**	**1.93**	**0.58**	**1.29**	**1.77**

the site in relatively high numbers for restoration. Pocketbook (*Lampsilis ovata*), which also was released in high numbers at the site, was not found during any of the four monitoring years. However, this species has been found at the Payne Property more recently (Tim Lane, Virginia Department of Wildlife Resources, personal observation).

Total mussel assemblage abundance and density at Sycamore Lane ranged from 1,590 individuals ($0.32/m^2$) in 2016 to 2,836 individuals ($0.67/m^2$) in 2017 (Tables 4 and 5; Fig. 2). *Cambarunio iris* was the most common species, followed by *P. fasciolaris* and *Ptychobranchus subtentus*. *Cambarunio iris* was released in the highest numbers during restoration at this site, followed by *L. fasciola* and Cumberland Moccasinshell (*Medionidus conradicus*).

Total mussel assemblage abundance and density at the Bennett Property ranged from 22,920 individuals ($2.73/m^2$) in 2016 to 42,360 individuals ($5.07/m^2$) in 2020 (Tables 4 and 5; Fig. 2). The most common species was Pheasantshell (*Actinonaias pectorosa*), which was not released during restoration and was already present at the site. *Epioblasma capsaeformis* and *Epioblasma brevidens*, which were not present at the site before being released there, were the third and fourth most abundant species, respectively. In addition, 1,547 individuals of the federally endangered Snuffbox (*Epioblasma triquetra*) were released from 2017–2019 (most in 2018). This species was found during monitoring in 2020 with an estimated abundance of 652 individuals ($0.8/m^2$).

Total mussel assemblage abundance and density at Artrip ranged from 4,423 individuals ($1.02/m^2$) in 2016 to 11,359 individuals ($2.58/m^2$) in 2015 (Tables 4 and 5; Fig. 2). The most common species in all years at the site was *A. pectorosa*. *Epioblasma brevidens* had the highest number of individuals released at the site, followed by releases of *E. capsaeformis*, which was detected during all monitoring years.

Total mussel assemblage abundance and density at the Whited Property ranged from 5,789 individuals ($1.04/m^2$) in 2016 to 14,454 individuals ($2.59/m^2$) in 2015 (Tables 4 and 5; Fig. 2). *A. pectorosa* was the most common species at the site in all years. However, mussel releases at the Whited Property were relatively low, and all occurred before 2013. *Epioblasma capsaeformis* was the species with the most individuals released but was only detected during the first monitoring year in 2015.

Total mussel assemblage abundance and density at Cleveland Islands in the right descending channel ranged from 2,423 individuals ($0.58/m^2$) in 2016 to 8,529 individuals ($1.93/m^2$) in 2015 (Tables 4 and 5; Fig. 2). *Actinonaias pectorosa* was the most common species, followed by *Eurynia dilatata* and *Pleuronaia* spp. *Epioblasma capsaeformis* had the highest number of released individuals at the site and was found in all monitoring years. *Epioblasma brevidens* was released in 2013 (N = 789) but was not detected during 2015 and 2016 monitoring. *Epioblasma brevidens* was released again in both 2017 and 2018 (>1,000 in both years) and was found in both 2017 and 2020.

Powell River.—Total mussel assemblage abundance and density at Upper Brooks Bridge ranged from 3,232 individuals ($0.63/m^2$) in 2016 to 8,392 individuals ($1.67/m^2$) in 2015 (Tables 6 and 7; Fig. 2). The most common species were *A. pectorosa*, followed by Mucket (*Ortmanniana ligamentina*), and *M. conradicus*. *E. capsaeformis* and *E. brevidens* had the most released individuals at the site and were last released in 2013, but only *E. capsaeformis* was found in 2021.

Lower Brooks Bridge had the highest abundance and density of the Powell River sites across all years. Total mussel assemblage abundance and density ranged from 8,860 individuals ($1.24/m^2$) in 2016 to 14,367 individuals ($2.04/m^2$) in 2015 (Tables 6 and 7; Fig. 2). *Actinonaias pectorosa* and *O. ligamentina* were the most dominant species. *Epioblasma capsaeformis* and *E. brevidens* were released as late as 2017. *Epioblasma capsaeformis* was not found in 2021, but *E. brevidens* still occurred at a density of 0.11 mussels/m^2.

Because of its small size, the Oakley site had the lowest abundance of the Powell River sites across all years but had the highest density in 2017 and 2021 and the second-highest density in 2016. Total mussel assemblage abundance and density ranged from 906 individuals ($0.80/m^2$) in 2016 to 2,426 individuals ($2.10/m^2$) in 2021 (Tables 6 and 7; Fig. 2). *Epioblasma capsaeformis* had the most released individuals at the site, almost all of which occurred in 2012. In 2016, abundances ranged from 32 individuals of Purple Wartyback (*Cyclonaias tuberculata*), *L. fasciola*, *L. ovata*, and *P. fasciolaris* to 259 individuals of *E. capsaeformis*, with densities ranging from 0.03–$0.23/m^2$ for these species, respectively. In 2017, abundances 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. Overall, *E. capsaeformis* was the most common species at this site.

Mark-Recapture Monitoring Data

During mark-recapture sampling in 2015, we collected and tagged 105 untagged mussels in the Clinch River at the Payne Property. We also collected 26 mussels that were tagged from previous releases. The total number of observations (including mussels collected on both sampling days) was 137 at the Payne Property (Table 8). In 2016, we collected and tagged 92 untagged mussels at the Payne Property. Including mussels that were already tagged (42) and mussels observed on both days, we had a total of 147 observations. Of these observations, only 11 were recaptures from 2015. In 2017, we collected and tagged 99 untagged mussels at the Payne Property. We also sampled 31 previously tagged mussels and had a total of 141 observations, 18 of which were recaptures from 2015 and 2016. An individual *E. 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, we sampled and tagged 101 untagged mussels in the Clinch River at the Sycamore Lane site. We also collected 84 mussels that were

Table 6. Estimated abundances of freshwater mussels at population restoration and monitoring sites based on quadrat sampling in the Powell River, Tennessee from 2015–2017, and again in 2021.

	Upper Brooks Bridge				Lower Brooks Bridge				Oakley			
Species (20)	2015	2016	2017	2021	2015	2016	2017	2021	2015	2016	2017	2021
Ortmanniana ligamentina	1,526	775	593	990	3,352	2,807	2,439	3,139	—	97	167	390
Actinonaias pectorosa	2,479	1,098	2,173	2,609	4,668	3,509	3,226	3,270	—	130	314	725
Amblema plicata	127	0	132	180	479	351	472	392	—	0	42	56
Cambarunio iris	191	0	132	720	0	88	79	654	—	65	42	167
Cyclonaias tuberculata	445	0	132	90	239	0	236	262	—	32	42	28
Dromus dromas	64	0	0	0	120	88	79	131	—	0	0	28
Epioblasma brevidens	572	65	263	0	599	263	708	785	—	65	21	251
Epioblasma capsaeformis	763	388	790	90	1,197	351	236	0	—	259	418	139
Eurynia dilatata	0	65	0	360	120	175	315	0	—	97	188	112
Fusconaia subrotunda	0	0	0	0	0	0	0	0	—	0	0	28
Lampsilis fasciola	381	65	132	630	599	263	157	916	—	32	188	112
Lampsilis ovata	127	65	66	180	120	0	0	131	—	32	42	84
Lasmigona costata	0	0	0	0	120	88	0	0	—	0	0	0
Leaunio vanuxemensis	0	0	0	90	120	88	0	131	—	0	0	28
Ligumia recta	0	65	0	0	0	0	0	0	—	0	0	0
Medionidus conradicus	1,462	517	527	1,260	1,317	526	787	1,700	—	65	63	139
Plethobasus cyphyus	0	0	0	0	239	0	0	262	—	0	0	0
Ptychobranchus fasciolaris	191	129	263	450	958	175	630	1,046	—	32	146	139
Ptychobranchus subtentus	64	0	0	180	120	88	0	0	—	0	0	0
Theliderma intermedia	0	0	0	0	0	0	79	0	—	0	0	0
Grand Total	**8,392**	**3,232**	**5,203**	**7,829**	**14,367**	**8,860**	**9,443**	**12,819**	—	**906**	**1,673**	**2,426**

already tagged from previous releases/studies for a total of 194 observations at Sycamore Lane (Table 8). During mark-recapture sampling in 2016, we sampled and tagged 184 untagged mussels at Sycamore Lane. Including sampled mussels that were already tagged (213), we had a total of 418 observations at Sycamore Lane. Of these mussels, only 13 were recaptures from 2015. During mark-recapture sampling in 2017, we sampled and tagged 253 untagged mussels at the Sycamore Lane site. Including sampled mussels that were already tagged (331), we had a total of 644 observations at Sycamore Lane, 49 of which were recaptures in 2015 and 2016. An individual *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 removed from the site.

We could not estimate abundance at Sycamore Lane using the robust design model (Pollock 1982), possibly due to lower recapture rates compared to the Payne Property, especially in 2016 when only 3% of observations were of previously observed mussels. Estimates using the Lincoln-Petersen estimator ranged from 976–1,872 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 (Fig. 3). For *C. iris* at Sycamore Lane, Lincoln-Petersen estimates ranged from 357–914 and were generally higher than surface quadrat estimates but lower than combined quadrat estimates (Fig. 3). We were unable to estimate apparent survival at Sycamore Lane for either the total assemblage or *C. iris*, despite having three years of data.

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 – likely due to captured mussels burrowing into the substrate after being returned to the stream). Abundance estimates for the total assemblage at the Payne Property ranged from 155–186 individuals and the estimate for apparent survival was 86% (95% CI [5%, 99%]). For *C. 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 *C. iris* at the Payne Property ranged from 113–135 individuals and the estimate for apparent

Table 7. Estimated densities of freshwater mussels at population restoration and monitoring sites based on quadrat sampling in the Powell River, Tennessee from 2015–2017, and again in 2021. Density is reported as individuals per m^2.

	Upper Brooks Bridge				Lower Brooks Bridge				Oakley			
Species (20)	2015	2016	2017	2021	2015	2016	2017	2021	2015	2016	2017	2021
Ortmanniana ligamentina	0.30	0.15	0.12	0.20	0.47	0.40	0.35	0.44	—	0.08	0.15	0.34
Actinonaias pectorosa	0.49	0.22	0.43	0.52	0.66	0.50	0.46	0.46	—	0.11	0.27	0.63
Amblema plicata	0.03	0.00	0.03	0.04	0.07	0.05	0.07	0.06	—	0.00	0.04	0.05
Cambarunio iris	0.04	0.00	0.03	0.14	0.00	0.01	0.01	0.09	—	0.06	0.04	0.15
Cyclonaias tuberculata	0.09	0.00	0.03	0.02	0.03	0.00	0.03	0.04	—	0.03	0.04	0.02
Dromus dromas	0.01	0.00	0.00	0.00	0.02	0.01	0.01	0.02	—	0.00	0.00	0.02
Epioblasma brevidens	0.11	0.01	0.05	0.00	0.08	0.04	0.10	0.11	—	0.06	0.02	0.22
Epioblasma capsaeformis	0.15	0.08	0.16	0.02	0.17	0.05	0.03	0.00	—	0.23	0.36	0.12
Eurynia dilatata	0.00	0.01	0.00	0.07	0.02	0.02	0.04	0.00	—	0.08	0.16	0.10
Fusconaia subrotunda	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	—	0.00	0.00	0.02
Lampsilis fasciola	0.08	0.01	0.03	0.12	0.08	0.04	0.02	0.13	—	0.03	0.16	0.10
Lampsilis ovata	0.03	0.01	0.01	0.04	0.02	0.00	0.00	0.02	—	0.03	0.04	0.07
Lasmigona costata	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00	—	0.00	0.00	0.00
Leaunio vanuxemensis	0.00	0.00	0.00	0.02	0.02	0.01	0.00	0.02	—	0.00	0.00	0.02
Ligumia recta	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	—	0.00	0.00	0.00
Medionidus conradicus	0.29	0.10	0.10	0.25	0.19	0.07	0.11	0.24	—	0.06	0.05	0.12
Plethobasus cyphyus	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.04	—	0.00	0.00	0.00
Ptychobranchus fasciolaris	0.04	0.03	0.05	0.09	0.14	0.02	0.09	0.15	—	0.03	0.13	0.12
Ptychobranchus subtentus	0.01	0.00	0.00	0.04	0.02	0.01	0.00	0.00	—	0.00	0.00	0.00
Theliderma intermedia	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	—	0.00	0.00	0.00
Grand Total	**1.67**	**0.63**	**1.04**	**1.57**	**2.04**	**1.24**	**1.33**	**1.82**	—	**0.80**	**1.46**	**2.10**

survival was 96% (95% CI [0%, 100%]). The lowest estimates of abundance for *C. iris* and the total assemblage at the Payne Property were calculated using the robust design model (Fig. 3). For the total assemblage, estimates of abundance based on surface quadrat data and the Lincoln-Petersen estimator of our mark-recapture data were similar, whereas the Lincoln-Petersen estimate of abundance was slightly higher for *C. iris*. At the Payne Property, estimates of abundance from combined quadrat data (surface and subsurface mussels) were comparatively higher for both the total assemblage and *C. iris*.

Expected vs. Estimated Mussel Abundance

Estimated abundance was lower than expected abundance across all years at all sites, although this effect was especially pronounced at sites in the impact zone of the Clinch River (Payne Property and Sycamore Lane) (Fig. 4). Overall, the percentage of expected mussels not found during quadrat monitoring across sites and years ranged from 42.6%–97.6%, with a mean of 75.4% (Table 9). Mean discrepancies were similar for the Clinch and Powell River sites (75.7% and 74.8%, respectively). The Payne Property had the highest mean discrepancy across all years (85.3%), followed by Cleveland Islands RDC (83.8%) and Sycamore Lane (81.8%). The Bennett Property had the lowest discrepancy at 57%.

Mussel Length and Growth Rates

Shell growth rates of mussels sampled during mark-recapture surveys were calculated only for *C. iris* at the Payne Property and Sycamore Lane sites due to low recapture rates of other species. The mean length of *C. iris* increased by 1.34 mm, or 3.7%, from 2015–2017, with a mean growth of 0.67 mm (1.85%) per year. No tagged mussels from 2015 were recaptured in 2020.

The mean lengths of *L. fasciola*, *P. fasciolaris*, and *L. vanuxemensis* from the 2013 release cohort all increased substantially from 2013–2015, with a much slower increase from 2015–2017 (Fig. 5). Only two individuals of *P. fasciolaris* and one individual of *L. vanuxemensis* from the 2013 cohort were observed in 2020. This 2013 cohort tracks mussels released in 2013 and later sampled during mark-recapture surveys from 2015–2017 (Fig. 5). Growth rates of *C. iris* from the 2015 mark-recapture cohort were similar to the other three species from 2015–2017 (3.8 mm) (Fig. 5). This cohort represents untagged mussels that were first sampled during mark-recapture

Table 8. Numbers of mussels sampled at two population restoration and monitoring sites in the impact zone for the Certus Inc. NRDAR case in the Clinch River, Tazewell County, Virginia, using transect guided mark-recapture sampling from 2015–2017. An asterisk (*) indicates inadvertent release and individual was removed from site. These values indicate observations during each pass, including observations of the same mussel during both passes.

		2015			2016			2017		
Site	Species	Pass 1	Pass 2	Total	Pass 1	Pass 2	Total	Pass 1	Pass 2	Total
Payne Property	*Ortmanniana ligamentina*	0	0	0	1	0	1	0	2	2
	Actinonaias pectorosa	5	2	7	6	7	13	5	2	7
	Cambarunio iris	71	29	100	56	36	92	66	47	113
	*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
	Leaunio vanuxemensis	4	2	6	3	1	4	1	1	2
	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
	Total Assemblage	**97**	**40**	**137**	**85**	**62**	**147**	**84**	**57**	**141**
Sycamore Lane	*Ortmanniana pectorosa*	0	1	1	0	0	0	0	0	0
	Cambarunio iris	39	55	94	117	86	203	141	163	304
	*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
	Leaunio vanuxemensis	1	5	6	10	9	19	14	11	25
	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
	Total Assemblage	**72**	**122**	**194**	**259**	**159**	**418**	**295**	**349**	**644**

surveys in 2015 and later sampled in 2016 and 2017. Based on confidence intervals, mean lengths of *P. fasciolaris* were significantly lower at the Payne Property and Sycamore Lane sites compared to most other monitored sites (Fig. 6).

DISCUSSION

There are several potential causes of lower estimated abundance relative to expected abundance. Estimates of mussel abundance from quadrat surveys were 57% to 85% lower than the expected number of mussels (based on past releases and expected survival and recruitment rates) at all restoration and monitoring sites for both the Certus and LMPI NRDAR cases (Table 9; Fig. 4). First, survival might be lower than we are currently assuming in the Leslie matrix model (e.g., 95% per year). Possibly, the release of propagated individuals into the wild might result in a higher-than-expected mortality. High initial mortality after releases for reintroduction are common for many taxa (Sarrazin and Legendre 2000). At the Payne Property, there were anecdotal reports of Canadian geese possibly feeding on mussels for several days after the mussels were released (Tim Lane, Virginia Department of Wildlife Resources, personal communication). However, estimates of apparent survival from our mark-recapture survey suggest survival is relatively high at the Payne Property (86%–96%),

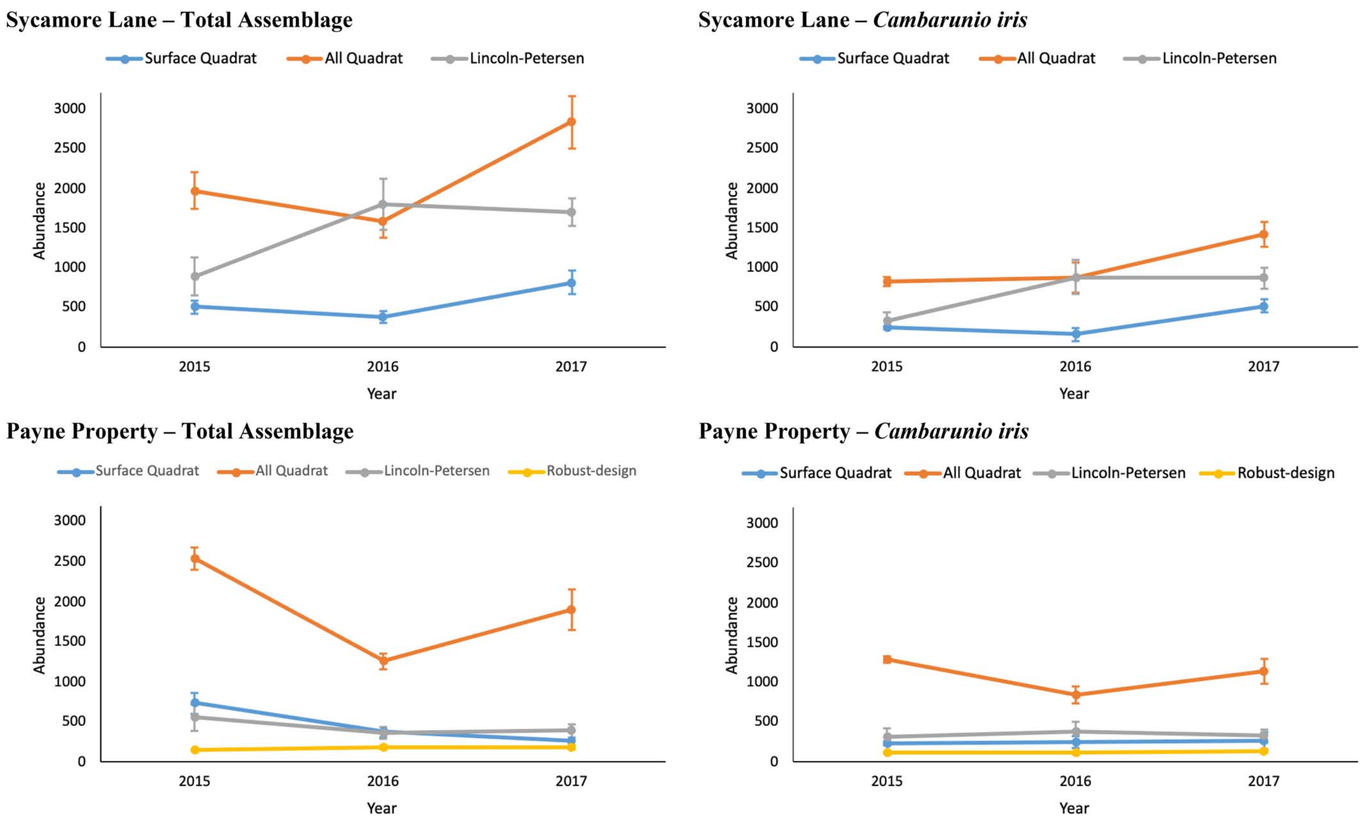

Figure 3. A comparison of abundance estimates for the total mussel assemblage and *Cambarunio iris* at the Sycamore Lane and Payne Property sites, Clinch River, Tazewell County, Virginia, from 2015–2017 based on quadrat and mark-recapture surveys. Surface quadrat abundance was calculated using only mussels 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 from data collected from mark-recapture surveys. Error bars represent standard error.

although these data do not cover the timeframe of initial release. Further, freshwater mussels typically have high annual survival rates. A study of naturally occurring Threeridge (*Amblema plicata*) in the Mississippi and Otter Tail rivers, Minnesota, found that annual survival was greater than 97% in natural habitats (Hart et al. 2001). Meador et al. (2011) found high annual survival of naturally occurring mussels in slack-water and pool habitats (>90%) in the Altamaha River, Georgia, in 2006 and 2007, although mussels in swift-water habitats had somewhat lower survival (75%). Villella et al. (2004) found annual survival was >90% for three species of naturally occurring adult mussels of Eastern Elliptio (*Elliptio complanata*), Northern Lance (*E. fisheriana*), and Yellow Lampmussel (*Lampsilis cariosa*) in the Cacapon River, West Virginia. Carey et al. (2015) found that 65–70% of laboratory-propagated *E. capsaeformis* released in 2010 and 2011 in the Clinch River at Cleveland Islands survived when the population was sampled in 2011 and 2012. A recovery survival rate of 82% also was observed a year after the release of laboratory-propagated *E. brevidens* into cages in the Powell River, Tennessee (Hua et al. 2011), although the cages may have contributed to high survival. However, recovery of PIT tagged *E. brevidens* also found high month-to-month survival (0.98) at this same site over a 2-year period (Hua 2015). Thus, available data suggest lower-than-expected annual survival is not the major contributor to the lower-than-expected abundance found at our sites. Regardless, given the potential for high initial mortality, it would be prudent to bury mussels when released, rather than spreading them on top of the substrate, especially if the presence of predators has been observed.

Another possibility is that mussels released at restoration sites are dispersing downstream from the immediate release and monitoring areas. For example, out of 100 mussels relocated in the Kishwaukee River, Illinois, 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, this study only included a buffer zone of 75 m downstream of their immediate sampling area. Other studies have found limited downstream movement. Balfour and Smock (1995) found that the mean net movement downstream of 84 naturally occurring *E. complanata* (out of 160 initially tagged) 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

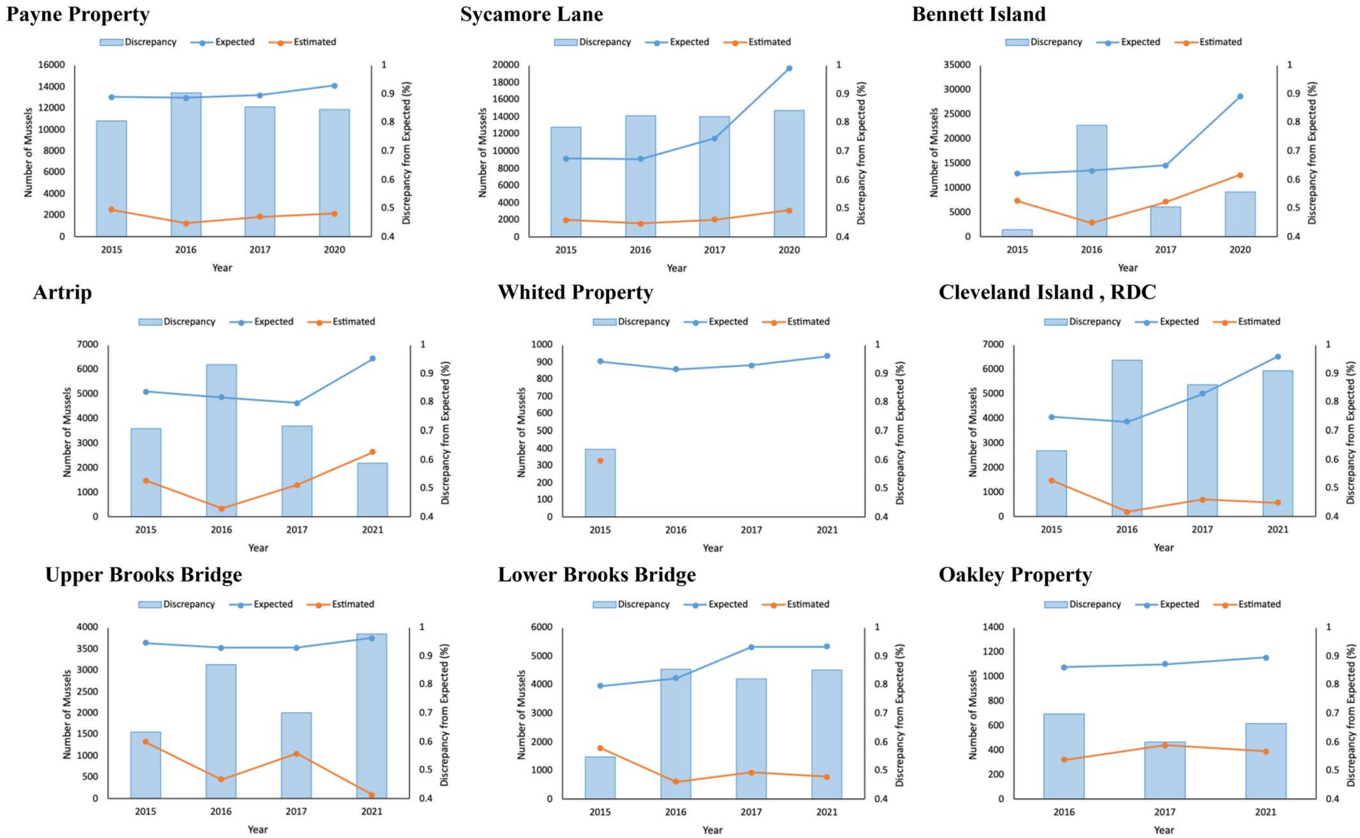

Figure 4. Comparison of expected versus estimated abundance of released mussels that did not previously occur at nine monitoring sites in the Clinch and Powell rivers, Virginia and Tennessee (see Table 3 for a list of mussels included). Expected abundance was determined using release data inputted to 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.

downstream). Another study found the probability of moving downstream among twelve 20-m sections of stream was less than 1% over a period of four years with most movement within 40 m (Villella et al. 2004). Increasing the recapture area compared to the initial sampling area can detect greater movement of mobile organisms such as fish (Albanese et al. 2003). This might also apply to mussels, although the effect would likely be less pronounced. None of the above studies were explicitly examining downstream dispersal. Further, both Balfour and Smock (1995) and Villella et al. (2004) were examining natural populations of mussels. Propagated mussels released into the wild or translocated mussels released at a different site might have higher downstream dispersal than natural populations, possibly due to a failure to burrow sufficiently and thus being more susceptible to high-flow events (Stodola et al. 2017). We also found some evidence of downstream dispersal in our study when qualitatively sampling (visual/snorkel) other potential mussel habitat in the impact zone of the Certus Inc. spill. Two tagged mussels were found at least a kilometer downstream from where they were released at the Sycamore Lane site (one *C. iris* and one *A. pectorosa*), and we observed a dead, tagged *L. fasciola* ~150 m downstream of its release location at the Payne Property. In 2015, we observed a tagged Flutedshell (*Lasmigona costata*) in the downstream section of Sycamore Lane, which was released at the Payne Property in 2009, approximately 2.5 km upstream. Several *E. capsaeformis* and *E. brevidens* released in the Powell River, Tennessee, in 2012 were observed alive 300 meters downstream of Upper Brooks Bridge in June 2022, and females were observed displaying their mantle lures (Tim Lane, Virginia Department of Wildlife Resources, personal observation). Finally, *P. collina* have been observed about 2 km downstream from release sites in Rock Island Creek (Brian Watson, Virginia Department of Wildlife Resources, personal communication). Future monitoring should include some form of sampling farther downstream of the immediate monitoring area to account for dispersal.

It is also possible that a high proportion of newly transformed juvenile mussels are excysting from host fish outside of the monitoring areas, i.e., fish that were infected with glochidia from mussels released at these restoration sites. For example, *C. iris*, *L. ovata*, and *L. fasciola* use mobile Centrarchids such as Rockbass (*Ambloplites rupestris*), Largemouth Bass (*Micropterus salmoides*), and Smallmouth Bass (*Micropterus dolomieu*) as hosts and their transformed glochidia wouldn't be expected to settle in the immediate areas where fish hosts were initially infected. Hence, setting

Table 9. Percentage 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 was likely a function of both emigration and additional mortality. Cleveland Islands was the lower-half of the right-descending channel.

	Clinch River, VA						Powell River, TN		
Year	Payne Property	Sycamore Lane	Bennett Island	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
2020	84.7	83.6	55.8	—	—	—	—	—	—
2021	—	—	—	58.7	—	91.1	97.6	85.3	66.3
Mean	85.3	81.8	57.0	73.6	63.8	83.8	79.5	76.9	65.5

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 possible explanation is that we failed to collect 100% of the individuals present in our sampling units (e.g., sampling lanes or quadrats) at the surveyed sites. For example, Balfour and Smock (1995) found that most mussels <3 years old 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

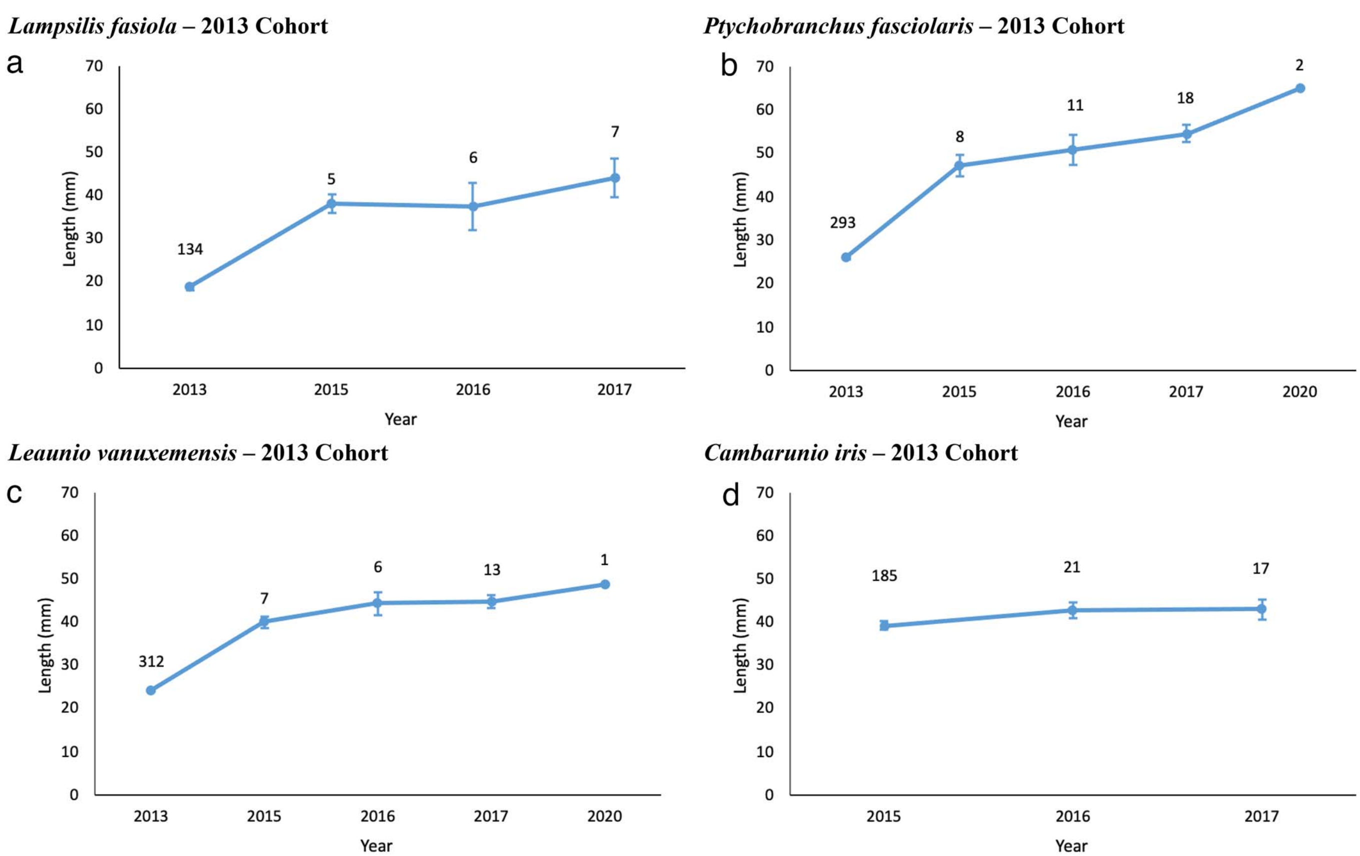

Figure 5. Mean lengths of selected mussel species at the Payne Property and Sycamore Lane sites in the Clinch River, Virginia, from 2013–2020. Subfigures a–c track cohorts of mussels released in 2013 and their mean lengths when recaptured during monitoring. Subfigure d tracks mussels tagged in 2015 during mark-recapture surveys and their mean lengths when captured during subsequent mark-recapture surveys. Numbers above means represent sample size and error bars show standard deviation. Standard deviation was not calculated for *Ptychobranchus fasciolaris* in 2020 because both mussels were the same length.

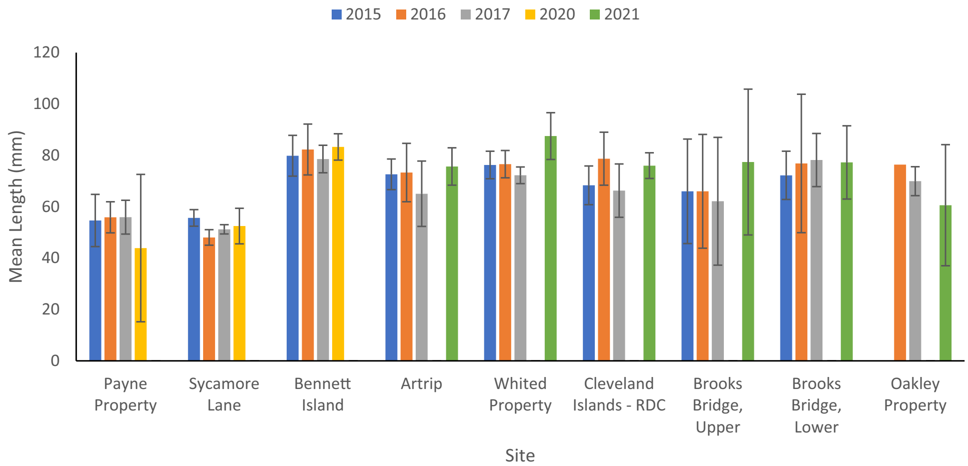

Figure 6. Mean lengths of *Ptychobranchus fasciolaris* at restoration and monitoring sites in the Clinch and Powell rivers, Virginia and Tennessee, from 2015–2017 and in 2020 and 2021. Error bars represent 95% confidence intervals. The error bar for Oakley property in 2016 was not calculated because only one mussel was sampled.

of mussels at the Payne Property and Sycamore Lane sites in 2017 suggests that 35% of the mussels might be <5 years old. This observation might have caused a negative bias in our mark-recapture estimates, given that we were searching only on the surface. However, if detectability was near 100% in the quadrat survey, buried juveniles should have been detected (and were in our study) and thus would not have affected our abundance. Collector experience can also affect detectability (Wisniewski et al. 2014), suggesting that differences in the experience of collectors may have influenced the survey results of both methods. For example, during quadrat surveys there was a consistent decline in estimated abundance across all sites in 2016 compared to 2015 (Fig. 2). 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 the discrepancy between expected and estimated abundance have substantial implications for future planning of mussel restoration via propagation. Based on Leslie matrix analysis and monitoring, up to 85% of the expected number of mussels (based on number released and expected survival) were unaccounted for. Mussels that emigrate downstream from a release site and are alive should still be credited toward restoration, even if they are no longer at the immediate restoration site, because they satisfy NRDAR's criterion of replacement and/or acquisition of equivalent natural resources. 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 for a more realistic estimate of the necessary yearly production to result in the targeted abundance. Future studies should examine dispersal rates of live mussels downstream. Survival also could be studied more thoroughly using PIT tags or estimates from the recovery of dead shells, especially in the period immediately following releases. Thus, future sampling designs should include assessment of areas downstream of the immediate release area to determine site-specific emigration and survival rates.

The large mussel assemblage present in the Clinch River at the Bennett Property is mostly due to one species (*A. pectorosa*) that was already naturally present at the site and was not released there as part of ongoing propagation efforts. However, *E. 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 collected there in 2015. Similarly, *E. brevidens* was detected only in 2017 in the right descending channel of Cleveland Islands in the Clinch River, although *E. capsaeformis* was collected 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 collected relative to the number of mussels released, suggesting survival was higher than expected at these sites.

Compared to quadrat sampling and the Lincoln-Petersen estimator, a robust design model tended to underestimate abundance. This outcome is likely due to the very low recapture rate, both within and among primary periods, making modeling difficult. For example, of the 39 *C. iris* sampled in 2015 on the first sampling day at Sycamore Lane, only six were recaptured the next day. Of the 88 *C. iris* that were sampled on both days in 2015, only 8 were sampled again in 2016, whereas 183 were sampled for the first time that year. Thus, one 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, which also would underestimate abundance.

The Lincoln-Petersen estimator provided a better, lower-bound estimate of abundance compared to the robust design model. It generally yielded higher estimates of abundance relative to abundance estimates made using the surface quadrat data because it accounts for <100% detectability. Both estimates accounted only for mussels found on the substrate surface. However, the mark-recapture surveys were typically conducted during the early fall when detectability at the substrate surface was expected to be higher, whereas the quadrat surveys were conducted in mid-to-late summer, when detectability at the substrate surface was likely lower (Carey et al. 2015).

The mean length of *P. fasciolaris* was significantly lower at the Payne Property and Sycamore Lane sites than at the other sites. Physicochemical factors, such as habitat, temperature and degree of eutrophication, can affect the growth rates and sizes of freshwater mussels via effects on productivity of the habitat and metabolism of mussels (Bauer 1992). However, the majority of *P. fasciolaris* released before monitoring in 2015 and 2016 was at the Payne Property and Sycamore Lane sites (741 and 608, respectively). Only 196 mussels 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*, *L. fasciola*, and *L. vanuxemensis* in the impact zone sites increased 20–28 mm from 2013–2017. Growth of *C. iris* from 2015–2017 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 period. Since three of the four species were released before 2013, it is likely that the much lower growth from 2015–2017 was a result of mussels reaching an age where overall growth rate begins to slow down.

Growth rates of *C. iris* at the Payne Property and Sycamore Lane sites were similar to comparably sized *C. iris* sampled at three sites in the Clinch River from 1988–1993 (Scott 1994). The same study found that growth rates of *L. fasciola* also were similar to comparably sized *L. fasciola* at four sites in the Clinch River. Hence, our results suggest that growth has not been negatively affected at the Payne Property and Sycamore Lane sites in the impact zone of the Certus Inc. chemical spill.

Overall, there is evidence of successful restoration for the Certus NRDAR case. Although current abundances in the impact zone have not reached baseline conditions (i.e., 18,621 mussels), we observed released mussels that had grown to breeding ages, displaying females, and recruitment of one species (*C. iris*) in all three monitoring years (length <20 mm). Because the local mussel assemblage at these sites was completely extirpated, released mussels are clearly breeding successfully. Collectively, our observations indicate moderate restoration success based on the criteria presented in Table 1. Abundance at the two monitoring sites in the immediate impact zone is lower than expected based on assumed survival, but there are populations of numerous species that have low-to-medium densities. Moreover, 37,101 mussels >6 months old, representing 14 species, were released in the impact zone, and another 60,486 mussels representing 20 species have been released at restoration and monitoring sites downstream in the Clinch River in Russell County (see Table 1.18 in Hyde and Jones 2021). Together, this total is far greater than the estimated 18,621 mussels killed during the spill, and therefore restoration at these sites satisfies the NRDAR criteria of recovering or acquiring equivalent natural resources as those injured. Further, the estimated kill was calculated by multiplying dead mussels by three to account for mussels buried in the substrate (U.S. Fish and Wildlife Service 2001). If the spill caused a significant number (e.g., 80%) of mussels to migrate to the surface before dying, then that 3x multiplier overestimated the injury. However, no quantitative sampling was conducted after the spill to validate the use of the 3x multiplier. Future spill assessment studies should include some quantitative sampling, such as excavation of quadrats, to more accurately determine the best multiplier for estimating injury. Although 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. In 2016 and 2017, 731 *E. aureola* were reintroduced in the Clinch River, 300 of which were released in the impact zone at Sycamore Lane. However, no individuals from this release have been observed alive since 2019 (Sarah Colletti, Virginia Department of Wildlife Resources, personal communication). Further, *E. aureola* surrogates–*E. capsaeformis* and *E. brevidens*–have been well established at other augmentation sites in the Clinch River, Virginia, and they are now the second and third most common species at the Bennett Property, despite not occurring there before restoration.

Restoration success for the LMPI NRDAR case was harder to measure as the impacts to mussels were potentially chronic and sub-lethal. Nevertheless, *E. capsaeformis*, one of the primary species released in the Powell River, Tennessee, and one which did not occur at restoration sites prior to release, is currently found at low-to-moderate densities at all three sites in the Powell River. These mussels include breeding-age individuals, and both gravidity and evidence of recruitment have been confirmed. Quantification of success for LMPI could have been improved by setting clear, explicit goals to define success.

To document full success, i.e., long-term presence of a stable population, in these and future NRDAR cases involving freshwater mussels, requires long-term monitoring well past the point of final restoration activities, i.e., >20+ years. This recommendation is due to many mussel species having periods of low recruitment punctuated by years with exceptionally high recruitment (Jones et al. 2012). More moderate strength of evidence could be obtained over the medium-term by documenting an increase in the number of recruits in the years (5-10) immediately following restoration activity. Ideally, this increase should be documented long enough after restoration activities that the increase could be attributed to successful breeding of 1st-generation recruits of released mussels, i.e., recruitment is not solely due to released mussels. Regardless, determination of restoration success in future NRDAR cases require both clear, concrete metrics for what constitutes baseline conditions, as well as medium- to long-term monitoring of restored populations.

It may not always be feasible to release enough mussels to reach baseline conditions or to recover the value of their lost ecosystem services. A case such as Certus may result in hundreds of thousands of lost mussel-years and associated services because of the services that would have been provided over the lifetime of the injured mussels and their offspring (Jones et al. 2012). Nonetheless, recruitment from released mussels must be sufficient to eventually reach restoration goals. To determine the appropriate amount of restoration, assumptions about survival and recruitment rates must be made. This study used a Leslie matrix with reasonable age-specific survival rates for long-lived species and sufficient recruitment to maintain a stable population to estimate the expected number of mussels at restoration and monitoring sites for two NRDAR cases. Monitoring of these sites suggested that either survival and/or recruitment of released mussels were lower than expected or that mussels are settling and/or recruiting downstream of the monitoring area. Further study is needed to determine the reasons for this discrepancy and to inform the amount of restoration needed for future NRDAR cases involving freshwater mussels.

Finally, we have developed a set of metrics that can be used to assess whether mussel restoration was successful (Table 1). These metrics range from easy, such as survival of mussels to breeding age, to difficult, such as determining the establishment of a self-sustaining population. They will be useful for designing monitoring programs for future restoration mussel-restoration activities, including determining what metrics are feasible given case-specific time and monetary restraints. Although the Certus Inc. and LMPI NRDAR cases did not assess all of these monitoring metrics, our study represents the largest evaluation to date of restoration of freshwater mussels in a NRDAR context, and we urge future monitoring programs to assess as many of these metrics as reasonable.

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