The conservation status of the brook floater mussel, *Alasmidonta varicosa,* in the United States: trends in distribution, occurrence, and condition of populations

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Museum Records

We obtained *A. varicosa* collection data from the: Academy of Natural Sciences of Philadelphia (ANSP); Carnegie Museum of Natural History, Pittsburg (CMNH); Canadian Museum of Nature, Ottawa, Ontario (CMN); Museum of Comparative Zoology, Harvard University, Cambridge (MCZ); University of Michigan Museum of Zoology, Ann Arbor (UMMZ); North Carolina Museum of Natural Sciences, Raleigh (NCSM); Smithsonian National Museum of Natural Sciences, Washington D. C. (NMNS); Ohio Sate University Museum, Columbus (OSUM); and Yale University Peabody Museum, New Haven (YPM).

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Executive Summary

The brook floater (*Alasmidonta varicosa*) occurs along the Atlantic slope from the Canadian Maritimes to Georgia. In Canada it is designated as a Schedule 1 Special Concern Species that is confined to 15 watersheds in Nova Scotia and New Brunswick where it is considered "never abundant, representing between 1-5% of the total mussels present" (Department of Fisheries and Oceans Canada 2016). In the United States it is listed as critically imperiled (S1) in 10 states: New Hampshire, Vermont, Massachusetts, New York, Connecticut, New Jersey, West Virginia, Virginia, North Carolina, and Maryland; imperiled (S1S2) in Pennsylvania; imperiled (S2) in Georgia; imperiled (S3) in Maine (in 2007 Maine amended the status of *A. varicosa* to threated from special concern); extirpated (SX) in two states (Rhode Island and Delaware), and unranked (SNR) in South Carolina. However, the South Carolina State Wildlife Action Plan 2015, lists *A. varicosa* as highly imperiled.

Here we report on: (1) the biology and life history of *A. varicosa*, (2) the distribution and condition of all known populations from Maine to Georgia, (3) the human impacts on populations (4) the results of models using environmental factors at both the HUC 12 level and stream level as predictors of population condition, and (5) the results of a survey sent to mussel biologists from Maine to Georgia concerning threats to this species.

Alasmidonta varicosa is a strict riverine species that favors low productivity streams and appears to have a low tolerance to eutrophication. It is a small mussel with a moderate life span, moderate age of reproductive maturity and low fecundity. Because it is a host fish generalist, *A. varicosa* populations are unlikely to be limited by the availability of a particular host fish. Our model results show a strong relationship between the rapid replacement of riparian forests with residential, commercial, agricultural and industrial development and the condition of *A. varicosa* populations. Protecting and restoring riparian forestlands may be our most practical tool for conserving this species. Survey respondents scored the loss of riparian forests, habitat fragmentation, agricultural runoff of nutrients and toxins, urbanization and development as the most spatially extensive and the most severe threats to *A. varicosa* populations. Captive propagation, reintroduction and population augmentation may be needed in order to maintain or rescue *A. varicosa* populations.

We document a dramatic contraction in the distribution range of this species. Surveys show that many populations consist of declining numbers of older animals and show little or no evidence of recruitment. Sharp declines in the size and spatial extent of populations as well as population extirpations have occurred throughout the range, however important populations persist in multiple states including Maine, which appears to hold the largest self-sustaining populations range-wide. Dams, impoundments and waters that are heavily polluted isolate many populations throughout the range. We note that current and projected increases in extreme precipitation and drought will seriously impact remaining *A. varicosa* populations.

Part 1. Biology and Life History of the Brook Floater (*Alasmidonta varicosa*)

Summary

Alasmidonta varicosa (Lamarck, 1819) is a small, thin-shelled strictly riverine species that inhabits sand, gravel and cobble substrates in areas of low to moderate current. Its distribution is restricted to the Atlantic slope region. It is considered a small river species but has been found in streams just a few meters wide to large rivers. It may be confused with *A. marginata* where the two species overlap in the Susquehanna River basin or young *A. undulata*, which like *A. varicosa*, occasionally bear small ridges or corrugations aligned perpendicular to growth lines on the posterior slope of the shell.

As a long-term brooder, *A. varicosa* fertilization occurs in late summer and glochidia are released the following spring. Gravid females discharge glochidia in mucus threads; potential host fish are exposed to glochidia through passive entanglement. Studies in New Hampshire show that the timing of glochidial release is temperature dependent. Glochidia are large and fecundity is low but strongly dependent on mussel size. Laboratory experiments show *A. varicosa* is a host generalist. Glochidia can metamorphose on fish from six families: Cyprinidae, Ictaluridae, Catostomidae, Cottidae, Percidae and Centrarchidae. Passive entanglement is considered a nonselective host fish infection strategy but bottom-feeding fish may be exposed at higher frequencies. Experiments show that glochidial metamorphosis success frequencies were highest on longnose dace, *Rhinichthys cataractae*, margined madtom, *Noturus insignis* and young white sucker, *Catostomus commersonii* – all of which share the same feeding niche. Moreover, successful metamorphosis also occurs on margined madtoms that were infected in the wild. This is the first record of the madtom identified as a primary host fish for a mussel. As small mussels with moderate life spans, moderate age of reproductive maturity and low fecundity, *A. varicosa* fits the profile of a periodic life history strategist.

Biology

Taxonomy and Nomenclature – The genus *Alasmidonta* Say, 1818 comprises 12 species (Graf, D.L. and K.S. Cummings 2013). Nine *Alasmidonta* species are considered extinct, endangered, or threatened and *A. varicosa* is being assessed for federal listing (Williams 1993, p. 10; S. Doran, USFWS, pers. comm.). Additionally, DNA sequencing suggests that individuals first identified as *A. varicosa* from the Uwharrie River basin in North Carolina may are actually be members of a separate and distinct species (Bogan et al. 2008). We look forward to the publication of this data in a peer-reviewed journal. In 1970, Johnson summarized the taxonomy of *A. varicosa*:

Unio varicosa Lamarck 1819, Hist. Nat. des Animaux sans Vertebres, 6, pp. 78-79 (Type locality: la riviere de Schuglkill [Schuylkill] pies de Philadelphie [Philadelphia Co., Pennsylvania]; Holotype, Geneva Museum, Johnson, 1953, Nautilus, 66, p. 95; aussi dans le l ac Champlain, [Vermont]).

Alasmodon corrugata De Kay 1843, 198, pl 24, Zool. New York, Moll. fig. 259 (Passaic River, New York; type, New York Lyceum of Nat. Hist, [destroyed by fire]).

Mya rugulosa Wood 1856, in Hanley, Index Test, p. 199, pi. 1 supp., fig. 7 (North America; type [probably lost]).

Alasmidonta varicosa (Lamarck). Simpson, 1914, Cat. Naiades, 1: 506.

Alasmidonta (Decurambis) varicosa (Lamarck). Ortmann, 1919, Mem. Carnegie Mus., 8: 190, pi. 12, fig. 5. Clarke and Berg, 1959, Cornell Univ. Exp. Sta. Mem. no. 367, p. 28, fig. 34. Athearn and Clarke, 1962, Natl. Mus. Canada, Bull. 183, p. 25, pi. 3, figs. 5, 6. (Johnson 1970, p. 354)

The Integrated Taxonomic Information System lists the species as *Alasmidonta varicosa* (Lamarck, 1819) (TSN 79920) (www.itis.gov, accessed May 2017).

Species Description – *Alasmidonta varicosa* is a small mussel usually less than 75 mm in length (Nedeau 2008, p. 76), rarely exceeding 80 mm. The average length of *A. varicosa* collected in Maine, was 55.3 mm (n = 1,917) (Nedeau and Swartz 2017) and 43.8 mm (n = 2929) in New Hampshire (Wicklow 2008, p. 24). Capture probability increases with mussel size; *A. varicosa* juveniles, less than 20 mm long, are difficult to find during visual searches.

Shell shape ranges from elliptical to trapezoidal (Figure 1). The center of the shell near the dorsal posterior ridge is inflated, giving the mussel a slightly swollen appearance. The ventral margin may be slightly rounded, but is usually straight or indented. The posterior slope is slightly concave dorsally then rises laterally to a rounded posterior ridge. From the beak, the posterior shell is elongate and curves gently to the ventral margin whereas the anterior shell curves abruptly from the beak to the ventral margin. The periostracum is yellowish green to yellowish brown with green rays, which may be obscured by a deep brown-black periostracum in older adults (Figure 2).

Figure 1. A handful of *A. varicosa* shows the variation in shape and the cantaloupe orange foot. The inset shows a 1.5-year old (length 10 mm) *A. varicosa* found during a mussel survey by Ethan Nedeau. Photo © Barry J. Wicklow, Inset Photo © Ethan J. Nedeau

A series of small ridges or corrugations is present on the posterior slope arranged perpendicular to the growth lines. The number of corrugations varies among individuals and the corrugations may be well defined, inconspicuous or absent. The corrugations begin to develop at about age four. The shell is thin, especially posteriorly. The pseudocardinal teeth are small and knob-like; the lateral teeth are absent. The nacre is bluish but more bluish white anteriorly and with pink or orange highlights in and around the beak cavity.

Figure 2. *Alasmidonta varicosa* shell retrieved from muskrat midden, Piscataquog River, NH (shell length 55mm, width 23 mm, height 31 mm). Photos © Barry J. Wicklow

The foot, like much of the internal soft tissue, is usually a bright cantaloupe color but it may be paler in some individuals. This species is known for relaxing its adductor muscles and gaping when removed from the water. (See also Ortmann 1919, p. 190; Johnson 1970, p. 354; Clark 1981, p. 75; Strayer and Jirka 1997, p. 46; Nedeau 2008, p. 76).

Similar Species – The elktoe *A. marginata* co-occurs with *A. varicosa* in the Susquehanna River basin. Like *A. varicosa*, *A. marginata* has corrugations along the posterior slope and an orange foot (Ortmann 1919, p. 182; Strayer and Jirka 1997, p. 44). The two species can be distinguished by *A. marginata'*s more angular posterior ridge and truncated posterior slope, which is usually lighter than the rest of the shell (Figure 3). Additionally, the green rays on the periostracum of *A. marginata* are usually flecked with small dark dots (Ortmann 1919, p. 182). Although Ortmann (1919, p. 192) and Clark and Berg (1959, p. 59) could readily separate these two species, Strayer and Fetterman (1997, p. 337) found intergrades to be common. They suggest that hybridization between these two species is possible and may be contributing to the sharp decline of *A. varicosa* observed in the upper Susquehanna River basin (Strayer and Fetterman 1999, p. 337).

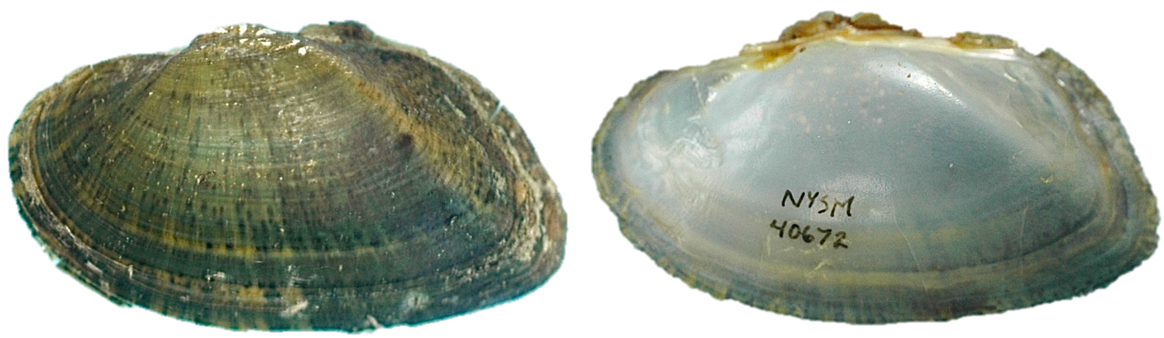

Figure 3. *Alasmidonta marginata*, courtesy of Denise Mayer, New York State Museum. Collected from the Susquehanna River, NY by Fetterman and Strayer, 1997. (Shell length, 48mm, width 20 mm, height 26 mm). Photos © Barry J. Wicklow

Mussel surveyors may misidentify the triangle floater *A. undulata* as *A. varicosa*. For example, in 2006 two mussels from the James River, VA, tentatively identified as *A. varicosa*, were proven through DNA analysis to be *A. undulata* (The Catena Group 2007, p. 11). Fine ridges or corrugations on the posterior slope perpendicular to growth rings – a feature shared with *A. varicosa* – are sometimes present on *A. undulata* (Figure 4). Corrugations in *A. undulata* are present in some very young animals making them more prone to misidentification (Figure 5). Corrugations may persist in older individuals including an individual 55 mm long (B. Wicklow, Saint Anselm College, pers. obs.). However, the ventral shell margin is clearly rounded and the posterior is bluntly pointed in *A. undulata*. Internally, the right pseudocardinal tooth in *A. varicosa* is small and knob-like, whereas the right pseudocardinal tooth in *A. undulata* is large and buttressed by a thick ridge extending from the nacre. In *A. undulata* the anterior third of the shell is distinctly thicker and whiter than the bluish-pink posterior portion of the shell (Figure 4).

Figure 4. *Alasmidonta undulata* shell retrieved from muskrat midden, Connecticut River, NH (shell length 36mm, width 18 mm, height 21 mm). Fine ridges or corrugations perpendicular to growth rings may sometimes also occur on the posterior slope of *A. undulata* as shown above. Photos © Barry J. Wicklow

Figure 5. Comparative shell morphology of young (2.5 to 4.5 years) *A. undulata* (left column) and *A. varicosa* (right column) showing the rounded ventral margin of *A. undulata* and the straight to slightly rounded ventral margin of *A. varicosa* (bar = 10 mm). Although not yet developed on the young shells shown above, small ridges or corrugations running perpendicular to growth lines along the dorso-posterior slope of *A. varicosa* are considered a diagnostic feature of this species. However, small ridges perpendicular to growth rings may sometimes also occur on the dorso-posterior slope of young *A. undulata* as shown above. Photos © Barry J. Wicklow

Habitat – Although present in larger rivers, Ortmann (1919, p. 193) and Clarke (1981, p. 78) considered *A. varicosa* more abundant in smaller rivers and streams; self-sustaining populations have been found in streams just a few meters wide to large rivers (Nedeau and Swartz 2017; W. Russ, NC Wildlife Resources Commission 2017, pers. comm.; B. Wicklow, Saint Anselm College, pers. obs.) This species is found in low to moderate current velocities in runs, pools and glides (Nedeau and Swartz 2017) often in areas of sand, gravel and small to large cobble that provides armoring and bed stability. In the Suncook River in New Hampshire, recruiting populations of *A. varicosa* were found in very fine to very course gravel among small cobble in areas of moderate current velocities and in flow refuges downstream of instream wood or boulders in areas of higher current velocities; few *A. varicosa* were found in unstable sections of sand (Wicklow 2008, p. 32). Biocriteria thresholds for macroinvertebrates collected at the *A. varicosa* population reach show that the stream is near or above reference condition for medium gradient rivers (Wicklow 2008, p. 21).

Life History

Brooding and Glochidial Release – *Alasmidonta varicosa* is a long-term brooder. In Pennsylvania, Ortmann (1919, p. 191) found female *A. varicosa* with eggs in August and with glochida in September, which were held overwinter and shed in early May. Studies in New Hampshire show the spring release of glochidia to be temperature dependent (Wicklow 2008, p. 22). Drift net sampling showed that females begin releasing glochidia as water temperature reached about 14^{0} C, stopped releasing as temperatures decreased, then resumed release as temperature rose above the 14^{0} C threshold (Figure 6).

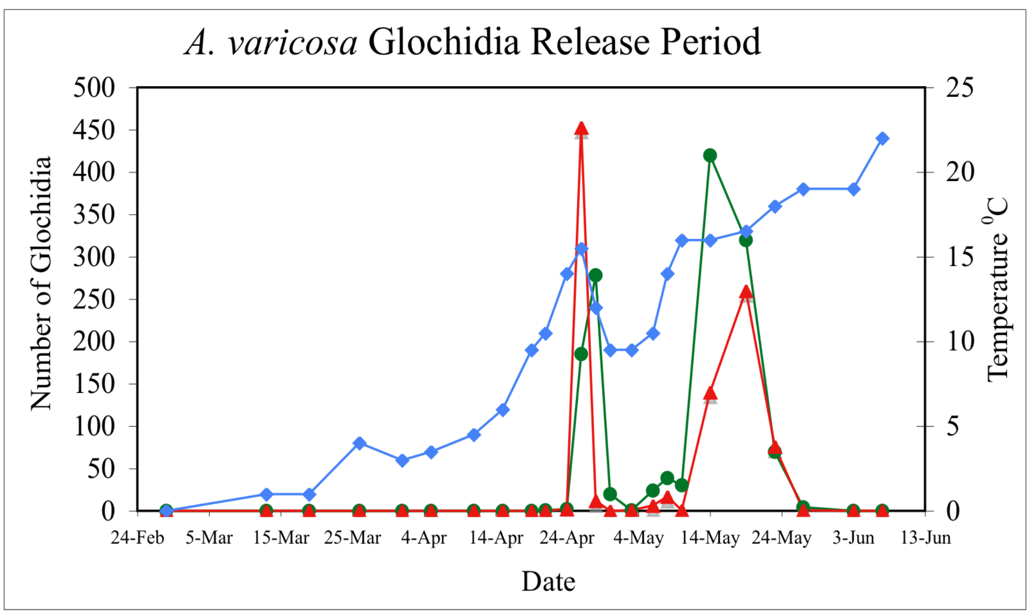

Figure 6. Drift net sampling shows the correlation between temperature and glochidial release for *Alasmidonta varicosa* in 2008 (blue is temperature, red and green are drift net replicates 1 and 2) (Wicklow 2008, p. 22).

In gravid females the mantle around the inhalant and exhalent apertures is prominently barred and tessellated and may play a role in attracting host fish (Figure 6). Species of *Alasmidonta*, including *A. marginata* (see Haag 2012, p. 158), *A. undulata*, and *A. varicosa* (B. Wicklow, Saint Anselm College, unpubl. data) use passive entanglement as a host fish infection strategy (see Haag 2012, p. 157). With a quick jet of water from the exhalent aperture, gravid *A. varicosa* females discharge long mucus threads with glochidia attached. The mucus threads may remain attached to the mussel or stick to other objects and may combine with other threads forming mucus webs (Figure 7). Currents keep the threads suspended in the water column. Fish are exposed to glochidia as they swim through the mucus webs. Passive entanglement is considered a nonselective host fish infection strategy used by host fish generalists, however bottom-feeding fish are more likely to be infected than water column feeding fish (Haag 2012, p. 158). For example, longnose dace, *Rhinichthys cataractae*, are exposed to *A. varicosa* glochidia as they feed near the stream bottom (Figure 7).

Figure 7. A gravid female *A. varicosa* showing barred and tessellated patterns of the mantle around the exhalent and inhalant apertures (left). In a lab experiment longnose dace, *Rhinichthys cataractae*, are exposed to mucus threads with attached glochidia (blue arrows) released from gravid *A. varicosa*. Note glochidia attached to the fins, lip and side of the longnose dace. Photos © Barry J. Wicklow

Fecundity – As in most mussels (see Haag 2012, p. 202), fecundity in *A. varicosa* is related to mussel size (Figure 8). Fecundity in *A. varicosa* is low, glochidia are large and the youngest observed gravid females are 3-4 years old. In the elktoe, *A. marginata*, mean annual fecundity is 47,298, glochidial length is 356 μm and maximum adult size is 102 mm (R. Mair, unpubl. data *in* Haag 2012, p. 423).

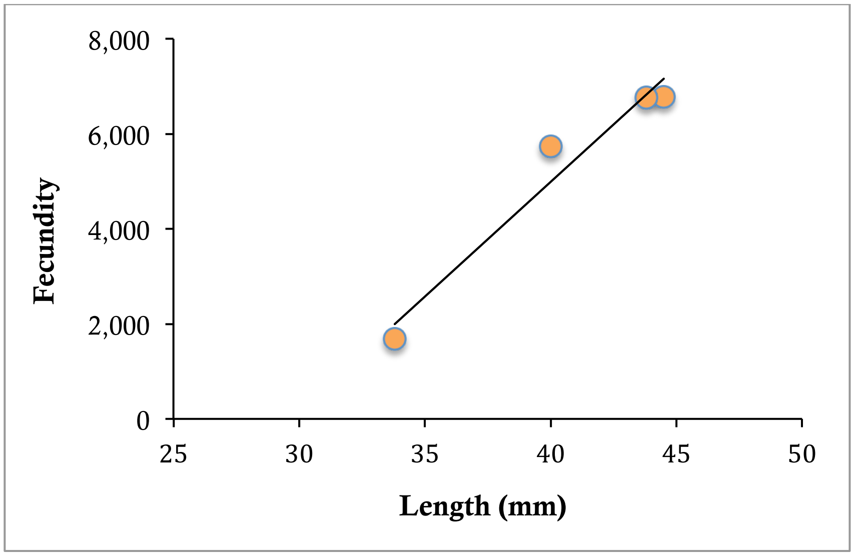

Figure 8. There is a direct relationship between fecundity and mussel length in *Alasmidonta varicosa,* $R^2 = 0.9545$ (B. Wicklow, Saint Anselm College, unpubl. data).

Glochidia morphology – Glochidia of *A. varicosa* are large (mean = 368 μm, SD = 7.7, n = 25), pyriform and like other Anodontini are hooked with the tip of each hook sharp and bare and the remaining portion bearing over 100 small teeth or microstyles (Figure 9). There are three tufts of sensory hairs extending from the mantle of each valve. Gochidia remain viable for at least 12 days at 15^0 C (B. Wicklow, Saint Anselm College, unpubl. data). The glochidia of *A. marginata* (Clarke 1981, p. 60) and *A. undulata* (B. Wicklow, Saint Anselm College, unpubl. data) are similar except those of *A. varicosa* are more asymmetrical (Figure 9). The glochidia of *A. viridis* (Clarke 1981, p. 23) and *A. heterodon* (B. Wicklow, Saint Anselm College, unpubl. data) are shaped differently.

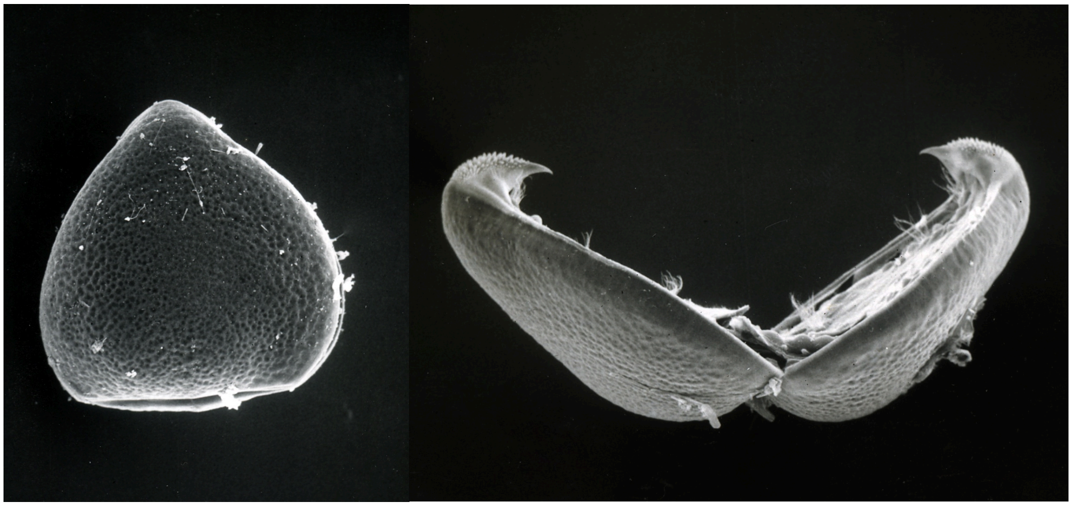

Figure 9. Scanning electron micrographs of *A. varicosa* glochidia. Lateral view of glochidium (left) showing asymmetrical valves and open glochidium showing tufts of sensory hairs and hooks bearing fine teeth. Photos © Barry J. Wicklow

Host Fish – *Alasmidonta varicosa* is a host generalist species. Lab experiments show that glochidia from streams in New Hampshire can encyst and metamorphose on fish species of several families: Cyprinidae, Ictaluridae, Catostomidae, Cottidae, Percidae and Centrarchidae (Wicklow and Richardson 1995; B. Wicklow, Saint Anselm College, unpubl. data). Glochidial metamorphosis success frequencies were highest on the longnose dace, *Rhinichthys cataractae*, margined madtom, *Noturus insignis* and young white sucker, *Catostomus commersonii* (glochidia were sloughed off from older white suckers). Slimy sculpin, *Cottus cognatus* and the common shiner, *Luxilus cornutus* also had relatively high metamorphosis success frequencies (Table 1). Successful metamorphosis has also occurred on margined madtoms that were infected in the wild (B. Wicklow, Saint Anselm College, unpubl. data). Glochidial trials with the fallfish, *Semotilus corporalis* and brown bullhead, *Ameiurus nebulosus*, showed the lowest frequencies of metamorphosis success.

The time required for metamorphosis ranged from 19-51 days at 15^0 C (Table 1). A study of the pearl mussel, *Margaritifera margaritifera,* showed longer periods of glochidial encystment had a positive effect on fitness of metamorphosed juveniles (Marwaha et al. 2017, p. 1380).

Additional potential host fish species include bluegill, *Lepomis macrochirus*, redbreast sunfish, *Lepomis auritus*, fantail darter, *Etheostoma flabellare*, johnny darter, *Etheostoma nigrum*, piedmont darter, *Percina crassa*, Roanoke darter, *Percina roanoka* and white shiner, *Luxilus albeolus* (Eads et al., 2007 *in* Bogan 2017, p. 32-33) and the ninespine stickleback, *Pungitius pungitius* (Beaudet 2006 *in* Department of Fisheries and Oceans Canada 2016).

Fish that did not produce juveniles during lab tests using New Hampshire glochidia include: redbreast sunfish, *Lepomis auritus*, largemouth bass, *Micropterus salmoides*, smallmouth bass *Microterus dolomieu*, adult white sucker, *Catostomus commersonii* and carp, *Cyprinus carpio*.

Table 1. Potential host fishes of *A. varicosa* as identified during laboratory infection trials at 15^0 C.

Fish Species	**Fish exposed**	**Number of glochidia used in infection**	**Shed glochidia**	**Number of juveniles**	**Metamorphosis success frequency**	**Number of days required for metamorphosis**
Cyprinidae						
Common shiner	5	135	108	27	0.2	29-35
Luxilus cornutus						
Golden pond shiner	5	160	133	27	0.17	37-44
Notemigonus crysoleucas						
Blacknose dace	5	68	64	4	0.06	24
Rhinichthys atratulus						
Longnose dace	4	100	49	51	0.51	21-44
Rhinichthys cataractae						
Fallfish	1	35	34	1	0.01	20
Semotilus corporalis						
Ictaluridae						
Margined madtom	4	128	86	42	0.33	21-44
Noturus insignis						
Brown bullhead	5	123	118	5	0.04	–
Ameiurus nebulosus						
Catostomidae						
White sucker	2	139	103	36	0.26	19
Catostomus commersonii (YOY)						
Cottidae						
Slimy sculpin	3	45	35	10	0.22	21
Cottus cognatus						
Percidae						
Tessellated Darter	3	68	64	4	0.06	19
Etheostoma olmstedi						
Yellow perch	4	205	188	17	0.08	21-28
Perca flavescens						
Centrarchidae						
Pumpkinseed	2	38	36	2	0.05	51
Lepomis gibbosus						

Lab studies show that glochidia of the triangle floater, *A. undulata*, may also use the white sucker, *Catostomus commersonii* as one of its host fish (McGrail and Wicklow 1995). The elktoe, *A. marginata* uses several catostomids as host fish suggesting it may be a catostomid specialist (Bloodsworth et al. 2013, p. 54).

As small mussels with moderate life spans, moderate age of reproductive maturity and low fecundity, *A. varicosa* fits the profile of a periodic life history strategist as characterize by Haag (2012, p. 210).

Part 2. Occurrences and Population Assessments of the Brook Floater (*Alasmidonta varicosa*)

Introduction

Alasmidonta varicosa is considered globally vulnerable (G3) and listed as critically imperiled (S1) in 10 states: New Hampshire, Vermont, Massachusetts, New York, Connecticut, New Jersey, West Virginia, Virginia, North Carolina, and Maryland); imperiled (S1S2) in Pennsylvania (M. Walsh 2017, PA Natural Heritage Program pers. comm.); imperiled (S2) in Georgia; imperiled (S3) in Maine; extirpated (SX) in two states (Rhode Island and Delaware), and unranked (SNR) in one state (South Carolina) (NatureServe 2016). South Carolina lists *A. varicosa* as highly imperiled (South Carolina State Wildlife Action Plan 2015). Maine has high regional responsibility for the conservation of *A. varicosa* (Maine State Wildlife Action Plan 2016).

Methods

Distribution Mapping and Population Condition – Data were delivered as spreadsheets or spatial files, with element occurrence (EO) location represented by points, lines, and/or polygons. Though there were many common attributes, they were stored differently between states. In order to create a unified dataset for mapping, and later, modeling, the data needed to be standardized. A standard schema was designed to hold the necessary data, and the data were manually transformed to fit that schema and loaded into a set of master tables, one for each geometry type, within our postgreSQL/postGIS database. The data were combined into one table by converting the line and polygon geometries to points.

As part of the field protocols for most states, each EO was delivered to us assigned with an element occurrence rank that conveys its viability, quality, or condition. Many states used the NatureServe ranking system, which was developed in cooperation with the National Heritage Network (Hammerson et al., 2008) and estimates the likelihood that, if current conditions persist, the EO will survive over a given period of time. For states that used a different ranking structure, we worked with state biologists to determine the appropriate EO rank under the NatureServe system in order to achieve a consistent and comparable set of EO conditions across all states. Additionally, EO data were reviewed for completeness and were updated based on communication with state experts.

The National Hydrography Dataset (NHD) was acquired for all states in the project area. A unified dataset of NHD Flowlines, HUC 8 watersheds, and HUC 12 watersheds was created for the project area. EO data, where a water body name was provided in the attributes, were linked with the closest NHD flowline segment with a matching name. If no water body name was provided, the EO data were linked to the closest NHD flowline segment. The master EO data tables were updated to include the information on the containing HUC 8 and HUC 12 watersheds.

Mapping Occurrences – We generated a series of maps showing the species range across all states in the project. The first map shows all HUC 12 watersheds that have present or historical EOs of any condition (Figure 1). The second map eliminates all watersheds with EOs that are ranked as non-viable, keeping only those EOs ranked as A, AB, AC, B, BC, and C (Figure 2). Finally, a third project-wide distribution map shows only those populations that are classified as excellent or good, defined as EOs ranked A, B, or AB (Figure 3). Populations ranked as C show "few aspects of size condition, landscape context, population size and/or quality and quantity of occupied habitat that are favorable and there may be some uncertainty about the long-term persistence of the EO" (Tomaino et al. 2008). Additionally, state-level maps were produced that show population condition at the HUC 12 level (Figures 4 - 18). Although the maps show the population condition at the HUC 12 level, the actual number of EOs in the watershed may be as low as one.

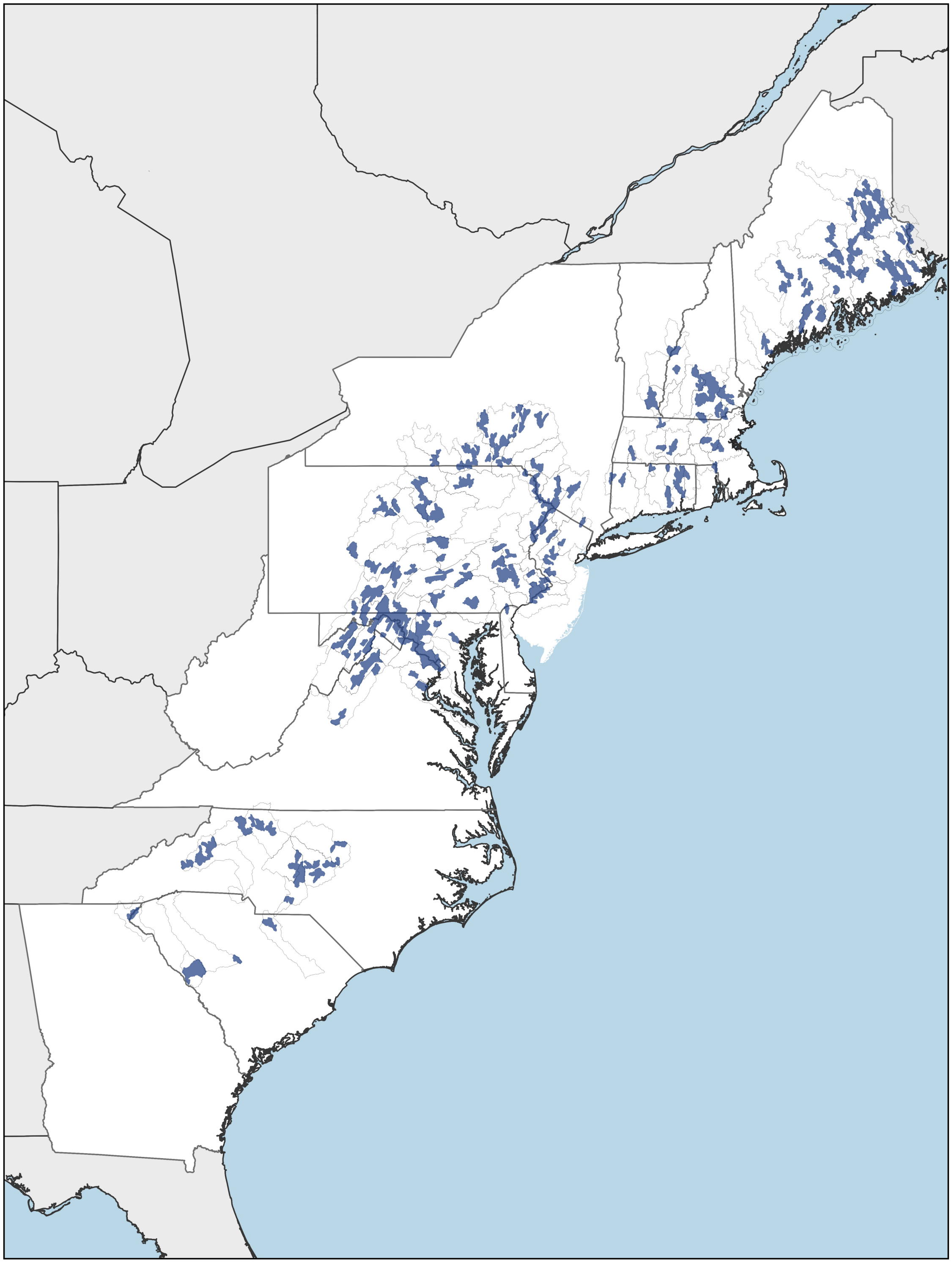

Figure 1. The distribution range of *A. varicosa* showing all HUC 12 watersheds that includes extant and historical EOs of any condition.

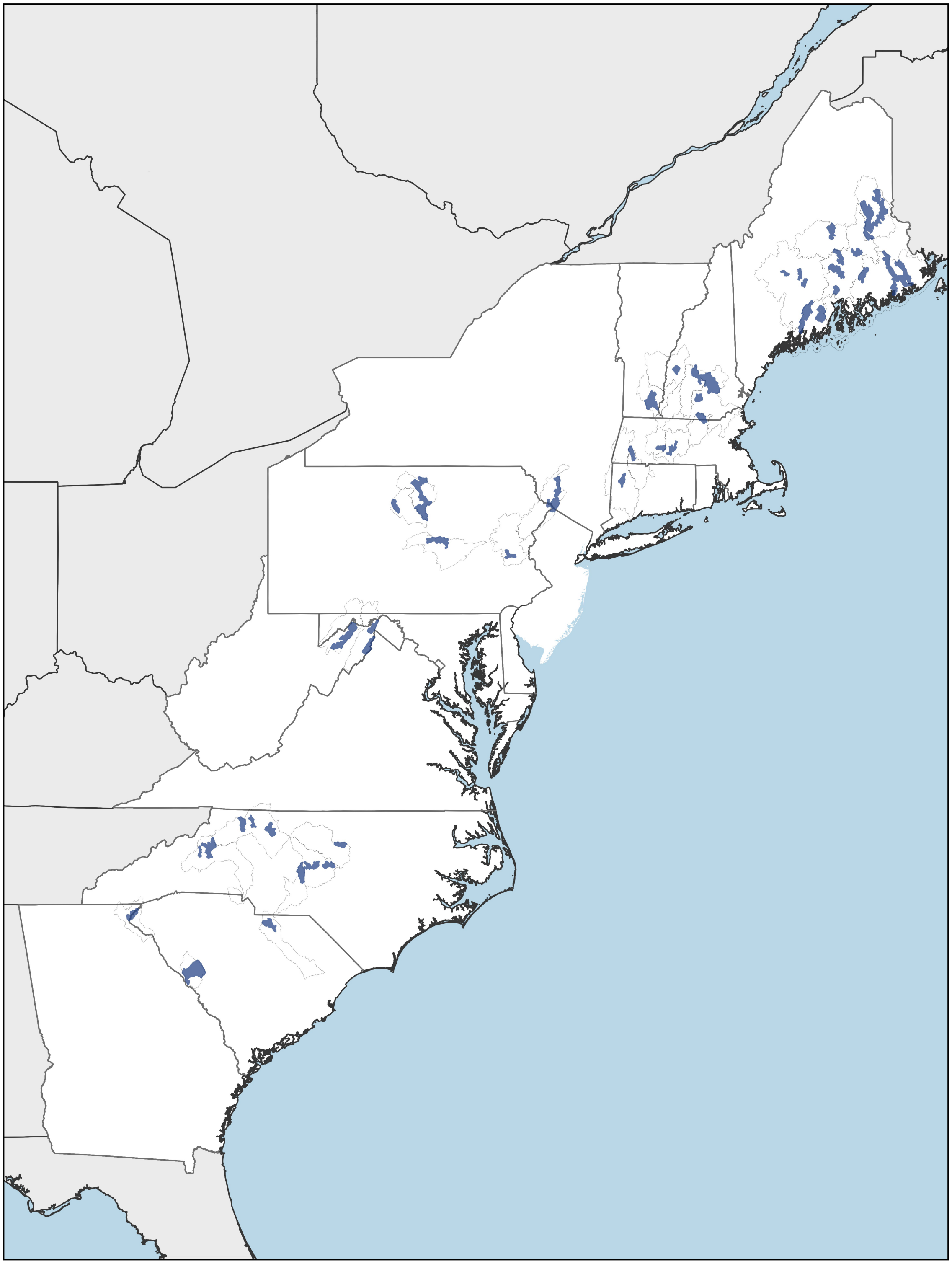

Figure 2. The distribution range of *A. varicosa* showing HUC 12 watersheds excluding watersheds with EOs that are ranked as non-viable, keeping only those EOs ranked as viable: A, AB, AC, B, BC, and C.

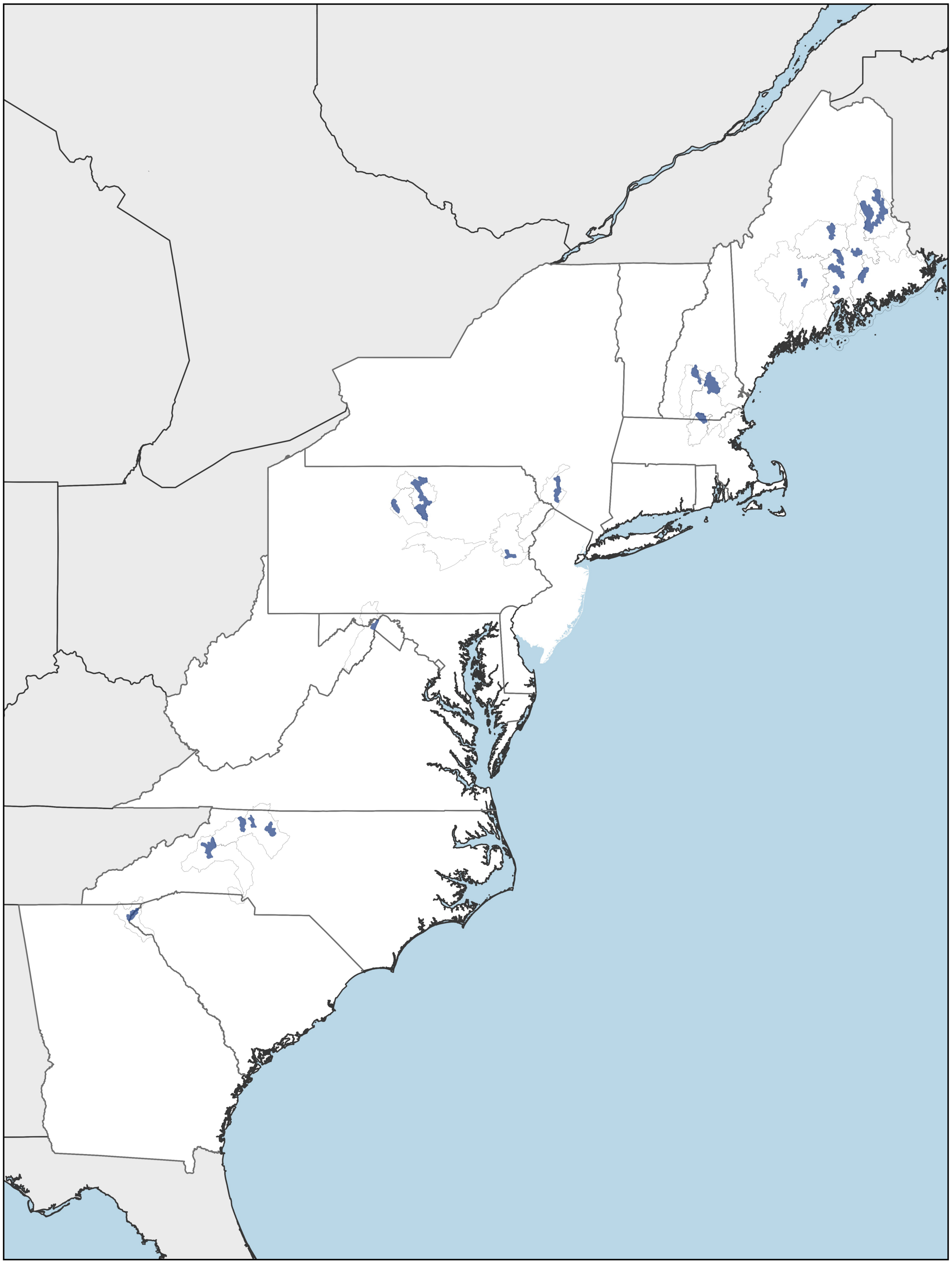

Figure 3. The distribution range of *A. varicosa* showing HUC 12 watersheds including only watersheds with EOs that are ranked as excellent or good: A, AB, or B.

Maine

Summary. Maine rivers support some of the largest self-sustaining *A. varicosa* populations known. This species has been documented in 41 streams of six Maine drainages: Casco Bay, Kennebec River, Central Coastal, Penobscot River, Eastern Coastal and Saint Croix River (Nedeau and Swartz, 2017). However, *A. varicosa* appears to be extirpated from at least one historic river (Presumpscot River), and nearly extirpated in several other rivers where extant populations comprise just a small number of aged individuals, show little or no evidence of recruitment and have fragmented distributions (e.g., Pleasant River in Cumberland Co., Dennys River) and in some rivers populations may already be non-viable. (e.g., Sandy River, Sebasticook River and possibly the West Branch Sheepscot River and Saint George River).

Additionally, it may have been extirpated from other rivers in Maine's central, mid-coastal and southern regions where it appears to be absent from most streams (Swartz and Nedeau 2007, p. 30). In 2007 the status of *A. varicosa* in Maine was changed to Threatened from Special Concern. Still, because of the number of healthy *A. varicosa* populations, Maine has a substantial role to play in conserving this species.

The Casco Bay basin holds the southernmost *A. varicosa* population in Maine. *Alasmidonta varicosa* is gone from the Presumpscot River – one of the most developed rivers in Maine – but a remnant population persists in one of its tributaries, the Pleasant River (Cumberland County). However, 18 of the 21 km (11 of 13.2 mi) Pleasant River are listed as impaired for aquatic life due to low levels of dissolved oxygen resulting from runoff containing phosphorus, nitrogen, sedimentation and sewage. The loss of riparian forests has accelerated the runoff of pollutants. Only six *A. varicosa* were found during extensive surveys in 2010-2011, a decline of nearly 90% from previous surveys, and the population is believed to be near extirpation.

Water quality, habitat suitability, and hydrology may limit *A. varicosa* distribution within the Kennebec River basin. Only one *A. varicosa* has been documented in the Kennebec River mainstem (although deep water and rapid currents have limited survey efforts), which is recovering from a history of dams and a legacy of 19th and 20th century industrial (including pulp wood and textile), agricultural and sewage discharge into the river. Just two and three individuals have been found in the Sandy River and the Sebasticook River respectively, and recent survey efforts failed to reconfirm the species' presence. Parts of both these rivers are listed as impaired for aquatic life. Although present in the Carrabassett River, all *A. varicosa* found were old with highly eroded shells; intense hydrologic forces appear to be responsible for eroded shells and may limit recruitment. However, self-sustaining populations of *A. varicosa* are present in Carrabassett Stream and Wesserunsett Stream, the latter supporting densities among the highest recorded in the state. Threats to water quality in these two streams include agricultural runoff, livestock access to the streams, extensive sedimentation and loss of riparian forests.

Three rivers support *A. varicosa* populations in the Central Coastal drainage: the Sheepscot, West Branch of the Sheepscot and Saint George Rivers. The results of qualitative and quantitative surveys of the Sheepscot River in 2011 show that the river supports a large, recruiting and broadly distributed population of *A. varicosa*. Much of the Sheepscot River is classified as having highest water quality (including a state designated Outstanding River Segment) but nine river segments do not meet Maine EPA standards due to low dissolved oxygen levels, high nutrient and sediment loads and elevated temperatures. A small isolated *A. varicosa* population is present in the West Branch of the Sheepscot River. The *A. varicosa* population in the Saint George River is considered to be in jeopardy due to its narrow distribution and apparent limited recruitment.

The Penobscot River watershed – the largest of Maine's watersheds – drains about one third of the state and holds the largest number of *A. varicosa* supporting streams in Maine. Most of the watershed is heavily forested but water quality in the lower mainstem has been impacted by discharges from tanneries, pulp and paper factories, industries, and municipal runoff and sewage. Small numbers of *A. varicosa* have been found in the Penobscot River and the East Branch of the Penobscot River although water depth and current have limited survey efforts. Small numbers of *A. varicosa* have also been found in Allen Stream, Great Works Stream, Fish Stream, Baskahegan Stream and Wytopitlock Stream but habitat suitability and/or survey effort is limited at these sites. Somewhat larger numbers have been found in Dead Stream, Molunkus Stream, Mattakeunk Stream and Pleasant River (Piscataquis County). Large self-sustaining populations of *A. varicosa* have been located in Marsh Stream, Kenduskeag Stream, Mattawamkeag River, Macwahoc Stream and the Passadumkeag River. The Passadumkeage River is considered the best *A. varicosa* river in the state and also supports one of the most dense *A. varicosa* populations known range-wide.

In the Eastern Coastal Basin, *A. varicosa* has been documented in eight rivers and streams. Just one to a few individuals have been found in the Dennys River, West Branch Machias River, Old Stream, and Chain Lakes Stream. Habitat suitability and extent may be limiting for *A. varicosa* in some of these streams and additional survey effort is needed. However, recent intensive surveys of the lower Dennys River yielded only one live *A. varicosa* and the population in this river may not be viable in the long-term. Somewhat higher numbers were found in the Pleasant River, Machias River, and East Machias River, but densities were low despite the presence of suitable habitat. A larger self-sustaining population was found in the West Branch Union River.

A few *A. varicosa* have been found in the Saint Croix River and one of its tributaries, Tomah Stream, within the Saint Croix River Basin. However, surveys have been limited and additional surveys are needed to determine the size and spatial extent of these populations.

In addition to dams and water and habitat quality changes (see Nedeau et al. 2008, pp. 25-34), climate change is considered a threat to *A. varicosa* in Maine. In a review of climate change and biodiversity in Maine, *A. varicosa* is listed as one of 14 invertebrate species that have a high vulnerability to climate change. The species is considered at risk to changes in hydrology and low summer water levels (Whitman et al. 2013, p. 81).

In a survey of mussel biologists, the Maine respondent reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats. In summary: (1) There are no streams that Maine is currently considering as reintroduction or augmentation sites. (2) Wesserunsett Stream, Carrabassett Stream, Passadumkeag River, Sheepscot River, Marsh Stream, Kenduskeag Stream, Mattawamkeag River, Macwahoc Stream, and West Branch Union River were ranked as conservation priority streams based on their healthy *A. varicosa* populations. (3) The Pleasant River (Cumberland County) was named as a conservation priority because of the immediate threat of extirpation to *A. varicosa*.

1. Casco Bay Basin

1.1 Presumpscot River

The 42 km (26 mi) Presumpscot River is the largest source of freshwater entering Casco Bay. Reverend E. C. Bolles collected *A. varicosa* in the Presumpscot River in the 1800s (Lermond, N. W. 1908). The Presumpscot River is now one of the most developed rivers in Maine. Numerous dams and impoundments and poor water quality due to urban and industrial development now make the potential for finding *A. varicosa* in the Presumpscot River unlikely. No *A. varicosa* had been found during seven surveys in the Presumpscot River mainstem and additional surveys on its tributaries in 1997 and 2001 (Maine Department of Inland Fisheries and Wildlife, unpublished data). It is considered extirpated from the river. However, a remnant population of *A. varicosa* does occur in Pleasant River, a tributary of the Presumpscot, where the population is thought to have once been continuous with a Presumpscot River population (McCollough et al. 1995, p. 7).

1.2 Pleasant River (Cumberland Co.)

The 21 km (13 mi) Pleasant River holds the last remains of a historic *A. varicosa* population that is believed to have once extended into the Presumpscot River from Standish to Westbrook (McCollough and Swartz 1997, p. 4). It is the southernmost river in Maine with an *A. varicosa* population. Fifty-nine *A. varicosa* were found in the Pleasant River in 1993 (Maine Department of Inland Fisheries and Wildlife, unpublished data). In 2001, biologists found 125 *A. varicosa* within a 1.2 km (0.75 miles) reach (CPUE 12.5 mussels/hr) but by 2009 biologists covering the same reach could find only 17 *A. varicosa* (CPUE 1.6 mussels/hr) (Nedeau 2010b, p. 6) – a decline of nearly 90%. Most mussel shells were badly eroded.

Nedeau (2013a, p. 4) conducted extensive surveys in the Pleasant River in 2010 and 2011. A total of 41.1 hours of search time along 9.6 kilometers (6 miles) of river was logged during 2009, 2010 and 2011. During the 2010 and 2011 surveys, just three species were found with only six *A. varicosa* (CPUE 0.17 mussels/hr) detected. There was no evidence of *A. varicosa* recruitment and most mussels showed highly eroded shells (Nedeau 2013, p. 7).

Eighteen km (11.2 mi) of the Pleasant River are listed as impaired for fish, shellfish and wildlife protection and propagation due to low levels of dissolved oxygen resulting from runoff containing phosphorus, nitrogen, sedimentation and sewage (Maine Department of Environmental Protection 2012).

Habitat analysis (Nedeau, 2013a, p. 10) showed the river to be generally degraded with *A. varicosa* absent even in areas that appeared to be suitable habitat. Results show: (1) Lack of, or insufficient width of riparian buffers appears to have contributed to heavy bank erosion and sedimentation during recent floods which, in turn, have led to deterioration of mussel habitat. (2) Agricultural runoff from cropland and livestock manure not only increases sedimentation but also adds a high nutrient load to the river. Nutrients may be released in pulses. For example, agricultural lands and manure stockpiles drain into and become concentrated in swales that connect to the river. The concentrated nutrients (including ammonia) are then discharged directly into the river during heavy rains and snowmelt. (3) New development and associated impervious surfaces increase the potential for additional contaminants to enter the river. Biologists noted high turbidity levels in all three survey years: poor water clarity influenced search efficacy in reaches where visibility was only 10 centimeters (Nedeau 2013a, pp. 10-12).

Maine's southernmost population of *A. varicosa* seems to be represented by just a handful of individuals. Small and isolated with no sign of recruitment, the population appears to be rapidly declining toward extirpation.

2. Kennebec River Basin

2.1 Kennebec River

The Kennebec River is recovering from a history of pollution from the pulp industry, textile mills, agriculture, and untreated sewage that severely impacted the water quality of the river during the 19th and 20th centuries (Michor 2003, pp. 27-28). One A. *varicosa* was located in the Kennebec River during a hydroelectric dam relicensing survey in 2007 (Maine Department of Inland Fisheries and Wildlife, unpublished data). The large size and deep water of the Kennebec River have limited survey efforts.

2.2 Carrabassett Stream

In 1966, A. H. Athearn collected two *A. varicosa* from Carrabassett Stream (13976 Museum of Fluviatile Mollusks) and in 1997, 24 individuals were found during survey of 3 sites (Maine Department of Inland Fisheries and Wildlife, unpublished data). In 2009, 135 *A. varicosa* were found in a 450-meter reach of the Carrabassett Stream (CPUE 14.8 mussels/hr) (Nedeau 2010b, p. 14).

2.3 Wesserunsett Stream

In 1997, eight *A. varicosa* were discovered in the Wesserunsett Stream, eight were also found during a survey in 2008 and 107 were found during surveys of two sites in 2009 (Maine Department of Inland Fisheries and Wildlife, unpublished data). Six mussel species including 438 *A. varicosa* were found during surveys of 25 sites in the Wesserunsett Stream in 2015 (Nedeau 2016, p. 5). *Alasmidonta varicosa* occurred in 18 of the 25 sites surveyed, often as the dominant species at the site with CPUE ranging from 1.7 to 57.6 mussels/hr (among the highest densities ever reported in Maine) with an average CPUE of 16.0 mussels/hr – the second highest average CPUE among the 12 rivers surveyed in 2014-2015 (Nedeau 2016,

p. 8, 12). Water quality in Wesserunsett Stream is threatened by agricultural runoff, livestock access to the river, extensive sedimentation and loss of riparian forests (Nedeau 2016, p. 12).

2.4 Sebasticook River

A total of three *A. varicosa* were found during surveys in 1998 and 2005 in the Sebasticook River, a tributary of the Kennebec River (Maine Department of Inland Fisheries and Wildlife, unpublished data). However, during a survey in 2016, no *A. varicosa* were located (Nedeau 2016, unpublished data). The East Branch of the Sebasticook was contaminated with chemicals from a woolen mill, which became an EPA superfund site in 1999 (EPA Superfund Program 2016). Twenty-nine km (18 mi) of the Sebasticook mainstem below Burnham is listed as impaired for fish, shellfish and wildlife protection and propagation due to elevated levels of dioxin and polychorinated biphenyls from industrial and municipal/sewage discharge (US EPA Water Quality Assessment Report 2002). Additionally, dioxin, polychorinated biphenyls and organic enrichment/oxygen depletion were listed as causes for water impairment in the Sebasticook River in 2012 (US EPA Water Quality Assessment Report 2012). Still, the Sebasticook River supports a high density of freshwater mussels, including large populations of the yellow lampmussel, *Lampsilis cariosa* and the tidewater mucket, *Leptodea ochracea*, both state-threatened species in Maine. Because there are few records of *A. varicosa* in the Sebasticook River it is uncertain whether the species was always rare or has declined. However, historical records show that in other streams with high diversity mussel assemblages, *A. varicosa* has been the first mussel to be extirpated.

2.5 Sandy River

Two *A. varicosa* were found in the Sandy River in 2006 (Maine Department of Inland Fisheries and Wildlife, unpublished data). However no *A. varicosa* were found during surveys of 11 sites in the Sandy River in 2013 (Nedeau 2014, p. 3).

2.6 Carrabassett River

In 1966, A. H. Athearn collected four *A. varicosa* in the Carrabassett River (13987 Museum of Fluviatile Mollusks) and nine individuals were found during one survey in 2008 (Maine Department of Inland Fisheries and Wildlife, unpublished data). In 2016, during a survey of the same reach, biologists found 22 *A. varicosa* – all with eroded shells, most between 60-80 mm (2.4-3.15 in) long and none below 55 mm (2.17 in) (Nedeau 2016, unpublished data). Hydraulic impacts including high shear stress may limit distribution and recruitment of the *A. varicosa* population in the Carrabassett River.

2.7 Gilman Stream

Two *A. varicosa* were found in Gilman Stream, a tributary of the Carrabassett River, in 1996 (McCollough and Swartz 1997, p. 4) and one was found during limited surveys in 2016 (Nedeau 2016, unpublished data).

3. Central Coastal Basin

3.1 Sheepscot River

In 1947 Athearn collected *A. varicosa* from the Sheepscot River in Alna (CMN 30856). During the late 1980s and early 1990s small numbers of *A. varicosa* were found in both Alna and Whitefield (Albright 1991, p. 6; McCollough and Swartz 1997, p. 5). In 2009, Nedeau (2010b, p. 6), during a survey limited in time and spatial extent, reported low numbers of *A. varicosa* (CPUE 1.7 mussels/hr). However, the low numbers were not representative of the *A. varicosa* population in the entire river.

In 2011, an intensive follow-up survey that included both qualitative and quantitative methods showed that the river supported a much larger and broadly distributed population of *A. varicosa*. Biologists found *A. varicosa* in 70 percent of survey sites sampled, extending along 18.8 kilometers (11.7 miles) of river with a total of 177 found (Nedeau 2013, p. 9). The mean CPUE was 7.91 mussels/hr, the range was from 0 to >10 mussels/hr depending on the site surveyed (Nedeau 2013, p. 8). A quantitative survey was conducted at the site that showed the highest density of *A. varicosa* during semi-quantitative surveys. Biologists sampled 165 0.25 m^2 quadrats, excavated to a depth of 10-15 centimeters (4-6 in) in a 2,500 m^2 (27,000 ft^2) area. Results showed a mean density of 0.46 *A. varicosa* per m^2 and a population estimate of 1,152 *A. varicosa* within the survey area (Nedeau 2013b, p. 7). Based on shell condition, the wide range of age classes present and the evidence of recruitment, the Sheepscot River population is healthy and self-sustaining.

Much of the Sheepscot River is classified as highest water quality (including a state designated Outstanding River Segment) but nine river segments do not meet Maine EPA standards due to low dissolved oxygen levels, high nutrient and sediment loads and elevated temperatures (McLean et al. 2007, p. 3). An increase of impervious surface from development and roads threaten water quality in the Sheepscot River (McLean et al. 2007, p. 3).

3.2 West Branch of the Sheepscot River

Thirty-two *A. varicosa* were found in the West Branch of the Sheepscot River in the 1990s (Maine Department of Inland Fisheries and Wildlife, unpublished data). In a 2009 survey 49 *A. varicosa* were found (CPUE 5.1 mussels/hr) (Nedeau 2010b, p. 11). The population in the West Branch appears to be confined to a 6.8 km (4.25 mi) reach with the most records of mussels located in the middle of the reach. The surrounding area is mostly agricultural, with a large electrical substation present and a section of channelized river located downstream (Nedeau 2010b, p.11). Because the population is small and confined in an area with multiple potential threats, Nedeau (2010b, p. 11) considered it to be vulnerable to extirpation.

3.3 Saint George River

Records of *A. varicosa* in the Saint George River are restricted to a 9.6 km (6 mi) section of river although suitable habitat appeared to be widespread from Quantabacook Lake downstream to Sennebec Pond (Albright 1991, p. 6; McCollough et al 1995, p. 31;

McCollough and Swartz 1997, p. 5; Nedeau 2010b, p. 11). In the mid-1980s only three *A. varicosa* were documented. Forty-five *A. varicosa* were found during a survey in 1992 (Maine Department of Inland Fisheries and Wildlife, unpublished data). Fourteen *A. varicosa* were reported in 2001 (Maine Department of Inland

Fisheries and Wildlife, unpublished data). In 2009, 91 *A. varicosa* (CPUE 8.0 mussels/hr) were found within a 650 m (2,133 ft) reach (Nedeau 2010b, p. 11). But because the population appeared to be narrowly restricted in distribution and showed little recruitment it was considered to be vulnerable to extirpation (Nedeau 2010b, p. 12).

4. Penobscot River Basin

4.1 Penobscot River
Water quality in the lower mainstem of the Penobscot River declined during the 20th century due to discharges from tanneries, pulp and paper factories, industries, and municipal runoff and sewage; contaminants included dioxins, mercury and chlorine compounds (McCollough and Swartz 1997, p. 6; Opperman et al 2011, p. 3). Dams fragmented the river (but, as part of the Penobscot River Restoration Project, the Great Works Dam and the Veazie Dam have been removed) (Opperman et al 2011, p. 5). *Alasmidonta varicosa* was found in the Penobscot River during surveys in 1997 (6 individuals), 1998 (25 individuals), 1999 (1 individual), 2006 (2 individuals), 2007 (3 individuals) and 2011 (1 individual) (Maine Department of Inland Fisheries and Wildlife, unpublished data). Eight *A. varicosa* were found during seven surveys of the East Branch of the Penobscot River between 1995 and 2009 (NCSM 41952) (Maine Department of Inland Fisheries and Wildlife, unpublished data). Deep fast moving water in both the mainstem and East Branch has limited survey efforts.

4.2 Marsh Stream
In 1995 two, 1997 two and 1998 three *A. varicosa* were found in Marsh Stream (Maine Department of Inland Fisheries and Wildlife, unpublished data). Survey records show that the *A. varicosa* population in Marsh Stream may be restricted to a 7.2 km (4.5 mi) reach between Frankfort and Winterport (McCollough and Swartz 1997, p. 5; Nedeau 2010b, p. 11). A total of 110 *A. varicosa* were detected during surveys within this reach in 2009 (CPUE 9.3 mussels/hr) and the sample showed a normal distribution of age classes including young animals (Nedeau 2010b, p. 7). The *A. varicosa* population in Marsh Stream shows evidence of recruitment, excellent shell condition and large size making it an important self-sustaining population. Threats to the population include its apparent isolation within the watershed and impacts from an adjacent golf course (Nedeau 2010, p. 12). The inactive Winterport Dam on Marsh Stream built in the 1800s and converted to hydropower in the 1980s was removed in 2010 thereby restoring 128 km (80 mi) of riverine habitat immediately upstream of this population (NOAA Habitat Conservation 2010).

4.3 Kenduskeag Stream
Based on survey records from 1993 and 1995, *A. varicosa* appears to inhabit suitable habitat in the Kenduskeag Stream from Bangor to its tributary Allen Stream in Corinth and Exeter

(Maine Department of Inland Fisheries and Wildlife, unpublished data). Forty-eight *A. varicosa* were found during these surveys. In a 2009 survey, 109 *A. varicosa* were detected within a 575 m (1,887 ft) reach in Corinth (CPUE 9.2 mussels/hr). Mussels showed excellent shell condition and a normal distribution of age classes with lengths ranging from 30-82 mm (1.18-3.23 in) (Nedeau 2010b, p. 12). Threats to this population include insufficient riparian buffers, sedimentation and nutrient loading from agricultural runoff (Nedeau 2010b, p. 12).

4.4 Allen Stream

Three *A. varicosa* were found in Allen Stream, a tributary of Kenduskeag Stream, in 1993 (Maine Department of Inland Fisheries and Wildlife, unpublished data). Biologists surveyed the same site in 2013 and found five mussels species including five *A. varicosa* (CPUE 1.2 mussels/hr) (Nedeau 2014, p. 3).

4.5 Dead Stream

Between 1995 and 2008 biologists found six *A. varicosa* at two sites in the Dead Stream in Alton and Lagrange (MCZ 361490) (Maine Department of Inland Fisheries and Wildlife, unpublished data). Nedeau (2010, p. 13) surveyed 1.6 km (1 mi) in 2009 finding 46 *A. varicosa* (CPUE 3.4 mussels/hr) with 87 percent located in just two areas. Deep pool and high gradient habitats separate these subpopulations (Nedeau 2010b, p. 13).

4.6 West Branch Dead Stream

In 1995, five *A. varicosa* were found in two sites on the West Branch of Dead Stream in Bradford (Maine Department of Inland Fisheries and Wildlife, unpublished data).

4.7 Passadumkeag River

During a 1995 survey of the Passadumkeag River, *A. varicosa* was considered "fairly common to common and often the most common species in the survey" but no mussels were found above the Lowell Tannery dam (Maine Department of Inland Fisheries and Wildlife, unpublished data). In a 2009 survey of the Passadumkeag, biologists found 272 *A. varicosa* within a 500 meter reach (CPUE 28.5 mussels/hr) – the highest *A. varicosa* density of nine rivers surveyed that year (Nedeau 2010b, p. 14). Mussels showed good shell condition and there was evidence of recruitment. The river supports all of Maine's 10 mussel species. Much of river is protected by extensive riparian buffers and contains a large amount of potentially suitable *A. varicosa* habitat. This is likely the best *A. varicosa* river in Maine (Nedeau 2010b, p. 14).

4.8 Great Works Stream

In 1995, one *A. varicosa* was found during one hour of searching at the mouth of Great Works Stream, a tributary of the Penobscot River (Maine Department of Inland Fisheries and Wildlife, unpublished data).

4.9 Pleasant River (Piscataquis River, Piscataquis Co.)
Just one *A. varicosa* was found during a two-hour search in the Pleasant River mainstem in 1997 (Maine Department of Inland Fisheries and Wildlife, unpublished data). However, during 1996 surveys *A. varicosa* was considered "common" in the East Branch of the Pleasant River (106 *A. varicosa* were found during four surveys in 1996 and three were found in 2009) (Maine Department of Inland Fisheries and Wildlife, unpublished data). Six *A. varicosa* were also found between Ebeemee Lake and Upper Ebeemee Lake. About 15 km (9.3 miles) of potential *A. varicosa* habitat exists in the East Branch most of it protected by undisturbed riparian buffer. The East Branch Pleasant River has the potential to support a large *A. varicosa* population and should be a priority river for additional survey work.

4.10 Mattawamkeag River
H. D. Athearn collected 17 *A. varicosa* from the mainstem of the Mattawamkeag River in 1953 (MCZ 199395); two were collected in 1957. During surveys, 32 were found in 1992, 13 were found in 1994, 15 were found in 1996 and six were found in 2006 (Maine Department of Inland Fisheries and Wildlife, unpublished data). Between 1992 and 1996, CPUE ranged from 1.0 to 18.5 mussels/hr (Maine Department of Inland Fisheries and Wildlife, unpublished data). In 2015 seven mussel species including 162 *A. varicosa* were found during surveys of 28 sites on the Mattawamkeag mainstem; CPUE ranged from 0.7 to 18.0 mussels/hr with an average CPUE of 4.4 mussels/hr (Nedeau 2016, p. 9).

4.11 Mattakeunk Stream (Mattawamkeag River)
In 1994, biologists found 13 *A. varicosa* during a survey of three sites on Mattakeunk Stream (Maine Department of Inland Fisheries and Wildlife, unpublished data). Biologists found eight mussel species including 16 *A. varicosa* (CPUE 2.6 mussels/hr) during surveys of seven sites on Mattakeunk Stream in 2015; much of the stream was inaccessible (Nedeau 2016, p. 9).

4.12 Molunkus Stream (Mattawamkeag River)
H. D. Athearn collected *A. varicosa* from Molunkus Stream in 1954 (CMN 005887, 020993; MCZ 200668). It was also found during surveys in 1985 (3 individuals), 1992 (17 individuals), 1996 (15 individuals) and 2006 (5 individuals) (NCSM 41949) (Maine Department of Inland Fisheries and Wildlife, unpublished data). Biologists found seven species including 43 *A. varicosa* (CPUE 3.4 mussels/hr) during surveys of 12 sites in Molunkus Stream in 2015 (Nedeau 2016, p. 10).

4.13 Macwahoc Stream (Molunkus Stream, Mattawamkeag River)
Two *A. varicosa* were found during one survey in Macwahoc Stream in 1996, and eleven were found at one site in 2006 (NCSM 41950) (Maine Department of Inland Fisheries and Wildlife, unpublished data). Biologists found five mussel species including 87 *A. varicosa* (CPUE 19.3 mussels/hr) during surveys of four sites on Macwahoc Stream in 2015 (Nedeau 2016, p. 9). Macwahoc Stream had the highest average CPUE of the 12 streams surveyed in 2015 (Nedeau 2016, p. 9).

4.14 Wytopitlock Stream (Mattawamkeag River)
Three *A. varicosa* were found during two surveys in 1994 (Maine Department of Inland Fisheries and Wildlife, unpublished data). Biologists located five mussel species – but found only one *A. varicosa* (CPUE 0.4 mussels/hr) – during surveys of three sites in Wytopitlock Stream in 2015. Mussel densities of all species appeared low in comparison with other streams surveyed (Nedeau 2016, p. 10).

4.15. Baskahegan Stream (Mattawamkeag River)
Nine *A. varicosa* were found during two surveys of Baskahegan Stream in 1994 (CPUE 0.75 and 1.0 mussels/hr) (Maine Department of Inland Fisheries and Wildlife, unpublished data). Biologists found four mussels species including seven *A. varicosa* (CPUE 2.3 mussels/hr) during surveys of two sites on the Baskahegan in 2015 (Nedeau 2016, p. 5).

4.16 West Branch Mattawamkeag River
Thirty-six *A. varicosa* were found in the West Branch during eight surveys conducted between 1986 and 2006 (Maine Department of Inland Fisheries and Wildlife, unpublished data).

4.17. Fish Stream (West Branch Mattawamkeag River)
Twelve *A. varicosa* were found in Fish Stream in 1996 (CPUE 24.0 mussels/hr); one individual was found in 2004 (Maine Department of Inland Fisheries and Wildlife, unpublished data).

4.18 East Branch Mattawamkeag River
Seventy-one *A. varicosa* were found during six surveys in the East Branch of the Mattawamkeag River between 1992 and 2004, (CPUE ranged from 1.0 to 9.7 mussels/hr) (Maine Department of Inland Fisheries and Wildlife, unpublished data).

5. Eastern Coastal Basin

5.1 West Branch Union River
In 1951 A. H. Athearn collected four *A. varicosa* from the West Branch Union River(1936 Museum of Fluviatile Mollusks). Three *A. varicosa* were found in the West Branch Union River in 1986, 31 were located during a canoe survey in 1992 and five were found in 1997 (Maine Department of Inland Fisheries and Wildlife, unpublished data). Biologists found five species including 120 *A. varicosa* during surveys of 23 sites in the West Branch Union River in 2015; CPUE ranged from 1 to 43.2 mussels/hr with an average CPUE of 7.4 mussels/hr (Nedeau 2016, p. 8).

5.2 Pleasant River (Washington Co.)
Three *A. varicosa* were found in the Pleasant River downstream of the Little River in 1994 (CPUE 1.5 mussels/hr), 7 were found in 1998 and 13 were found in 1999 (Maine Department of Inland Fisheries and Wildlife, unpublished data). Cordeiro et al. collected *A. varicosa* from the Pleasant River in 2008 (MCZ 361499). In 2011, 3,785 liters (1000 gal) of

diesel oil spilled into the Little River, a tributary of the Pleasant River. In order to assess the effects of this spill on the *A. varicosa* population, biologists surveyed 16 sites on the lower Pleasant River and Little River in 2011 – three and one half months after the spill. An additional 19 sites were surveyed upstream of the spill to assess the overall status of A. varicosa in the Pleasant River. Accept for depositional areas and shorelines no impacts from the spill were evident. A total of 76 *A. varicosa* (mostly older animals) were found in the Pleasant River (CPUE 2.6 mussels/hr) but none were found in the Little River (Nedeau 2011, p. 4).

5.3 Machias River (Washington Co.)

Few *A. varicosa* have been found in the Machias River. D. Cameron collected *A. varicosa* from the Machias River in 1961 (OSUM 8069). During surveys of five sites on the Machias River in 1994, biologists found 26 *A. varicosa* (CPUE 2.2, 0.5, 2.3, 1.0, and 5.0 mussels/hr); four *A. varicosa* were found in 1997 and one in 2006 (Maine Department of Inland Fisheries and Wildlife, unpublished data). Four species including 13 *A. varicosa* were found during surveys of 12 sites in 2014; the average CPUE was 1.2 mussels/hr – the second lowest CPUE of the 12 rivers surveyed in 2014-2015 (Nedeau 2016, p. 5). Although much of the Machias River was inaccessible and therefore not assessed, Nedeau observed that habitat conditions within the surveyed areas seemed optimal for Brook Floater and their scarcity was a surprise.

5.4 Old Stream (Machias River)

Four *A. varicosa* were found during two surveys of Old Stream in 1994 (CPUE 1.4 and 1.0 mussels/hr) (Maine Department of Inland Fisheries and Wildlife, unpublished data).

5.5 Chain Lake Stream (Old Stream, Machias River)

One *A. varicosa* shell was found in Chain Lake Stream during a 1.6-hour search in 1994 (Maine Department of Inland Fisheries and Wildlife, unpublished data). Biologists located just three mussel species and failed to find *A. varicosa* in Chain Lake Stream during surveys in 2014 (Nedeau 2016, p. 4).

5.6 West Branch Machias River (Machias River)

Four *A. varicosa* were found during two surveys on the West Branch Machias River in 1994 (Maine Department of Inland Fisheries and Wildlife, unpublished data). However, only three mussels species were discovered and no *A. varicosa* were found in the West Branch Machias River during four surveys in 2014 (Nedeau 2016, p. 4, 11).

5.7 East Machias River

Biologists found 24 *A varicosa* (CPUE 1.6, 6.7 and 0.4 mussels/hr) during surveys of three sites on the East Machias River in 1994, (Maine Department of Inland Fisheries and Wildlife, unpublished data). Six mussel species including 20 *A. varicosa* were found during surveys of 16 sites in the East Machias River in 2014; the average CPUE was 1.8 mussels/hr – the third lowest CPUE of the 12 rivers surveyed in 2014-2015 (Nedeau 2016, p. 5, 11).

5.8 Dennys River

H. W. Haynes collected *A. varicosa* from the Dennys River (MCZ 154573, no date), listed in Johnson (1915, p. 27). Albright found one live *A. varicosa* and one shell during surveys of two sites on the Dennys River in 1985 (Albright 1991, p. 6) but biologists failed to relocate *A. varicosa* during seven surveys in the 1990s (McCollough and Swartz 1997, p. 8). During a four-day search of suitable habitat in 2013, only one live *A. varicosa* and one shell were located (Nedeau 2014, p. 3). Although the outlook for finding a self-sustaining *A. varicosa* population in the Dennys River is not optimistic, a large amount of the upper Dennys River still needs to be surveyed (Nedeau 2014, p. 5).

6. Saint Croix River Basin

6.1 Saint Croix River

The Saint Croix River marks the eastern boundary between Maine and New Brunswick. During two surveys on the Saint Croix River in 1994, biologists located four *A. varicosa* and 17 *A. varicosa* were found in 2006 (NCSM 41953, 14955, 14956) (Maine Department of Inland Fisheries and Wildlife, unpublished data).

6.2 Tomah Stream

Five *A. varicosa* were found during a survey of Tomah Stream in 1994 (CPUE 1.7 mussels/hr) and two were located in 2006 (Maine Department of Inland Fisheries and Wildlife, unpublished data).

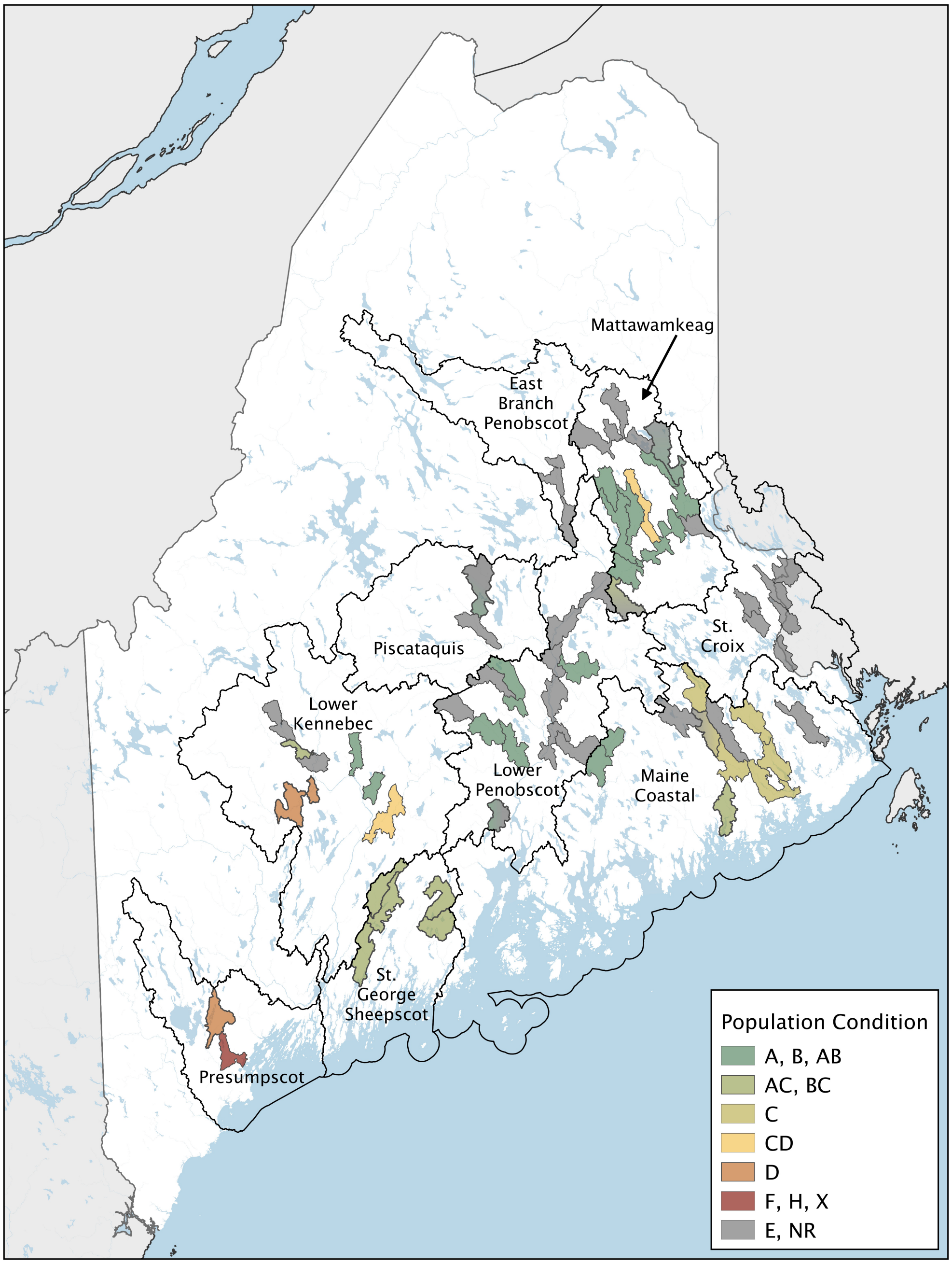

Figure 9. State-level condition map for Maine showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

New Hampshire

Summary. *Alasmidonta varicosa* occurs in three major drainages in New Hampshire: the Merrimack River, the Connecticut River, and Great Bay Estuary watersheds. The Merrimack River watershed was heavily impacted by industrial development, pollution (including toxins, heavy metals and untreated sewage) as well as habitat fragmentation and degradation during the 19th and 20th centuries. By the mid-20th century, the Merrimack River was among the most polluted rivers in the nation. During surveys of the lower Merrimack River in the 1960s, no pollution sensitive benthic species were found from Manchester to the Atlantic Ocean. There was less of an impact to water and habitat quality in the heavily forested and rural upper Merrimack River north of Concord, New Hampshire. In 2009, because of the projected loss of forestland to residential development, the National Forest Service ranked the Merrimack River watershed as the most threatened in the nation. Then, in 2016, the conservation group American Rivers – citing the replacement of riparian forests with suburban development – listed the Merrimack River as among the most endangered rivers in America. (The National Forest Service projects that by 2030, 40-60% of forestland in the watershed will be replaced by development and impervious surfaces with associated increased pollution.) Dams, altered hydrology, flooding and pollution from the increase in impervious surfaces are high-ranking threats to *A. varicosa* in New Hampshire (NH Sate Wildlife Action Plan 2015, A-27).

Still, large self-sustaining populations of range-wide importance are thought to persist in the Merrimack River watershed. Survey data show that the upper Merrimack River in Concord and a small section of the Pemigewasset River in Franklin support *A. varicosa* populations. Additional surveys are needed to determine the spatial extent of these populations. Three tributaries of the Merrimack River north of Manchester: the Suncook, Soucook and Blackwater Rivers, appear to hold the largest most robust populations of *A. varicosa* in New Hampshire. However, there have been no surveys in the Blackwater River, Soucook River and most of the Suncook River since the 1990s. The Suncook River is the site of a long-term, mark-recapture study that began in 2006 after a 100-year flood caused an avulsion that dewatered two river miles, stranding over 1000 *A. varicosa*. Rescued mussels were tagged and translocated to suitable habitat upstream of the avulsed channel. High mussel mortality was documented at the study site during a second 100-year flood in the spring of 2007. The floodwaters washed mussels downstream and onto banks. A drought during the summer of 2007 also caused heavy *A. varicosa* mortality as opportunistic predators gained access to mussel beds. In the early 1990s, both the Blackwater and Soucook Rivers supported dense recruiting populations of *A. varicosa* but new surveys are needed to assess the size and spatial extent of these populations. A fourth tributary of the Merrimack River, the Piscataquog River, supported large self-sustaining populations in the 1990s but these populations have since declined sharply. Long-term quantitative studies in the Piscataquog River show precipitous declines in *A. varicosa* abundance during the last one to two decades (a 98% decline in one once large population between 1996 and 2008 in a section of river where flow is regulated by a hydroelectric dam). Few records exist for *A. varicosa* in three Merrimack River tributaries in the southern tier of the state: Beaver Brook, Golden Brook, and the Nissitissit River (although Massachusetts portion of the Nissitissit River supports a viable population of *A. varicosa*).

The North Branch of the Sugar River supports the only New Hampshire *A. varicosa* population in the Connecticut River watershed. Although a dense *A. varicosa* population was documented in the North Branch in the 1990s, survey data from 2006 and 2009 suggests that this insular population has declined and become fragmented with no evidence of recruitment. Water quality of the North Branch was cited as impaired for aquatic life in 2010 (see below).

Populations of *A. varicosa* in the Great Bay Estuary watershed are imperiled. *Alasmidonta varicosa* appears to be gone from the Exeter River. Despite surveys in 1993, 2001 and 2010, no *A. varicosa* have been found in the Exeter River since 1953. In the 1990s the Lamprey River supported self-sustaining populations of *A. varicosa.* However, summer low flows, high temperatures and non-point source pollution resulting from rapid development and the replacement of riparian forests with impervious surfaces has impaired the water quality in the Lamprey River (see below). An extensive survey in 2010 that included a resurvey of all historic sites showed the population had declined precipitously: only a small number of aged individuals scattered along long stretches of river was found. This relict population will likely be extirpated within the next five to ten years.

In a survey of mussel biologists, New Hampshire respondents reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats. In summary: (1) Water quality and habitat may have improved enough to consider reintroduction or augmentation of *A. varicosa* in parts of the Piscataquog River, however extreme flood events have severely impacted or extirpated *A. varicosa* populations in this river. (2) The Merrimack River and Suncook River are considered conservation priorities because of their healthy populations of *A. varicosa*. (3) The Merrimack River and Suncook River are named as conservation priorities because of immediate threats to *A. varicosa*. New surveys are needed to determine the size, condition and spatial extent of *A. varicosa* populations in the Blackwater, Soucook and Suncook Rivers.

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1. Merrimack River Watershed

Originating in the White Mountains, the Pemigewasset River flows south, joining the Winnipesauke River to form the Merrimack River. The Merrimack flows south through central New Hampshire and northeast Massachusetts before emptying into the Atlantic Ocean. About 75 percent of the 12,976 km^2 (5010 mi^2) Merrimack River watershed is located in New Hampshire (Normandeau Associates 2011, p. 1). The Merrimack River supports a population of *A. varicosa* in Concord that may extend upstream to Boscawen depending on the presence of suitable habitat. In 2013 *A. varicosa* was also found in the Pemigewasset River in Franklin (see below). However, of all the tributaries of the Merrimack River in New Hampshire and Massachusetts, only the Blackwater, Suncook, Soucook, and Nissitissit Rivers are thought to support large self-sustaining *A. varicosa* populations.

The Merrimack River watershed has a history of industrial development, pollution and habitat fragmentation and degradation. Beginning in the 19th century and through much of the 20th century, dams were built on the Merrimack River and its tributaries to power mills for wool, textile, wood and paper processing, and other industries. New Hampshire cities such as Manchester and Nashua as well as Lowell, Lawrence and Haverhill in Massachusetts expanded as industries developed along the river. Industrial wastes (including toxins and heavy metals) and untreated sewage from growing urban populations were discharged directly into the Merrimack River. By the mid-20th century, the Merrimack River was among the most polluted rivers in the nation (Robinson et al. 2003, p 2). Over 454 million liters (120 million gal) of untreated or minimally treated wastewater was discharged into the Merrimack River through the 1960s (U.S. Department of the Interior 1968 *in* Robinson et al. 2003, p. 2). During biological surveys in 1964 and 1965, no pollution sensitive benthic species were found from Manchester to the Atlantic Ocean (Oldaker 1966, part 3, p. 35). Trends in water quality show that concentrations of chloride (related to road salt use) and nitrate (from atmospheric deposition, agriculture, livestock and municipal and industrial waste) increased ten fold during the 20th century while total phosphorus concentrations decreased (Robinson et al. 2003, pp. 14, 15, 17). Although scheduled for eventual elimination, effluents from combined sewage outflows continue to impact water quality of the Merrimack River (B. Wicklow, Saint Anselm College, pers. obs.). Nonpoint source pollution due to increased development and agriculture has also impaired water quality (Army Corps of Engineers 2006, p. 8-3).

There was less of an impact to water and habitat quality in the heavily forested and rural upper Merrimack River north of Concord, NH. For example, upstream of Manchester, dissolved oxygen levels increased and total nitrogen levels decreased from 1965 to 2011 (Normandeau Associates, Inc. 2011, p. 17, 19). However, in 2009, because of the projected loss of forestland to residential development, the National Forest Service ranked the Merrimack River watershed as the most threatened in the nation (Stein et el. 2009, part 2 p.14). Additionally, in 2016, the conservation group American Rivers – citing the replacement of riparian forests with suburban development – listed the Merrimack River as among the most endangered rivers in America (American Rivers 2016).

1.1 Pemigewasset River

The Pemigewasset River originates at Profile Lake in the White Mountains then flows south about 104 km (65 mi) to its confluence with the Winnipesauke River. During a 2013 survey of the lower Pemigewasset River downstream of the Franklin Dam, 106 *A. varicosa* were detected of a total of 2,610 mussels encountered – *Elliptio complanata* comprised 92.3 percent of the total (Kleinschmidt Group 2014, p. 7-57). This was the first discovery of *A. varicosa* in the Pemigewasset River.

1.2 Merrimack River

In the upper Merrimack River in an area north of Concord, *A. varicosa* was first observed in the 1970s. K. Wright collected *A. varicosa* from this site in 1982 (OSUM 52833). In 1993, Cutko reported a large *A. varicosa* population in this area (CPUE 37.7 mussels/hr) (Cutko 1993a, p. 4; Craig 1996, p. 3). During separate surveys in 1995, Craig also documented a large recruiting population of *A. varicosa* in the area (CPUE 15.0 and 32.0 mussels/hr) (Craig 1996, p. 3). In 2001, 183 *A. varicosa* were found north of Concord (Normandeau Associates, Inc. 2001) and during surveys of the same site in 2014 and 2015, 126 *A. varicosa* were located (Nedeau 2015, p. 3).

Upstream in Boscawen, one *A. varicosa* was found in 1992 and, during a three-hour search, another was located in 1995 (Craig 1996, p. 3; NH Natural Heritage Bureau, unpublished data).

Few *A. varicosa* have been found in lower sections of the Merrimack but search time and survey area have been limited. McLain found six *A. varicosa* during a SCUBA survey of the Merrimack River in Manchester (McLain 2004).

1.3 Blackwater River

The Blackwater River is a tributary of the Contoocook River, which empties into the Merrimack River. It was known in the 1990s to support *A. varicosa* populations upstream and downstream of the Blackwater Dam in Webster. In 1994, Gabriel located *A. varicosa* in the Blackwater River in both Salisbury and Webster, NH (NH Natural Heritage Bureau, unpublished data). Large numbers of *A. varicosa* were found in the lower reaches of the river above the dam (CPUE 13.8 in 1994, 8.6 in 1995, and 7.1 in 1996 mussels/hr) (NH Natural Heritage Bureau, unpublished data). Fewer mussels were found farther upstream. For example, in 1996 the NH Natural Heritage Bureau located one mussel upstream in Salisbury (CPUE 2.0 mussels/hr) and one upstream in Andover (CPUE 2.0 mussels/hr) – the farthest upstream record in the Blackwater River. Additionally, one *A. varicosa* was found during a bridge replacement survey in Salisbury (Nedeau 2010c) but no *A. varicosa* were found during a bridge replacement survey in Andover (Geiger 2012).

Also in the 1990s, a large recruiting population of *A. varicosa* was located downstream of the Blackwater Dam in Webster: surveys yielded CPUEs of 40.5 in 1994, 94.8 in 1995, and 53.4 in 1996 mussels/hr (NH Natural Heritage Program, unpublished data). These numbers show a robust *A. varicosa* population present in the 1990s, however no new survey data has been gathered during the last 20 years.

1.4 Piscataquog River

The 92 km (57 mi) Piscataquog River drains an area of 565 km^2 (218 mi^2); 96 percent of the river is free flowing (NH department of Environmental Services; Sundquist 2014, p. 4). It empties into the Merrimack River in Manchester. In the 1990s *A. varicosa* was first discovered in the mainstem and in all three branches of the Piscataquog River with the largest populations found in the mainstem and the South Branch (B. Wicklow, Saint Anselm College 1996, unpublished data).

North Branch of the Piscataquog River: Small numbers of *A. varicosa* were found downstream of the Everett Dam and in the lower section of the North Branch in 1993 (Perrone, Pothier, and Wicklow 1994, unpublished data).

Middle Branch of the Piscataquog River: The first *A. varicosa* population found in the Piscataquog Watershed was located downstream of a historic dam in 1993. Just 12 *A. varicosa* were detected. Lengths ranged from under 40 mm (3 individuals), between 60 -70 mm (8 individuals); one individual was 78 mm in length and showed very little shell erosion (B.

Wicklow, Saint Anselm College 1993, unpublished data). A second population on the Middle Branch, discovered in 1994, was noteworthy for the wide range of age classes present (B. Wicklow, Saint Anselm College 2011, unpublished data). However, this population has declined by 94%, in the last 20 years and there is no evidence of recruitment. It may be eliminated from the site within the next five years (B. Wicklow, Saint Anselm College 2015, unpublished data).

South Branch of the Piscataquog River: Three *A. varicosa* were found upstream of the Paradise dam in New Boston (B. Wicklow, Saint Anselm College 1993, unpublished data). Also in 1993 an *A. varicosa* population was discovered upstream of the confluence with the Middle Branch (CPUE 15.0 mussels/hr). This population had been stable through the 1990s but began to decline, as an eroded bank adjacent to the population became a recreational "beach" used for swimming, fishing, and wading. During this period, several *A. varicosa* were found crushed, as they lay partially embedded in the substrate in this area (B. Wicklow, Saint Anselm College 1998, unpublished observation.). During an intensive search in 2011 no *A. varicosa* were found at this site (B. Wicklow, Saint Anselm College 2011, unpublished data). At a third site, 70 *A. varicosa* were found within a 4 m x 50 m section of river in 1994. The population was resurveyed and appeared stable through the 1990s but was severely impacted by floods in 2006 and 2007 – both of which exceeded the 100-year recurrence interval. No *A. varicosa* were detected during careful searches of the site in 2010 and 2011 (B. Wicklow, Saint Anselm College 2011, unpublished data).

Mainstem Goffstown: In 1993 a large, robust population of *A. varicosa* was located downstream of Greggs Falls Hydroelectric Dam, which regulated stream flow to accommodate peak electricity usage. At that time, 75 dead mussels were found stranded among cobbles in a section of river that was dewatered during low flow; some dead mussels were still embedded in the substrate (B. Wicklow, Saint Anselm College 1993, unpublished data). Ten *A. varicosa* shells were sent to the North Carolina Science Museum (NCSM 7486). Still, *A. varicosa* was the dominant species in the mussel community. Prior to a bridge replacement project in 2006, the population was quantitatively surveyed using distance sampling to determine the abundance of *A. varicosa*: 180 *A. varicosa* were detected along 20 transects across the stream spaced five meters apart. However, in July 1997, high mussel mortality occurred when subdaily discharge from the hydroelectric dam fluctuated from below 0.5 m^3/s (18 ft^3/s) to over 8.5 m^3/s (300 ft^3/s) as water was held then released during peak usage. At extreme low flows mussels are susceptible to mortality from desiccation and opportunistic predation: 193 *A. varicosa* were predated during the low flow periods in July 1997. Subsequent sampling detected only 65 *A. varicosa* – a 64% decrease after the predation (Figure 1). The number of *A. varicosa* found decreased to 41 by 2004, then to three by 2008 (B. Wicklow, Saint Anselm College 2008, unpublished data). This last drastic decline appears to have resulted from severe flooding in 2006 and 2007. Fluctuations in flow from hydroelectric dams and successive 100-year floods appear to have caused catastrophic declines in this population.

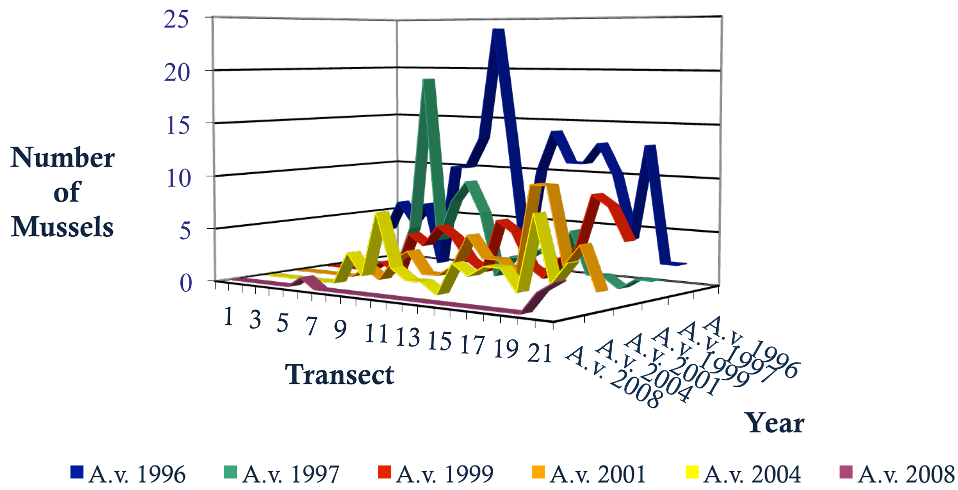


Figure 1. Piscataquog mainstem population: numbers of *A. varicosa* per transect from 1996 to 2008 (B. Wicklow, Saint Anselm College 2008, unpublished data).

Mainstem Manchester: During a 1994 survey, only five *A. varicosa* were found in the segment of river between Kelly Falls Dam and the confluence of the Piscataquog and Merrimack Rivers in Manchester (B. Wicklow, Saint Anselm College 1994, unpublished data).

1.5 Suncook River

The Suncook River originates from a series of lakes in central New Hampshire then flows about 48 km (30 mi) to its confluence with the Merrimack River between Manchester and Concord (Flynn 2009, p. 1). In 1953 Clarke collected *A. varicosa* from the Suncook River in Pittsfield (CMN 021046). In 1993, large numbers *A. varicosa* were found in Chichester, Pittsfield, and Epsom with the highest densities (CPUE 72.0 mussels/hr) found in Pittsfield (Cutko 1993a, p. 5). Craig (1995, p. 5) also reported large numbers (CPUE 32.0 mussels/hr). In 1996, a total of three *A. varicosa* were found during 3.5 hours of surveying in Barnstead and Gilmanton (Albright 1996, p. 3). Ostaudelafont et al. collected *A. varicosa* from the Suncook River in 2006 (NCSM 45040). Most of the Suncook River has not been surveyed since the 1990s.

In 2006, a 100-year flood caused an avulsion in the Suncook River in Epsom. After up to 36 cm (14 in) of rain during a four-day period in May, the estimated peak flow of the Suncook River was 215 m^3/s (7,600 ft^3/s) (Flynn 2009, p. 1). During the flood the Suncook River breached a glacial ridge, cut a new channel and dewatered a 3.2 km (2 mi) section of river leaving a large number of *A. varicosa* stranded. About 1,100 mussels were rescued, marked with numbered tags, and translocated to suitable habitat upstream of the avulsion where a resident *A. varicosa* population was present (Wicklow 2008, p. 12). The translocated mussels along with marked resident mussels are part of a long-term mark-recapture study – hundreds of mussels have been marked during this study. The surveys show *A. varicosa* is the dominant resident mussel at this site. However, high mortality occurred during a second 100-year flood in 2007 when large numbers of mussels were washed downstream and onto banks. Later that year, during a prolonged summer drought opportunistic predators preyed heavily on mussels (Wicklow et al. 2009). Predation during extreme low water in 2010 also caused high mortality from predation. Moreover, extreme low water in 2007 and 2010 provided easy ATV access across sensitive mussel habitat. In a 2013 survey, 35 *A. varicosa* were found upstream of the avulsion site in Epsom (Nedeau 2013, p. 4).

1.6 Soucook River
The Soucook River originates at Rocky Pond then flows about 45 km (28 mi) to its confluence with the Merrimack River between Manchester and Concord (Merrimack Watershed Assessment Study 2003, p. 2-1). A large recruiting population of *A. varicosa* was located in the Soucook River in Concord and Loudon in 1992 (CPUE 26.88 mussels/hr) and 1993 (CPUE 56.5 mussels/hr) (Cutko 1993a, p. 5). Survey results showed the Soucook River at Currier Road to be the farthest upstream extent of the *A. varicosa* population. In 1995, *A. varicosa* was found at three sites on the Soucook (CPUE 1.3, 13.0, 6.0 mussels/hr) (Craig 1996, p. 4). Water quality in the Suncook was listed as impaired for aquatic life upstream of Currier Road due to low pH (EPA Water Quality Assessment Report 2010). No additional survey data has been gathered during the last twenty years.

1.7 Beaver Brook
Few *A. varicosa* records exist for Beaver Brook. In 1952, Athearn and Clark collected specimens north of Pelham (OSUM 24678; CMN 037798). Specimens were also found in 1994 (Gabrielle 1995, p. 44). One *A. varicosa* was found in Pelham in 2003; downstream no mussels of any species could be found (Geiger 2008, p. 2). During a site assessment survey of 150 m (492 ft) of Beaver Brook, 77 *Elliptio complanata* were located but no *A. varicosa* were found (Normandeau Associates 2016, p. 2).

1.8 Golden Brook
Athearn collected ten *A. varicosa* at the mouth of Simpson Pond in 1952. In 1994, six live and several dead *A. varicosa* were found downstream of Cobbetts Pond (CPUE 4.0 mussels/hr) (Gabriel 1995, p. 37). No mussels were found during a site assessment survey on Golden Brook in 2015. However, the extent of the survey was limited to 150 m (492 ft) and the habitat appeared to be unsuitable for mussels (Normandeau Associates 2016, p. 1).

1.9 Nissitissit River
The Nissitissit River originates at the outflow of Potanipo Pond in southern New Hampshire then flows south into Massachusetts where it empties into the Nashua River. An important *A. varicosa* population is present in the Massachusetts section of the river (see Massachusetts report). In 1994, two *A. varicosa* were found in the Nissitissit River in Hollis (NH Natural Heritage Bureau, unpublished data). Nedeau found that the *A. varicosa* population in the Nissitissit River in northern Massachusetts extended into Hollis, New Hampshire (Nedeau 2009, Principal, Biodrawversity, pers. comm.).

2. Connecticut River Watershed

2.1 Connecticut River

Only one historic record exists. Over 100 years ago, C. W. Johnson collected *A. varicosa* from the Connecticut River mainstem at Hanover, (Johnson 1915, p. 27). Efforts to confirm this record have been unsuccessful.

2.2 North Branch of the Sugar River

The North Branch is a 16 km (10 mi) tributary of the Sugar River. The North Branch holds the only New Hampshire *A. varicosa* population in the Connecticut River Watershed. In 1993 *A. varicosa* were found infrequently from south of Spectacle Pond in Croyden to the confluence of the North Branch with the Sugar River in Newport. The highest density was found near the mouth of the North Branch (CPUE 92) (Cutko 1993a, p. 3). Also in 1993 a rectangular monitoring plot was established in the North Branch yielding CPUEs of 50.5 in 1993 and 45.5 in 1996 mussels/hr (Craig 1996, p. 6).

In 2006 Nedeau located *A. varicosa* at three of five sites surveyed (Nedeau 2006, p. 8). During an extensive survey in 2009, Nedeau found that the *A. varicosa* population in the North Branch of the Sugar River was restricted to two small areas comprising just 25 percent of the total stream survey length of four and one half miles (Nedeau 2009a, p. 5). The population appeared to have lower densities than documented in 1993 and 1995, comprising older individuals with a high degree of shell wear – no juveniles were located (Nedeau 2009a, p. 7). In 2010 the water quality of the North Branch was listed as impaired for aquatic life including fish, shellfish, and wildlife protection and propagation due to pH/acidity/caustic conditions (EPA Water Quality Assessment Report 2010).

The Sugar River does not support mussels and is therefore a barrier to dispersal. In 2010 the water quality the Sugar River was listed as impaired for aquatic life including fish, shellfish, and wildlife protection and propagation due organic enrichment/oxygen depletion (EPA Water Quality Assessment Report 2010). Thus, the *A. varicosa* population in the North Branch is insular, fragmented and may be declining making it vulnerable to stochastic demographic, genetic, and environmental events.

3. Great Bay Estuary Watershed

3.1 Lamprey River

The Lamprey River flows about 76 km (47 mi) through 14 towns in southeast New Hampshire before emptying into Great Bay Estuary (Lamprey River Watershed Association 2016). An 18 km (11.5 mi) segment of the Lamprey River was designated as a Wild and Scenic River in 1996 and a 19 km (12 mi) segment was added in 2000 (Lamprey River Advisory Committee 2007, p. i). In 1952, Athearn and Clark collected *A. varicosa* from the Lamprey River in Raymond. These specimens, (MCZ 198927), range in length from just under 60 mm to 70 mm and each shows extensive shell erosion (B. Wicklow, Saint Anselm

College 2013, pers. obser.). Forty-one years later, no *A. varicosa* were found in either Raymond or upstream in Deerfield (Cutko 1993a, p. 6; Gabriel 1996, p. 4). However, downstream in Lee, a large recruiting population was found in 1993 (CPUE 21.0 mussels/hr) (Cutko 1993b, p. 3). Gabriel surveyed the same area in 1996 (CPUE 9.1 mussels/hr) (Gabriel 1996, p. 5). In 2000, 15 *A. varicosa* were observed (CPUE 12 mussels/hr), none under 48 mm in length (average length 54.8 mm), indicating little or no recruitment (B. Wicklow, Saint Anselm College 2000, unpublished data).

In 1994, *A. varicosa* were found in West Epping and Epping (CPUE 20.0 mussels/hr), although most were found crushed due to instream ATV use (Albright 1994, p. 4). Gabrielle noted extensive algal growth on the streambed during her survey of the area in 1996 (CPUE 8.4 mussels/hr), (Gabriel 1996, p. 8).

Farther downstream, *A. varicosa* were found intermittently between Lee and Newmarket: CPUE of 12 mussels/hr in 1993 (Cutko 1993a, p. 6), CPUE of 20.6 mussels/hr in 1995 (Craig 1996, p. 7), and CPUE of 8.4 mussels/hr in 1996 (Gabriel 1996, p. 8).

The results of extensive surveys in 2010 and 2014 (including all historic sites as well as previously unsurveyed sites) show the *A. varicosa* population in the Lamprey River to be critically imperiled and near extirpation. The population is severely fragmented, consisting of older mussels with highly eroded shells; there was no evidence of recruitment. Only 17 *A. varicosa* were found during 150 hours of searching between 2010 and 2014 – 11 in 2010, one in 2011 and five in 2014 (CPUE 0.11 mussels/hr) (Nedeau 2011, pp. 10, 14; Nedeau 2015, pp. 5).

Rapid development and the replacement of riparian forests with impervious surfaces threaten water quality in the Lamprey River. Pollution during summer low flows and high temperatures results in excessive algal growth and oxygen levels that are below standards for class B waters; moreover, at extreme low flows concentrations of copper and zinc (toxic to mussels) reach levels that are harmful to aquatic life (Lamprey River Advisory Committee 2007, p. 6). There was also a significant decrease in pH from 1990 to 2013 but pH may have stabilized between 2004 and 2013 (Kotowski 2016, p. 23). The lack of recruitment, increased isolation of individuals, and deterioration of water quality and stream habitat have resulted in a relict *A. varicosa* population consisting of diminishing numbers of aged adult mussels. It is likely that the *A. varicosa* population in the Lamprey River is non-viable.

3.2 Exeter River

The 66 km (41 mi) Exeter River, located in southeast New Hampshire, becomes the tidal Squamscott River before emptying into Great Bay Estuary. Athearn and Clark collected five *A. varicosa* from the Exeter River in 1953 (CMN 005885). However no *A. varicosa* were found during surveys in 1993 (Cutko 1993a, p. 6), in 2001 (Geiger 2001, p. 1) and in 2010 (Nedeau 2011, p. 9). We believe *A. varicosa* has been extirpated from the Exeter River.

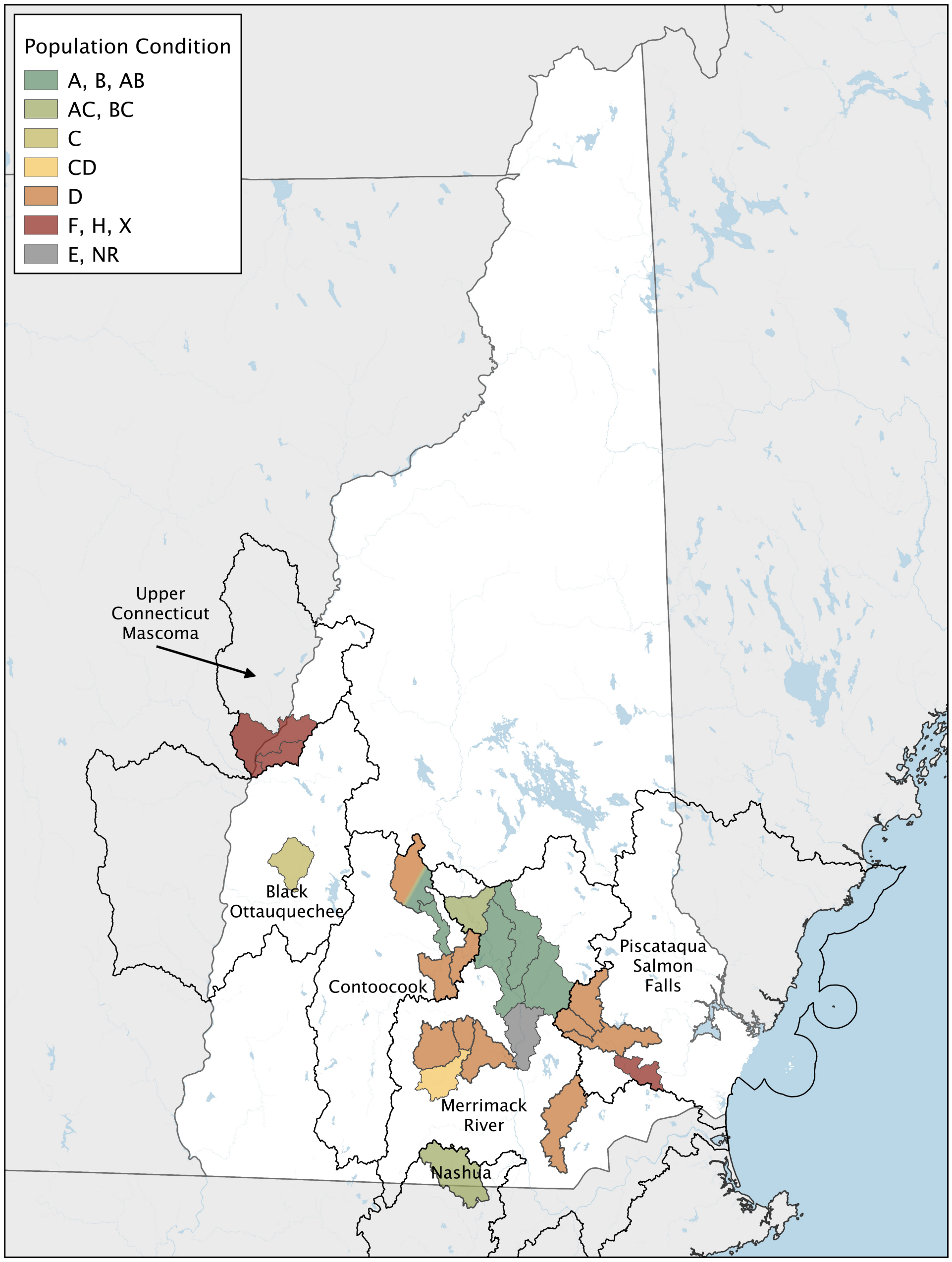


Figure 11. State-level condition map for New Hampshire showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

Vermont

Summary. The West River supports the only *A. varicosa* population in Vermont and the largest population in the Connecticut River watershed. However, numbers of *A. varicosa* have declined sharply since the early 1990s. Two dams dominate the upper West River watershed. One low density *A. varicosa* population is confined between the Ball Mountain Dam in Jamaica and the Townshend Dam in Townshend. No *A. varicosa* have been found upstream of the Ball Mountain Dam. The second population, which extends downstream of the Townshend Dam to Brattleboro, is spatially discontinuous with *A. varicosa* scattered in low densities between aggregates of higher densities. Two mussel beds have been the focus of several surveys since 1991: the Scott Covered Bridge Site, Townshend and the Green Bridge Site, Newfane. It appears that *A. varicosa*, the once dominant species in these multispecies mussel communities, has declined dramatically while *Elliptio complanata* has steadily increased in abundance. *Alasmidonta varicosa* were also found at moderate densities in a high-diversity and high-density mussel bed that was discovered during a recent survey along the State Forest Road in Townshend. While the West River still holds what is considered the largest and most expansive *A. varicosa* population in the Connecticut River Watershed, high mortality and unsustainably low recruitment appear to be putting this population in jeopardy. There has been a striking community shift in the West River, in which in *Elliptio complanata*, a habitat generalist has increased dramatically while *A. varicosa*, a habitat specialist, has declined sharply. This shift suggests a change in watershed-wide influences and although the cause(s) remain unknown, an increase in the number of American eels – an important host fish of *E. complanata* – in the West River during the mid to late 1990s likely led an increase in density and abundance of *E. complanata*. In 2011 Hurricane Irene caused severe damage and extensive flooding of rivers and streams in Vermont. Its effects on the mussel community in the West River have not been assessed.

Connecticut River Watershed

1. Connecticut River, Hanover

C. W. Johnson collected *A. varicosa* from the Connecticut River at Hanover, (Johnson 1915, p. 27). Efforts to confirm this historic record have been unsuccessful.

2. West River

The West River – a 74 km (46 mi) tributary of the Connecticut River – holds the only *A. varicosa* populations in Vermont and the largest population in the Connecticut River watershed. The 1095 km^2 (423 mi^2) West River watershed is 83% forested, 8% agricultural and 7% developed (Vermont Department of Environmental Conservation 2011, 15-1). First discovered by D. Smith, University of Massachusetts, in 1979 (MCZ 280673), *A. varicosa* have since been found from Jamaica downstream to Brattleboro (Fichtel 1992, p. 2; Fichtel and Smith 1995, p. 44). A low-density population in Jamaica is confined between the Ball Mountain Dam and the Townshend Dam where it is has been exposed to heavy siltation from water releases from the Ball Mountain Dam (Fichtel 1992, p. 5). Surveys in 2011 show the community between the Ball Mountain and Townshend dams to be of low diversity and dominated by *Margaritifera margaritifera* with few *A. varicosa* present (Nedeau 2014, p. 11). Despite surveys in 1993 and 2011 no *A. varicosa* have been found above the Ball Mountain Dam. The Army Corps of Engineers built the Ball Mountain Dam between 1957 and 1961 and the Townsend Dam between 1959 and 1961.

The population downstream of the Townshend Dam is spatially discontinuous, with mussels scattered in low densities between high-density beds where *A. varicosa* was the dominant species in the early 1990s. High-density beds surveyed in the 1990s include the Scott Covered Bridge Site in Townshend and the Green Bridge Site in Newfane. Additional mussel beds were found between Scott Covered Bridge and Green Bridge (Fichtel 1993, p. 2) including two sites surveyed by Nedeau (Nedeau 2008, p. 10) along the State Forest Road, Townshend. However, surveys in 2008 and 2011-2012 show that density and abundance of *A. varicosa* have declined substantially since the 1990s while density and abundance of *Elliptio complanata* has increase dramatically (Nedeau 2014, p. 10). *Elliptio complanata* is now the dominant species downstream of the Townshend Dam.

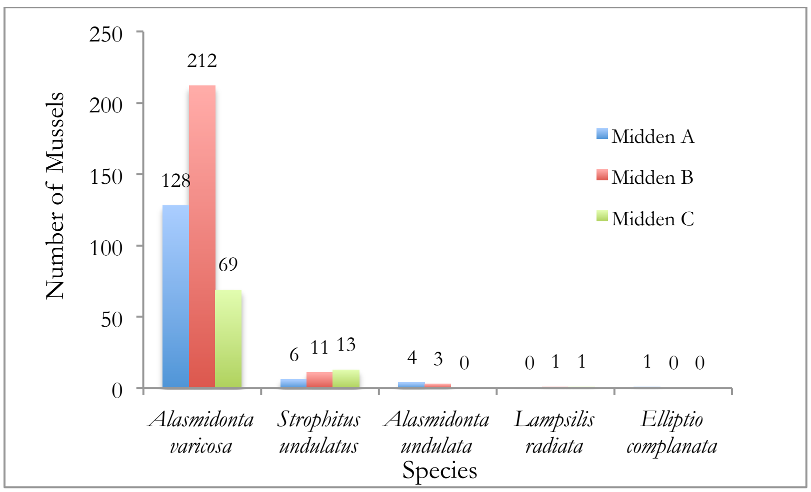


Figure 1. Numbers of mussels collected from three shell middens on July 1, 1992 in the West River, Scott Covered Bridge Site (S. von Oettingen, USFWS memo, 1992).

Scott Covered Bridge Site: A high density of *A. varicosa* was discovered below the Townshend Dam, just downstream of Scott Covered Bridge in Townshend. Fichtel (1991, p. 1) originally reported 13 *A. varicosa* at this site but in the following year the population was found to be much more extensive: 44 *A. varicosa* were found, however the population was considered much larger than counts indicated and extended farther downstream (Fichtel 1992, p. 3). Moreover, 1,097 freshly dead *A. varicosa* were found in shell middens on June 10th, July 1st and August 13th in 1992 (S. von Oettingen, USFWS memo 1992, Fichtel 1992, p. 4). *Alasmidonta varicosa* represented the largest proportion of total mussels in all middens (Figure 1): *A. varicosa* (91.1%), *Strophitus undulatus* (6.7%), *Alasmidonta undulata* (1.6%), *Lampsilis radiata* (0.05%) and *Elliptio complanata* (0.02%).

Fichtel (1992, p. 3), established a strip transect survey at the Scott Covered Bridge Site, which he resurveyed the following year (Fichtel 1993, p. 4). Later, surveys were repeated and numbers of mussels detected were converted to mussels per square meter (Ferguson 2002, Nedeau 2008). In 1992 and 1993, strip transect surveys produced 0.23 and 0.42 *A. varicosa* per square meter, respectively, then decreased to 0.02 and 0.06 *A. varicosa* per square meter in 2002 and 2008, respectively (Ferguson 2002, p. 1; Nedeau 2008, p. 8).

Comparisons between surveys should be made cautiously, nevertheless, there appears to be a dramatic shift in the relative abundance of species from the early 1990s to 2008 (Figure 2): *A. varicosa*, a specialized riverine species and the dominant species in the 1990s, declined sharply while the *Elliptio complanata*, a generalist species and less common in the 1990s, increased dramatically (Ferguson 2002, p. 1; Nedeau 2008, p. 8). Of the 82 mussels found in strip transects in 2008, 81.7 percent were *E. complanata* while just 6.1 percent were *A. varicosa* (Nedeau 2008, p. 8). Of 200 mussels found during surveys in 2011 at Scotts Covered Bridge, 83.5 % were *E. complanata* and 2 % were *A. varicosa* (Nedeau 2014, p. 10).

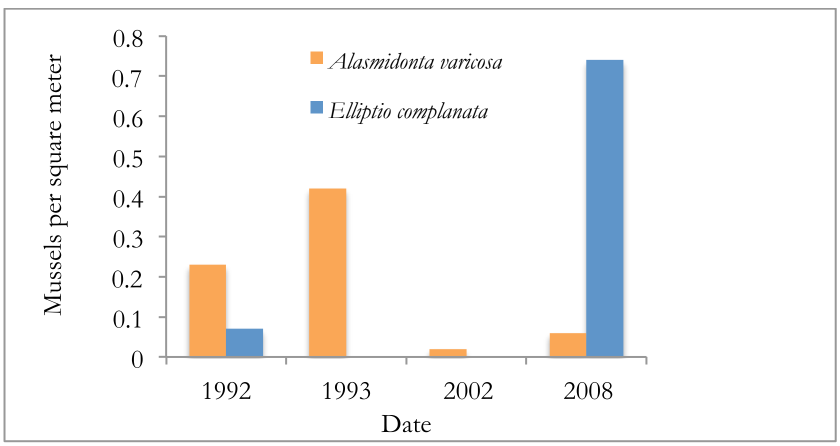


Figure 2. Numbers of mussels per square meter along transects in the West River Scott Covered Bridge Site during a 16-year span (Fichtel 1992, Fichtel 1993, Ferguson 2002, Nedeau 2008).

Green Bridge Site: This site once held the highest density of *A. varicosa* known in the West River (CPUE 42.8 mussels/hr) (Fichtel 1992, p.4). An even larger number of *A. varicosa* were found during a 1993 survey (CPUE 80.4 mussels/hr) (Fichtel 1993, p. 3). Transect surveys produced estimated *A. varicosa* densities of 0.97 and 1.26 *A. varicosa* per square meter in 1992 and 1993, respectively (Ferguson 2002, p. 5) but densities decreased to 0.28 *A. varicosa* per square meter in 2002 then to 0.20 *A. varicosa* per square meter in 2008 (Figure 3) while densities of *Elliptio complanata* increased during the same period (Ferguson 2002, p. 5; Nedeau 2008, p. 9) (Figure 3). As at the Scott Covered Bridge Site, the mussel assemblage at the Green Bridge Site appears to have changed dramatically since the early 1990s: *A. varicosa* has decreased in relative abundance from 78.1 percent in 1992 to 2.9 percent in 2008 while *Elliptio complanata* have steadily increased in relative abundance from 18.3 percent in 1992 to 94.7 percent in 2008 (Nedeau 2014, p 10).

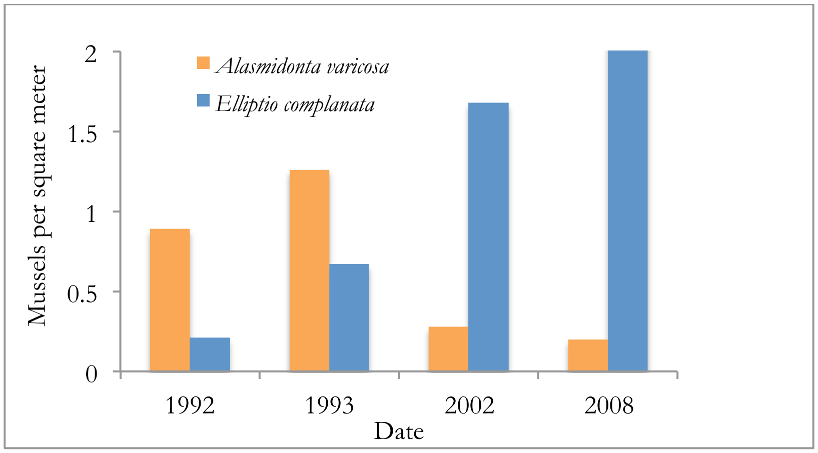


Figure 3. Numbers of mussels per square meter along transects in the West River Green Bridge Site during a 16-year span (Fichtel 1992, Fichtel 1993, Ferguson 2002, Nedeau 2008).

Along with the surprisingly low numbers of live *A. varicosa* encountered at the Green Bridge site in 2002, Ferguson also noted a large number of empty *A. varicosa* shells in the river channel that he suggested indicated recent mortality (Ferguson 2002, p. 5). Little recent recruitment was observed.

State Forest Road Sites: Nedeau (2008) surveyed two sites along the State Forest Road. The first area held high densities of *Lampsilis radiata*, *Elliptio complanata*, *Alasmidonta undulata*, and *A. varicosa* with smaller numbers of *Strophitus undulatus* and *Margaritifera margaritifera*. The largest densities of *A. varicosa* encountered during the 2008 surveys were detected at this site (CPUE 30.6 mussels/hr). Individuals were in good condition and there were signs of some recruitment (Nedeau 2008). Fewer *A. varicosa* (CPUE 15.2 mussels/hr), and lower mussel diversity and densities in general, were observed in the second area (Nedeau 2008, p. 11).

Mussel Community Shift in the West River: There are many possible interrelated factors that may have influenced the rapid and dramatic shift from an *A. varicosa* dominated community to a community dominated by *E. complanata* in the West River. For example:

(1) Climate change: Species have different tolerance levels to elevated stream temperatures. Laboratory experiments show that small increases in temperature can sharply decrease the survival of juvenile mussels (Pandolfo et al. 2010, p. 965). Additionally, because *A. varicosa* is less mobile than *E. complanata*, it is much more likely to be stranded during periods of extreme low water such as experienced in 2016 (B. Wicklow, Saint Anselm College, personal

observation). The projected increase in the frequency and magnitude of floods is expected to cause increased mortality in *A. varicosa* populations. In 2011, Hurricane Irene caused extensive flooding and erosion in Vermont. The high flows deposited large amounts of sediment (over one meter of sediment at some sites) near the mouths of tributaries of the West River (Ethan Nedeau, Nedeau, personal communication 2016). While the dams on the West River may adversely affect downstream water temperatures, they also may to have dampened the impact of Hurricane Irene floodwaters on *A. varicosa.*

(2) Changes in stream chemistry including nutrient input: *A. varicosa* is found in nutrient-poor streams whereas *E. complanata* may thrive in more productive streams (Strayer 1993, p. 242).

(3) Changes in the fish community: for example, Atlantic salmon, once the dominant salmonid in the West River, have been nearly extirpated while the smallmouth bass (a warm water species) has become the top predator (Nedeau 2011, p. 11).

(4) Predation: large numbers of *A. varicosa* shells have been recovered from predator middens in the West River. Additionally, low water levels allow opportunistic predators access to mussel beds and cause extensive mussel mortality (B. Wicklow, Saint Anselm College, unpublished data).

(5) Changes in recruitment may have profound effects on mussel assemblages. For example, laboratory experiments show that the American eel is the most effective host fish for glochidia of *E. complanata* and the likely primary host for *E. complanata* in the wild within the mid-Atlantic region (Lellis et al. 2013, p. 82). During fish surveys in the West River between the 1980s and the mid-1990s, American eels were almost never encountered, but starting in the mid-1990s, the number of eels increased (apparently due to the opening of the fish ladder at the Vernon Dam) (Ken Cox and Lael Will, VT Fish and Wildlife Department, personal communication 2016). The increase lasted just a few years. The subsequent decline of eels in the West River coincided with a decline in the eel population along the eastern seaboard. The increase in the number of host fish in the West River from the mid to late 1990s may have led to an increase in density and abundance of *E. complanata.* In laboratory studies, mussel recruitment was shown to be "strongly and positively dependent on host abundance" and this relationship continued even at low host abundance (Haag and Stoeckel 2015, p. 1165).

Nevertheless, assuming there is no competition for space or resources between the two species, the increase in *E. complanata* abundance does not necessarily explain the decrease in *A. varicosa* abundance. Although *A. varicosa* is a host fish generalist (Wicklow 2004), its low recruitment has been noted in the West River. Moreover, a second host fish generalist, *Strophitus undulatus*, which shares some of the same host fish with *A. varicosa* (Wicklow and Beisheim 1998) has shown a similar sharp decline in the West River (Nedeau 2011, p. 11).

Figure 17. State-level condition map for Vermont showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

Massachusetts

Summary. The distribution range of *A. varicosa* in Massachusetts has contracted sharply. Occurrence records from the late 20th century and earlier show *A. varicosa* once present in 11 major watersheds within the state. It's now known to exist in only four watersheds. Threats to large and medium rivers in Massachusetts include: residential and commercial development, agriculture, altered hydrology due to channelization and dams, sewage effluent, urban runoff and climate change with associated increased prevalence and intensity of drought and severe storms (MA Wildlife Action Plan 2015, pp. 122-124).

In surveys of 19 streams in the Connecticut River Basin between 2007 and 2011, *A. varicosa* was found in just Bachelor Brook (Connecticut River Watershed) and the Ware River and its tributaries Beaver Lake Brook and Muddy Brook (Chicopee River Watershed). Sixty-eight *A. varicosa* were found in Bachelor Brook during surveys in 2008 with most of the population occupying two discreet patches separated by unsuitable habitat. However, the population showed a variety of age classes and evidence of recruitment. Forty *A. varicosa* were detected during a survey of nearly 11 km (7 mi) of the Ware River. This population extends into Ware River tributaries, Beaver Lake Brook where one animal was found and in Muddy Brook near its confluence with the Ware River where five animals were found. Although more *A. varicosa* are likely to found with additional survey effort, urban, industrial and resident runoff threatens the water quality of the Ware River. Extensive surveys of the Westfield River and its tributaries between 2007 and 2009 failed to find *A. varicosa*. The West Branch of the Farmington River was once thought to harbor the most viable *A. varicosa* population in Massachusetts but recent surveys suggest it is now highly imperiled: 80 animals were found during surveys along a 16 km (10 mi) reach of the river in 2008. The population was patchy with low abundance and showed little evidence of recruitment.

Because of polluted water, the conservation group American Rivers listed the Merrimack River as among the most endangered American rivers in 2016. Historical records show *A. varicosa* was once present in the Shawsheen River (Merrimack River Watershed) but surveys in 2011 failed to find any animals. The Nissitissit River (Nashua River Watershed) may support a population of *A. varicosa* in the 100s. However, the partly forested and rural landscape is facing intense development pressure. The Millie Turner Dam, the only intact dam on the Nissitissit River, was dismantled and removed in 2015 thus removing a barrier that had separated *A. varicosa* into two populations since 1750. In 2008, 68 *A. varicosa* were found in a 5 km (3.1 mi) survey upstream from the Millie Turner dam to the New Hampshire border. Additionally, in an intensive search prior to and during dam removal in 2015, 75 *A. varicosa* were discovered within a reach that extended 2 km upstream and downstream of the dam; animals showed a variety of size classes and evidence of recruitment. Further downstream, 28 *A. varicosa* were found during a survey in 2009 but during a careful search of the same reach prior to dam removal in 2015 only three *A. varicosa* were detected. Upland fuel spills may have been responsible for the sharp decline.

Surveys in 2010 and 2011 failed to find *A. varicosa* where they were historically present in the Blackstone River, West River and Abbott Run. In a survey of mussel biologists, respondents reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats in Massachusetts. In summary: (1) Portions of the Ware River and

Nissitissit River may be areas where water quality and habitat have improved enough to consider reintroduction or augmentation (however, one respondent reported that no streams were ready for reintroduction or augmentation). (2) The Ware River and Nissitissit River were ranked as conservation priority streams based on their relatively healthy *A. varicosa* populations. (3) The West Branch of the Farmington River, Ware River, Nissitissit River and Bachelor Brook were named as conservation priorities because of immediate threats to *A. varicosa* populations.

1.0 Connecticut River Basin

During surveys between 2007 and 2011 sections of 19 streams in the Connecticut River Basin were searched for rare mussels: Bachelor Brook, Flat Brook, Fort River, Hop Brook, Ware River, Beaver Lake Brook, Great Brook, Muddy Brook, Quaboug River, Swift River, Chicopee Brook, Sevenmile River, Twelve Mile Brook, Little River, Mill River, Scantic Brook, Stony Brook, Millers River and the Westfield River. *Alasmidonta varicosa* was found in only Bachelor Brook and the Ware River (and its tributaries Beaver Lake Brook, and Muddy Brook) (Nedeau 2008 p. 4; Nedeau 2009b p. 2; Nedeau 2011 p. 3) (see below).

1.1 Connecticut River Mainstem

Historical records show that both Field, in Turner's Falls, no date (NMNS 86197), and C. B. Adams, in South Hadley, collected *A. varicosa* in the Connecticut River, no date (MCZ 154265).

1.2 Bachelor Brook

In 1950 H. D. Athearn collected a single juvenile *A. varicosa* (Athearn Collection) (Cordeiro 2006, p. 8) and a second individual (UMMZ 182587) from Bachelor Brook; there is a third record from 1994 (MA Natural Heritage Program, unpublished occurrence data). In 2008, Nedeau found 63 *A. varicosa* in Bachelor Brook within a total survey distance of 1.9 km (1.3 miles) and 36.5 person hours (Nedeau y 2009b, p. 3). Most (78%) of the population was confined to two spatial clusters with a combined distance of 280 m (919 ft) (CPUE of 3.1 and 3.75 mussel/hr) but separated by 1.6 km (1 mi) of unsuitable habitat including a dam and former impoundment. The remaining 22% of individuals were scattered – mostly as isolated animals – along 1.6 km (1 mi) of the brook. The population showed a variety of age classes and evidence of recruitment Nedeau 2009a, p. 13). Water quality impairment due to impoundments and agricultural runoff poses a significant threat to mussels in Bachelor Brook (Nedeau 2009a, p. 20). In spring of 2015, the low level outlet of the Quinneyville Dam failed ~ 2 km (3.2 mi) upstream from the highest density cluster of *A. varicosa* in Bachelor Brook. MA Natural Heritage biologists conducted transect-based surveys in July 2015 at four 100 m (328 ft) sites downstream of the dam. They found *A. varicosa* during transect searches at only one of the high density locations surveyed by Nedeau in 2008, with a mean density of 0.015 animals per m^2 (10.8 ft^2). At the second highest ranking area from 2009 surveys, only one *A. varicosa* was observed outside of transects during a 15 min unconstrained search. Water clarity conditions were not optimal during searches and a combination of visual and tactical searching was used (Hazelton 2015, MA Division of Fish and Wildlife, Natural Heritage and Endangered Species Program, unpublished data). The effect of the dam breach on the Bachelor Brook on the *A. varicosa* population is unknown.

1.3 Westfield River (Westfield Watershed)
C. B. Adams collected *A. varicosa* in the Westfield River in 1942 (MCZ 154274; NMNS 1020075). However, Nedeau failed to find *A. varicosa* during surveys of 51 sites in the Westfield River and its tributaries between 2007 and 2009 (Nedeau 2009c, p.5).

1.4 Ware River (Chicopee Watershed)
Alasmidonta varicosa was found in surveys of the Ware River in 1996 (MA Natural Heritage Program, unpublished occurrence data). In a larger effort, Nedeau (2009a, p.8) surveyed 11.9 km (6.75 mi) of the Ware River detecting 40 *A. varicosa* within a survey distance of 6.3 km (3.9 miles) during 30.75 person hours in 2008. The Ware Brook *A. varicosa* population extends into Beaver Lake Brook (one animal) and Muddy Brook (five animals) (see below). Impairment of water quality due to industrial, urban and residential runoff is a major threat to mussels in the Ware River (Nedeau 2009a, p. 21).

1.5 Beaver Lake Brook (Chicopee Watershed)
Nedeau (2009b, p. 3) found just one *A. varicosa* in Beaver Lake Brook, a small tributary of the Ware River. Nedeau suggested that the single individual is likely a recruit from the Ware River *A. varicosa* population.

1.6 Muddy Brook (Chicopee Watershed)
H. D. Athearn collected *A. varicosa* (Athearn Collection) from Muddy Brook (Cordeiro 2006, p. 8). Nedeau (2009b, p. 3) found five *A. varicosa* in Muddy Brook near its confluence with the Ware River.

1.7 Flat Brook (Chicopee Watershed)
There is one 1982 record of *A. varicosa* in Flat Brook (MA Natural Heritage Program, unpublished occurrence data).

1.8 West Branch Farmington River (Farmington Watershed)
Most of the Farmington River's 1,559 km^2 (602 mi^2) watershed lies in Connecticut; the West Branch of the Farmington River makes up the major portion of the 25% of the watershed located in Massachusetts (Duerring 2005, p. iv). *Alasmidonta varicosa* was first found in the West Branch during surveys in 1979 (MA Natural Heritage Program, unpublished occurrence data). In 2007, a total of 80 *A. varicosa* were found at 9 different sites along a 16 km (10 mi) reach in the West Branch (Nedeau and Low 2008, p. 7). Their distribution was patchy with most animals found at three locations in clusters of 24, 23 and 10 animals; no *A. varicosa* were found in the Connecticut portion of the West Branch (Nedeau and Low 2008, p. 7). Nedeau and Low (2008, p.14) considered *A. varicosa* as highly imperiled in the West Branch due to its patchy distribution, low abundance, high shell erosion and apparent low recruitment. Although 85% of the West Branch Watershed in Massachusetts is forested, nonpoint source pollution such as salt and sedimentation from roads, effluents from storage

tanks and dumps, septic leakage and storm water runoff threaten water quality (Duerring 2005, p. 14).

2.0 Merrimack River Basin

2.1 Merrimack River Mainstem

In 1866 John Bartlett collected *A. varicosa* in Haverhill – we presume from the Merrimack River (MCZ 151601).

2.2 Spicket River

There are two historical records of *A. varicosa* collected from the Spicket River in Lawrence: one with no date (MCZ 154572) and one from 1942 (MA Natural Heritage Program, unpublished occurrence data).

2.3 Shawsheen River (Shawsheen River Watershed)

Historical records show that *A. varicosa* was once present in the Shawsheen River (Report on the Invertebrata of Massachusetts 1841). Additionally, C. B. Adams collected *A. varicosa* in the Shawsheen River circa 1942 (MCZ 154269). In 2011 Marea Gabrielle surveyed two sites totaling 518 m (1,700 ft) in the Shawsheen River but the only species observed was *Elliptio complanata* (Gabrielle 2011, MA Division of Fish and Wildlife, Natural Heritage and Endangered Species Program, unpublished data).

2.4 Nissitissit River (Nashua River Watershed)

Flowing from a relatively undeveloped forested headwaters in New Hampshire, the 14.8 km (9.2 mi) Nissitissit River is considered cold and well oxygenated with approximately 50% of its shore protected by a 91 m (300 ft) vegetated buffer; however, the watershed faces intense development pressure (Nashua River Watershed Association). Until its removal in September 2015, the Millie Turner Dam in Pepperell separated *A. varicosa* in the Nissitissit River into two populations: the upstream population – extending northwest into New Hampshire – and a downstream population. In a 1986 survey upstream of the dam, D. Schweitzer observed dozens of *A. varicosa* in one hour; dozens of animals were also found during surveys in 1988 (MA Natural Heritage Program, unpublished occurrence data). Nedeau (2009a, p.17) in a survey of 5 km (3.1 mi) and 36 person hours found a total of 68 *A. varicosa* in the Nissitissit River in 2008. Most (78%) of the mussels were confined to two spatial clusters separated by 1.9 km (1.2 mi) while the remaining 22% of animals – usually isolated individuals – were scattered over 4 km (2.5 mi) of river.

A 1986 survey recorded *A. varicosa* downstream of the Millie Turner Dam (MA Natural Heritage Program, unpublished occurrence data). In a 2009 survey Nedeau (Nedeau 2009a, p. 2) found 28 *A. varicosa* in a 50-60 m (164-197 ft) reach between the dam and the Nashua River. This same reach was carefully surveyed prior to dam removal in 2015 but only 3 *A. varicosa* were detected; it is speculated that upland fuel spills may be responsible for the steep decline (Hazelton 2015, MA Division of Fish and Wildlife, Natural Heritage and Endangered Species Program, unpublished data).

Before and during the removal of the Millie Turner Dam, volunteers led by Peter Hazelton, MA Division of Fish and Wildlife, Natural Heritage and Endangered Species Program, searched for target mussels: *Margaritifera margaritifera*, *Strophitus undulatus*, *Alasmidonta undulata*, and *A. varicosa*. Of the 200 target mussels detected, 75 were *A. varicosa*. There was evidence of recruitment and preliminary estimates suggest the population may be in the 100s of individuals (Hazelton 2015, MA Division of Fish and Wildlife, Natural Heritage and Endangered Species Program, unpublished data).

2.5 Gates Pond (Concord River Watershed)
E. L. Wheeler collected *A. varicosa* from Gates Pond in 1859 and there is a second historical collection by Tracy, no date (NMNS 452075) and a third with no date or data (MCZ 150653). It is possible that these records are from Gates Pond Brook near Gates Pond. There have been no recent surveys (MA Natural Heritage Program, unpublished occurrence data).

2.6 Cochituate Aqueduct (Concord River Watershed)
There is an undated historical record for *A. varicosa* in the Cochituate Aqueduct (MA Natural Heritage Program, unpublished occurrence data). Built in the mid-1800s this now defunct aqueduct conveyed water from an impounded tributary of the Sudbury River (forming Cochituate Lake) to the Brookline Reservoir then to Boston (Massachusetts Water Resources Authority 2015).

2.7 Course Brook (Concord River Watershed)
There is one undated occurrence record (circa 1800) of *A. varicosa* in Course Brook. This brook flows into Cochituate Lake (see above).

2.8 Sudbury Aqueduct (Concord River Watershed)
C. A. Frost collected *A. varicosa* in the Sudbury Aqueduct in 1931 (MCZ 154574).

3.0 Mystic River Watershed

3.1 Aberjona River
C. B. Adams collected *A. varicosa* in Woburn, MA in 1942 (MCZ 154174). We speculate that the collection was from the Aberjona River that runs through Woburn.

4.0 Blackstone River Watershed

4.1 Blackstone River
Although not considered common, *A. varicosa* was historically present in the Blackstone River and its tributaries (Gould 1841). However, Nedeau (2011, p. 8) failed to find *A. varicosa* in the Blackstone River or its tributaries, the West River, Mumford River and Quinsigamond River, during surveys in 2010.

4.2 West River
In 1944 H. D. Athearn collected one *A. varicosa* (Athearn Collection) in the West River, a tributary of the Blackstone River (Cordeiro 2006, p. 8). Nedeau (2011) failed to find *A. varicosa* during surveys in 2010.

4.3 Abbott Run
In 1946 H. D. Athearn collected eight *A. varicosa* (Athearn Collection and MCZ 176673) including juveniles from Abbott Run (Cordeiro 2006, p. 8). In 1999 B. Reid and D. Pugh, working independently, surveyed four sites in Abbott Run. Search times ranged from 40-190 minutes but only *Alasmidonta undulata* and *Elliptio complanata* were detected (MA Natural Heritage Program, unpublished occurrence data).

5.0 Charles River Watershed

5.1 Bogle Brook
Bogle Brook flows from Nonesuch Pond. There are historical occurrence records (no date or data) of *A. varicosa* in Bogle Brook (MA Natural Heritage Program, unpublished occurrence data). Additionally, there are two historical records by S. W. Denton (MCZ 154575) of *A. varicosa* in the Nonesuch Pond. We speculate that these records could be from the outflow of Nonesuch Pond that forms Bogle Brook.

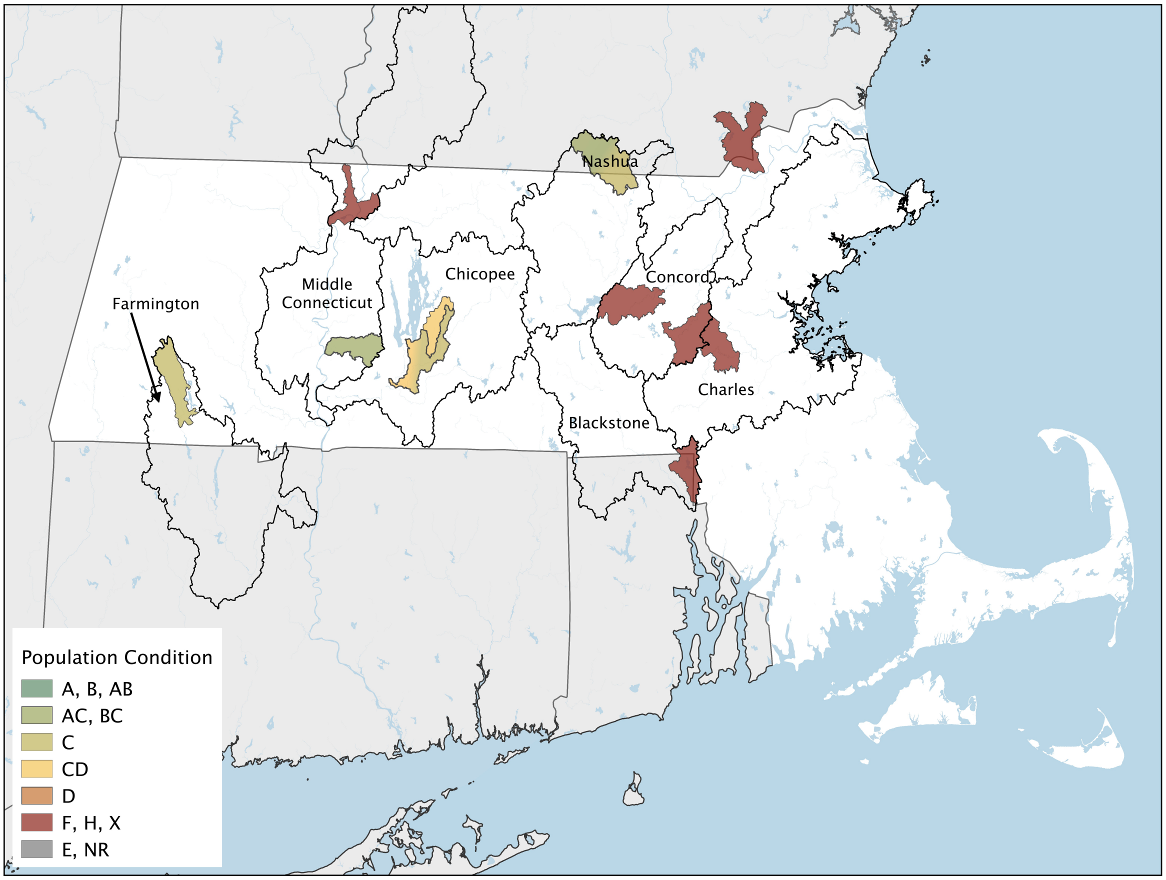


Figure 7. State-level condition map for Massachusetts showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

Connecticut

Summary. The Shepaug River may hold the last viable population of *A. varicosa* in Connecticut. Altered hydrology, industrial and sewage discharge, pollution from agricultural and urban runoff, residential development and the loss of riparian forests and wetlands have impaired water quality and stream habitat in Connecticut. Of the 12 native freshwater mussel species in Connecticut, six are listed as special concern, threatened or endangered (Nedeau and Victoria 2008, p. 2). There are occurrence records of *A. varicosa* from three watersheds in Connecticut: the Thames River, Connecticut River and the Housatonic River watersheds. Few *A. varicosa* have been found during recent surveys in the Thames River Watershed. A total of 17 *A. varicosa* were found during surveys from 2008-2012 in the Shetucket River, Natchaug River, Edson Brook, Mount Hope River and Bungee Brook. During surveys from 2006-2012 in the Connecticut River watershed biologists found a total of 24 *A. varicosa* in the Jeremy River, Eightmile River and Stony Brook; 15

of the 24 *A. varicosa* were found in the Jeremy River. Despite extensive surveys in 2007-2009, no *A. varicosa* have been found in the Connecticut River or the Farmington River in Connecticut. However, during a survey of 73 sites in the Housatonic River watershed in 2010, 41 *A. varicosa* were located in the East Branch of the Shepaug River. (The number is equal to the total of *A. varicosa* found in all other watersheds within the state from 2006-2012.) Moreover, this is the only known population remaining in the Housatonic River basin. The East Branch of the Shepaug River, which flows through a heavily forested and sparsely populated landscape, may be the last hope for a self-sustaining *A. varicosa* population in Connecticut.

In a survey of mussel biologists, respondents reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats in Connecticut. In summary: (1) While one respondent named the Eightmile River, another respondent said there are no streams where water quality and habitat have improved enough to consider reintroduction or augmentation at this time. (2) The Shepaug River was ranked as conservation priority stream based on its healthy *A. varicosa* population. (3) All streams with extant *A. varicosa* populations in Connecticut were named as conservation priorities because of immediate threats to the species.

1.0 Thames River Watershed

1.1 Mashamoquet Brook (Quinebaug River Watershed)

E. H. Reed collected *A. varicosa* in Mashamoquet Brook in 1966 (REED) as well as in the Town of Abington (probably in Abington Brook, a tributary of Mashamoquet Brook) (YBM IZ 051978, no date or data). Riseman (1995) failed to find *A. varicosa* in Mashamoquet Brook. Further, in an effort to confirm Reed's 1966 record, Nedeau (2009d, p. 17) surveyed 3.6 km (2.24 mi) of Mashamoquet Brook and a short distance near its confluence in the Quinebaug River. Few mussels were found and no *A. varicosa* were located. However, in an upstream reach of Mashamoquet Brook, S. Johnson and D. O'Halloran found six *A. varicosa* in 2012 (CT Natural Diversity Database, unpublished data). Low water, channelization, agriculture, roads and residential development are threats to mussels in Mashamoquet Brook (Nedeau 2009d, p.17).

1.2 Shetucket River (Shetucket River Watershed)

The Natchaug River joins the Willimantic River to form the Shetucket River. Point source pollution from industrial and sewage treatment sites as well as nonpoint source pollution from agricultural and urban runoff and the loss of riparian forests and wetlands has impaired the water quality in the Willimantic River (Willimantic River Alliance). During a five-day survey two SCUBA divers found one *A. varicosa* upstream of an impoundment in the Shetucket River (Nedeau 2009d, p. 16). In 2012 S. Johnson and D. O'Halloran found just one *A. varicosa* in the Shetucket River (CT Natural Diversity Database, unpublished data). Nedeau (2009d, p. 17) suggested that if a source population exists, it would be upstream in the Natchaug River.

1.3 Natchaug River (Shetucket River Watershed)
Both W. H. Clench (MCZ 217134) and S. L. H. Fuller (REED) collected *A. varicosa* in the Natchaug River in 1957. Cordeiro also found it in 2002 (MCZ 375175). In 2008, during extensive surveys of over 6.8 km (4.2 mi) of the Natchaug River, only two, isolated *A. varicosa* were found (Nedeau 2009d, p. 15-16). Threats to the Natchaug River include low flow, agricultural runoff, nearby roads and residential development (Nedeau 2009d, p. 26)

1.4 Edson Brook (Shetucket River Watershed)
In 2012 S. Johnson and D. O'Halloran found four adult *A. varicosa* in Edson Brook (CT Natural Diversity Database, unpublished data).

1.5 Mount Hope River (Shetucket River Watershed)
S. Johnson and D. O'Halloran found two adult *A. varicosa* in the East Branch of Mount Hope River in 2012 (CT Natural Diversity Database, unpublished data).

1.6 Bungee Brook (Shetucket River Watershed)
Both in 1994 and 1995, Riseman (1995) found two live *A. varicosa* in Bungee Brook, a tributary of the Natchaug River and D. Smith and J. Victoria collected two specimens in 1993 (CT Natural Diversity Database, unpublished data). However, in a 2008 survey of the same reach surveyed in 1995, Nedeau (2009d, p. 15) failed to find any *A. varicosa* – only one live animal was detected further downstream. Nedeau (2009d, p. 26) lists low flow, agricultural runoff, nearby roads and residential development as threats to Bungee Brook.

2.0 Connecticut River Watershed

2.1 Connecticut River
Nedeau (2009d, p. 8) surveyed 17 sites totaling 11 river miles of the Connecticut River but failed to locate *A. varicosa*.

2.2 Farmington River
Although it occurs in the upper West Branch of the Farmington River in Massachusetts, no *A. varicosa* were found in the Farmington River in Connecticut despite extensive surveys in 2007 (Nedeau and Low 2008, p. 5) and 2008 (Nedeau 2009d, p. 9-10).

2.3 Stony Brook
In 1959, W. A. and S. H. Fuller collected *A. varicosa* from Stony Brook (REED) and D. Smith collected it in 1979 (UMASS). Nedeau (2009d, p. 11) surveyed the same area in 2006 but no *A. varicosa* were found. During additional surveys in 2008 only one *A. varicosa* was found (Nedeau 2009d, p. 11). No *A. varicosa* were located during a survey of Stony Brook in 2014 (CT Natural Diversity Database, unpublished data).

2.4 Muddy Brook
S. L. H. Fuller collected *A. varicosa* in Muddy Brook, a tributary of Stony Brook, in 1959 (REED). E. H. Reed also collected it in 1960 (two sites) (NMC 040236, OSUM 75062 and

REED) and again in 1961 (REED). D. Smith collected it in 1977 (UMASS). It's likely that there was once a continuous *A. varicosa* population extending from Muddy Brook into Stony Brook. No *A. varicosa* were located during surveys of Muddy Brook in 2013 and 2014 (CT Natural Diversity Database, unpublished data).

2.5 Jeremy River

Cordeiro collected *A. varicosa* in the Jeremy River, a tributary of the Salmon River, in 2003 (MCZ 375062). Nedeau (2006) found a sparse population of 14 *A. varicosa* within a stream reach that extended 1.2 km (0.75 mi); one additional animal was found in 2008 (Nedeau 2009d, p. 14).

2.6 Eightmile River

Riseman (1995) located one *A. varicosa* in the Eightmile River. Nedeau (2009d, p. 13) found a highly insular population of just eight *A. varicosa* in 2008 in a 100 m (328 ft) reach frequented by fishermen thus making trampling a cause of concern.

2.7 Trout Brook (Noyes River)

In 1957, N. W. Leamond collected *A. varicosa* in the Noyes River (now called Trout Brook) in West Hartford (MCZ 71164). Trout Brook is a tributary of the South Brook Park River, which flows through Hartford before joining the Connecticut River. The Trout Brook watershed is 63% urban and much of the brook is listed as impaired for aquatic life (Connecticut Department of Energy and Environmental Protection 2012, p. 2).

3.0 Housatonic River Watershed

There is one historical collection by Jacot of *A. varicosa* from the Housatonic River in Connecticut (YPM no date). There was also a small – now extirpated – population of *A. varicosa* in Tenmile River and its tributary Webatuck Creek within the Housatonic Watershed in New York (Strayer 2010).

3.1 Shepaug River

In a 2010 mussel survey of 73 sites in the Housatonic River and its tributaries, Nedeau (2011, p. 10) found 41 *A. varicosa* (CPUE 6.2 mussel/hr) in the East Branch of the Shepaug River. The river flows through a heavily forested landscape that may have contributed to the persistence of this population. This appears to be the state's last remaining viable population of *A. varicosa* (Nedeau, 2011, p. 16). This is the only known living population of *A. varicosa* remaining in the entire Housatonic River watershed.

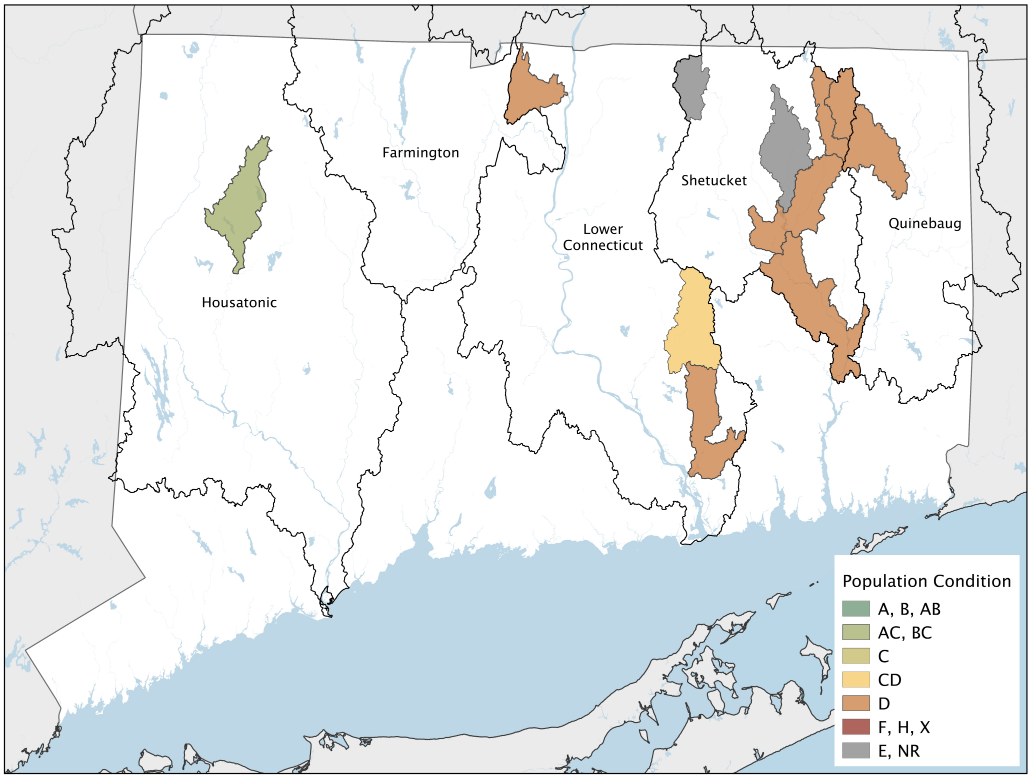


Figure 4. State-level condition map for Connecticut showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

Rhode Island

Summary. *Alasmidonta varicosa* has been extirpated from Rhode Island. The only occurrence records known are from the Blackstone River, which originates in Massachusetts and flows south through Rhode Island to empty into Narragansett Bay. An 1841 record shows that *A. varicosa* was present in the Blackstone River and its tributaries in Massachusetts and an 1889 record shows it was present in small numbers in the Blackstone River in Rhode Island. The Blackstone River was extensively dammed and heavily polluted during an intense period of industrialization in the 18th and 19th centuries. The Blackstone River was considered to be one of the most polluted rivers in the country.

Blackstone River

The Blackstone River begins at the confluence of the Mill and Middle Rivers in Worchester Massachusetts then flows south through Rhode Island to become the tidal Seekonk River, which empties into Narragansett Bay. The river has a long history dams and legacy of pollution beginning with the first textile mill built on the river in 1793. By the end of the 1800s there were as many dams as river miles on the Blackstone River (Rhode Island Department of Environmental Management 2013, p. 24). During the 1900s, the river became heavily polluted from industrial and municipal discharges. By 1900 it was considered the most polluted river in New England (Leighton 1903, p. 63). In an EPA-sponsored report, the Blackstone River was considered "the most polluted river in the country with respect to toxic sediments" (Blackstone River Valley National Heritage Corridor Commission 1998, p. 6). The river is considered impaired because of high levels of lead and cadmium as well as low levels of dissolved oxygen, high levels of total phosphorus, elevated levels of polychlorinated biphenyls, mercury and poor biodiversity indicators (Rhode Island Department of Environmental Management 2013, p. 11). Dams and pollution decimated mussel populations in the Blackstone River including stretches in tributaries where there are no mussels despite the appearance of suitable habitat (Raithel and Hartenstine 2006, p. 115).

Alasmidonta varicosa was historically present in the Blackstone River and its tributaries in Massachusetts (Gould 1841). H. F. Carpenter reported that *A. varicosa* (referred to as *A. marginata*) was "found very sparingly in the Blackstone River just above the Tin Bridge in Central Falls" in Rhode Island (H. F. Carpenter 1889). In 1905, C. Abbott Davis referred to Carpenter's *A. varicosa* occurrence record – probably mistakenly – as from Cunliff Pond (Davis 1905, p. 8). Because no Rhode Island specimen of *A. varicosa* is known to exist, there is a question as to whether the species ever occurred in Rhode Island (Chris Raithel 2013, personal communication). Based on 19th century records from the Blackstone River in both Massachusetts and Rhode Island we believe that a population of *A. varicosa* occurred in the Blackstone River in both states but was eliminated during the industrial revolution.

New York

Summary. *Alasmidonta varicosa* may be disappearing from the upper Susquehanna River basin. Listed in New York as a "high priority species of greatest conservation need" (NY State Wildlife Action Plan 2015, p. 79), it survives in small numbers or has been extirpated from the Unadilla River, Chenango River, Otselic River, Tioughnioga River, Sangerfield River and Oaks Creek. Catatonk Creek is considered to hold the best remaining *A. varicosa* population in the upper Susquehanna basin but numbers appear to be decreasing with little evidence of recruitment. In 1996-1997, Strayer and Fetterman (1999, p. 333) surveyed 67 sites in the upper Susquehanna River basin where mussel communities had been previously surveyed between 1955-1965. They found that average species richness and the range of most mussel species had not changed significantly except the range of *Lampsilis cariosa* had expanded while the range of *A. varicosa* had contracted sharply. There are few records of *A. varicosa* from the Chemung River basin; for example, seven *A. varicosa* were detected during surveys of 54 sites in the Cohocton River. It appears to be gone from the Hackensack-Passaic River basin (Mahwah River), the New York portion of the Housatonic River basin (Webatuck Creek and Tenmile River) and possibly, the Hudson River basin (Shawangunk River) as well. Only small numbers of widely scattered *A.*

varicosa were located in the Delaware River. However the Neversink River, a tributary of the Delaware River, supports two important *A. varicosa* populations. The Neversink, which along much of its length flows through a sparsely populated forested landscape, may be the last hope for a self-sustaining *A. varicosa* population in New York. However, extensive flooding in 2005 and 2006 appears to have impacted the *A. varicosa* populations in the Neversink. Additionally, intense flooding caused by Hurricane Irene in 2011 followed by Tropical Storm Lee a week later may have also caused extensive damage to *A. varicosa* populations in the Neversink River.

In a survey of mussel biologists, the New York respondent reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats. In summary: (1) The East and West Branches of the Delaware River above the reservoirs may be sites to consider for introduction and augmentation of *A. varicosa*. (2) The Neversink River and Catatonk Creek were ranked both as conservation priority streams based on their healthy *A. varicosa* populations and as conservation priorities because of the immediate threat to those populations. (3) In addition to threats such as impaired water quality (high nutrient and sediment loads), altered flow and invasive species, *A. varicosa* may be in danger of genetic extinction through hybridization with the more abundant *Alasmidonta marginata*. Genetic studies are needed to confirm this hypothesis.

1.0 Upper Susquehanna Basin

Alasmidonta varicosa, once widespread in the eastern half of the Susquehanna basin (Strayer and Jirka 1997, p. 46), is now rare and declining. In 1996 and 1997, Strayer and Fetterman surveyed 115 locations within the upper Susquehanna basin including 67 sites previously surveyed between 1955-1965 by Arthur Clarke and Clifford Berg (1959), Willard Harman (1970) and Carol Stein (unpublished notes held at the Ohio State University Museum) (Strayer and Fetterman 1999, p. 330). In their resurvey of historic sites they found that the range of *A. varicosa* had contracted sharply and was limited to Catatonk Creek, the West Branch of the Tioughnioga River and the Otselic River. But even those populations are in jeopardy of disappearing or may be already gone (see below). Within the last ten years small numbers of *A. varicosa* have been found in both the Chenango and Sangerfield Rivers. Strayer and Fetterman suggested that hybridization with *Alasmidonta marginata* might be a major reason for the decline of *A. varicosa* (Strayer and Fetterman 1999, p. 337). Although intergrades between the two species were not observed in in the early to mid-1900s (Ortmann 1919, p. 192; Clarke and Berg 1959, p. 59) in areas where *A. varicosa* and *A. marginata* populations overlapped, intergrades between the two species now appear to be prevalent. Genetic studies are needed to confirm this possibility.

1.1 Susquehanna Main Stem

There are few records of *A. varicosa* in the Susquehanna River: one shell in 2008, two live animals, in 2009 (NY Natural Heritage Program, unpublished data) and one live animal and one shell were found in 2009 (Lord et al. 2010).

1.2 Cripple Creek

There is one record from the 1800s of *A. varicosa* collected from Cripple Creek, Otsego Lake Watershed (OSUM 68235).

1.3 Unadilla River

In 1965 Carol Stein (OSUM 20953, 21934) found 16 *A. varicosa* in the Unadilla River. However, surveys in 1988, 1995, 1996, 1997 (NY Natural Heritage Program, unpublished data) and most recently 2010 (Maricle 2010) and 2012 (Zemken et al. 2012) failed to find one live individual.

1.4 Chenango River

Clarke and Berg (1959) found *A. varicosa* in the Chenango River. One live animal was reported in both 2006 and 2007 and eight live animals in 2009 (NY Natural Heritage Program, unpublished data). Lord and Harman (2010, p. 4) found one live animal in 2010.

1.5 Sangerfield River

The Sangerfield River is a tributary of the Chenango River. One live animal was found in 2008 and three were found in 2009 (NY Natural Heritage Program, unpublished data).

1.6 Tioughnioga River

Both Stein in 1965 (OSUM 21929) and Clarke and Berg in 1959 (CMN 024933) found *A. varicosa* in the West Branch of the Tioughnioga River; Strayer and Fetterman (1999) reported two animals during a 3.35-hour search in 1996. In 1908, C. J. Maury (CMN 023285) collected six *A. varicosa* in the Tioughnioga River, one collection with no data (MCZ 260938), H. H. Smith also collected it, no date (CMNH 61.1988) and van der Schalie in 1955 (UMMZ 247032) but surveys in the mainstem in 1991, 1997 (NY Natural Heritage Program, unpublished data) and 2009 (Lord and Harman 2010) failed to find live animals.

1.7 Otselic River

Karlin and Vander Schalie (1955 MCZ 217046), Karlin and Berg (1965) and Clarke and Berg (1959, p. 29) all found *A. varicosa* in the Otselic River. However, just one live adult was reported in 1991 and in 1996 but no live animals were discovered during surveys in 1997 and only a shell was found in 2009 (NY Natural Heritage Program, unpublished data).

1.8 Catatonk Creek

A. H. Clark, no date (MCZ 214320), E. J. Karlin in 1955 (UMMZ 247030), Clifford Berg in 1955 (MCZ 217024, 217018; UMMZ 197843, 197844), Carke and Berg (1959 p. 29) and Strayer and Fetterman (1999, p. 332) all found *A. varicosa* in Catatonk Creek. Just ten adult animals were located during an extensive 2011 survey by Lord and Pokorny (2012, p. 4). Even though the survey included the excavation and sieving of 105 randomly placed 0.1 m^2 quadrats, no young *A. varicosa* were found. Although considered the best remaining population in the upper Susquehanna drainage, the Catatonk Creek population appears to be small and sparse (Paul Lord, State University of New York-Oneonta, pers. comm. 2015).

1.9 Oaks Creek
Although reported in 1935 (UMMZ 101155), surveys in 1997 and 2010 failed to find *A. varicosa* in Oaks Creek(NY Natural Heritage Program, unpublished data). A collection from Canandaigua Lake outlet (UMMZ 101159) was most likely from Canadarago Lake outlet, Oaks Creek (Strayer and Jirka 1997, p. 46).

2.0 Chemung River Basin

2.1 Chemung River
Two live *A. varicosa* were reported in the Chemung River in 2009 (NY Natural Heritage Program, unpublished data) and one found by Lord and Harman (2010).

2.2 Cohocton and Conisteo Rivers
In 2015, a total of seven *A. varicosa* were found at five of 54 sites sampled in the Cohocton River and tributaries; no *A. varicosa* were discovered in 21 sites sampled in the Conisteo River (Amy Maher, NY Department of Environment Conservation, unpublished data)

3.0 Hackensack-Passaic Basin

3.1 Mahwah River
One relic shell was found in a 1994 survey of the Mahwah River (NY Natural Heritage Program, unpublished data).

4.0 Delaware River Basin
There is a historical record, Marsh Collection (UMMZ 101158) from the Delaware River. Few *A. varicosa* have been found in the Delaware River. In a 2000-2001 survey of 201 continuous kilometers (125 mi), only 24 widely scattered *A. varicosa* were found of over 307,000 total mussels discovered (W. Lellis, USGS Ecosystems Mission Area, unpublished data). All the *A. varicosa* were old animals. Lellis listed siltation, nutrient enrichment, invasive species and altered hydrology as potential threats to mussel populations.

4.1 Neversink River
Perhaps the last hope for a self-sustaining *A. varicosa* population in New York lies in the Neversink River. Gathering water from its headwaters in the Catskill Mountains and tributaries such as the Basha Kill, the Neversink River flows through a relatively undeveloped, sparsely populated landscape of hemlock, spruce and hardwood forests with only two cities in the watershed, Monticello and Port Jervis. However, the construction of the Neversink Reservoir has altered the flow (a decrease in mean annual flow by nearly 6 m^3/s), sedimentation and the thermal regime of the river (Strayer 1999, p. 469; Fitzhugh and Richter 2004, p. 447; Baldigo 2002, p. 3; Baldigo et al. 2003-2004. p. 30). The Neversink River holds by far the largest populations of *A. varicosa* in New York (Strayer and Jirka 1997, p. 46). The Neversink Gorge divides *A. varicosa* into two populations: the upstream population is smaller and sparser than the downstream population but still occupies about two miles of river; the larger downstream population is spatially more extensive (D. Strayer,

Cary Institute of Ecosystem Studies, pers. comm.; Baldigo et al. 2007, p. 4). Surveys in the 1990s show *A. varicosa* populations in the Neversink to be healthy – both stable and reproducing (Strayer and Ralley 1991, p. 23; Strayer and Jirka 1997, p. 46).

Flow refuges help to explain mussel distribution (Strayer 1999, p. 472; Allen and Vaughn 2010, p. 392) but even populations within flow refuges can be disrupted by intense storms and flooding. Extensive flooding from storms in 2005 and 2006 may have seriously impacted mussel populations in the Neversink River (D. Strayer, Cary Institute of Ecosystem Studies, pers. comm.; J. Cole, USGS, Leetown Science Center, pers. comm.). During the 2005 flood three to five inches of rain fell within a 36-hour period and water levels in some areas exceeded 500-year flood elevations (Suro and Firda 2006, p. 21). In post-flood surveys *A. varicosa* populations appeared to have declined sharply (J. Cole, USGS Leetown Science Center, pers. comm.) but survey locations were different from the locations surveyed in the 1990s. Further, in 2011 heavy rain from Hurricane Irene followed a week later by rain from Tropical Storm Lee caused extensive flooding in the Neversink. Additional surveys would help determine whether the floods significantly diminished *A. varicosa* populations and if so, are the populations rebounding to their pre-flood levels.

4.2 Beaver Kill

Four *A. varicosa* have been found in the lower Beaver Kill: two animals in 1990, no animals in 1995 and two animals in 2011 (NY Natural Heritage Program, unpublished data).

5.0 Hudson River Basin

5.1 Hudson River

There is one historical collection of *A. varicosa* from the Hudson River, no data (CMNH 61.11284).

5.1 Rondout – Shawangunk Kill

If still extant, the Shawangunk Kill holds the only known population of *A. varicosa* in the Hudson River basin. In 1985 Strayer found fresh *A. varicosa* shells during the first mussel survey of the stream; surveys in 1986, 1990 and 1992 yielded several live animals and dead shells but no live animals were found in 2001 (Strayer 2001, p. 7). Rapid development near the stream, water diversion for agricultural and municipal use, increased sediment load, and predation by the invasive rusty crayfish threaten the mussel community of this stream (Strayer 2001, pp. 7-8). This small, sparse population appears to be disappearing from the stream or may already be extirpated (D. Strayer, Cary Institute of Ecosystem Studies 2015, pers. comm.).

6.0 Housatonic River Watershed

6.1 Tenmile River and Webatuck Creek

Tenmile River and its tributary Webatuck Creek likely held the last *A. varicosa* populations in the New York portion of the Housatonic River basin. Webatuck Creek water is turbid and high in calcium and nutrients (Strayer 1999, p. 469). During surveys of 32 sites within the Tenmile watershed – including Webatuck Creek – no live *A. varicosa* were found (Strayer 2010, p. 5). In fact, Strayer found no mussel species in the Tenmile River whereas, seven mussel species were observed in Webatuck Creek. However, the dozens of old *A. varicosa* shells found in the lower reaches of Webatuck Creek show that *A. varicosa* was once common in the creek – and likely in the Tenmile River. Strayer (2010, p. 7) suggests that lack of mussels in Tenmile River may be due to past pollution events and that the mussel community in Webatuck Creek shows signs of decline. A small population of *A. varicosa* still lives in a tributary of the Housatonic River in Connecticut (Nedeau 2011, p. 12).

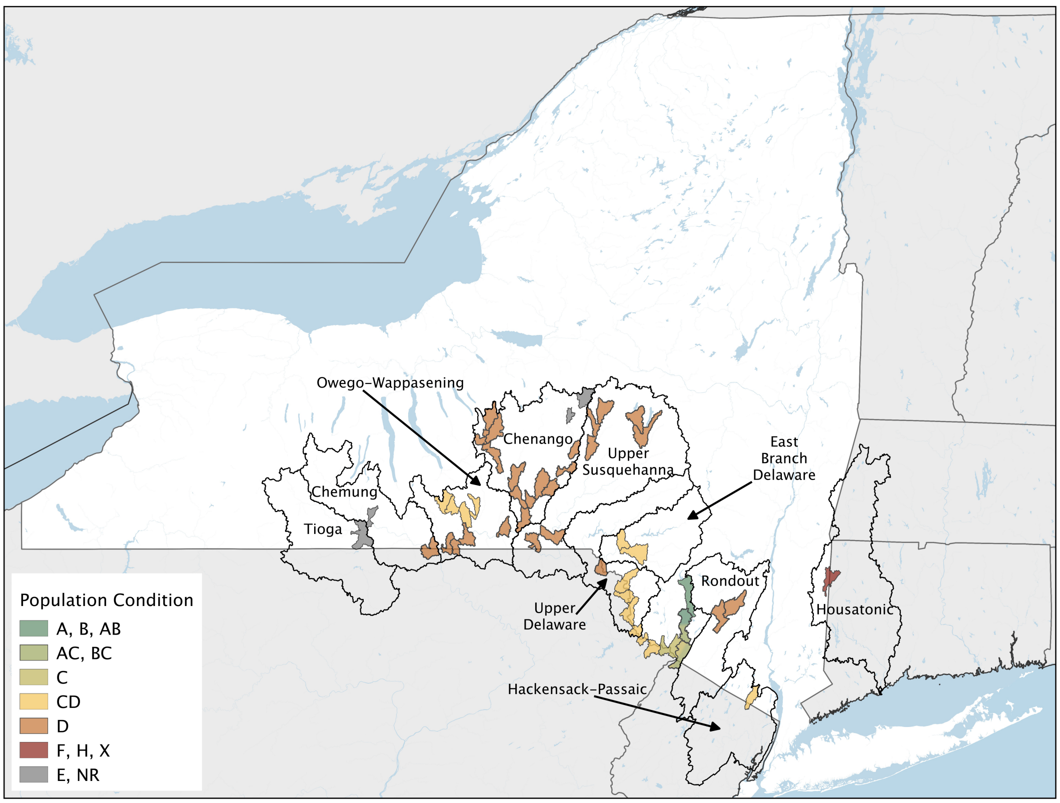


Figure 13. State-level condition map for New York showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

New Jersey

Summary. It is uncertain whether viable *A. varicosa* populations remain in New Jersey. Threats to *A. varicosa* in New Jersey include agricultural, residential, commercial and urban runoff, lack of riparian buffers, sewage and contamination from past pollution events. Few *A. varicosa* have been found during recent surveys and little or no recruitment has been detected (the only young animals found were one in the Lamington River in 2004 and one in the Musconetcong in 2013). They appear to be gone from the Hackensack-Passaic basin where only relict shells have been found in the Mahwah River in New York and the Whippany River in New Jersey. Surveys from 2002-2014 failed to find live *A. varicosa* in the North Branch or the South Branch of the Raritan River; however, a total of two *A. varicosa* were found during 2001 and 2004 surveys in the Lamington River, a tributary of the North Branch. Just seven *A. varicosa* were found in surveys of Stony Brook during the past 23 years; no juveniles or young animals were detected. However two fresh shells were found in 2016. One live *A. varicosa* was found in the Musconetcong River in 2002 and two more were found in 2013 but surveys in 2014 failed to find *A. varicosa*. Although the Paulins Kill harbors a high diversity of mussel species, *A. varicosa* appears to have been extirpated. In 2001, three *A. varicosa* were found in Flat Brook, which flows through a rural forested area of the state and holds a high diversity of mussel species. In a survey of mussel biologists, the New Jersey respondent reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats. In summary: (1) Water quality and habitat have improved enough to consider reintroduction or augmentation of *A. varicosa* in Flat Brook and the upper Pequest River. (2) Flat Brook, the Musconetcong River and the Lamington River were considered conservation priorities because of their extant populations of *A. varicosa*. (3) Stony Brook was named as a conservation priority because of the immediate threat to *A. varicosa*.

1.0 Hackensack-Passaic Basin

The Hackensack-Passaic basin drains a portion of southeastern New York and most of northern New Jersey. Sixty-nine percent of the watershed is urban; agricultural areas are found in the western part of the drainage (Natural Resource Conservation Service). Only relict shells of *A. varicosa* have been found in the Hackensack-Passaic basin: two in the Whippany River in NJ (see below) and one relic shell found in a 1994 survey the Mahwah River in the NY portion of the basin (NY Natural Heritage Program, unpublished data).

1.1 Whippany River

The 32 km (20 mi) Whippany River drains 186 km^2 (72 mi^2) before joining the Rockaway River near its confluence with the Passaic River. The water quality of the Whippany River has greatly improved from what was in the middle of the 20th century, "a dumping ground for sludge from paper mills that lined the river" (Silber 2014). In 1884, a relict shell of *A. varicosa* was collected from the Whippany River (NMNS 451975); another relict shell was found in 2005 (New Jersey Dept. of Environmental Protection Biotics Database 2015).

2.0 Raritan River Basin

The Raritan River basin covers 2,849 km² (1,100 mi²) in north-central New Jersey. The 50 km (31 mi) Raritan River begins at the confluence of the North and South Branches of the Raritan River and flows through a landscape of urban, suburban, industrial and commercial development before emptying into Raritan Bay (Natural Resource Conservation Service New Jersey).

2.1 North Branch Raritan River

The North Branch of the Raritan River drains a rural area of forested and agricultural lands that are facing increased development pressure (Natural Resource Conservation Service New Jersey). J. Wallace collected *A. varicosa* from the North Branch in 1935 (ANSP 166775). A relict shell was found in 1996 and a fresh shell was found in 2002; a survey in 2008 failed to find any shells or live animals (New Jersey Dept. of Environmental Protection Biotics Database 2015).

2.2 South Branch Raritan River

The South Branch flows through an area dominated by agriculture but is experiencing rapid suburban and commercial development (Natural Resource Conservation Service New Jersey). Surveys in 2002, 2004, 2005 failed to find *A. varicosa*, however one fresh shell was found in 2009, 2010 and 2014 surveys failed to find any animals (New Jersey Dept. of Environmental Protection Biotics Database 2015).

2.3 Lamington River

One live *A. varicosa* was found in the Lamington River, a tributary of the North Branch of the Raritan River, in 2001 and another (a young mussel) in 2004; one fresh shell was found in 2009 (New Jersey Dept. of Environmental Protection Biotics Database 2015).

2.4 Stony Brook

Stony Brook is a tributary of the Millstone River, which flows into the Raritan River. Ortmann collected *A. varicosa* from Stony Brook in 1898 (CMNH 61.896), as did Dahlgren in 1910 (CMNH 61.4964). Only seven live *A. varicosa* have been found in Stony Brook during the last 23 years: one fresh dead animal in 1992, one relict shell and one live animal in 1994, one fresh dead animal in 1995, one live animal in 1996, one fresh shell and one live animal in 1998, one relict shell in 2001, one live animal during surveys of two sites in 2002, and one live animal in 2005. No *A. varicosa* were found during surveys of two sites in 2006, three sites in 2007, and three sites in 2008. Two live animals and one shell were found during surveys of two sites in 2011; one shell was found in 2014 and two fresh shells were found in 2016. No juvenile or young animals were detected in any of the surveys. Water quality assessment using the NJ High Gradient Macroinvertebrate Index for Stony Brook showed three sites ranked as fair and one site ranked as poor in 2014 (Stony Brook Millstone Watershed Association 2014).

3.0 Delaware River
Very few *A. varicosa* have been found in the Delaware River recently. For example, in a 2000-2001 survey of 201 continuous km (125 mi) of the Delaware River in NY, only 24 widely scattered *A. varicosa* were found out of over 307,000 total mussels discovered (W. Lellis, USGS Ecosystems Mission Area, unpublished data). All the *A. varicosa* were old animals. Lellis listed siltation, nutrient enrichment, invasive species and altered hydrology as potential threats to mussel populations. In 2011, Walsh (2015) failed to find *A. varicosa* during surveys at 32 sites on the Delaware River in Pennsylvania. In New Jersey, S. N. Rhoads collected *A. varicosa* from the Delaware River in 1893 (ANSP 64197). Two relict shells were also found at two different sites circa 1909. Surveys at three different sites in 2001 yielded one relict shell and two live *A. varicosa* (New Jersey Dept. of Environmental Protection Biotics Database 2015).

3.1 Musconetcong River
The 74 km (46 mi) Musconetcong River, a major tributary of the Delaware River, drains a rural and agricultural region, however an increase in impervious surfaces and poor riparian buffers has impacted the water quality of the river (Musconetcong Watershed Association 2015). One live *A. varicosa* was found in 2002 and in 2007 but only one shell was found in 2008 and another in 2009; two animals, including one young, were located in 2013 but a survey in 2014 failed to find *A. varicosa* (New Jersey Dept. of Environmental Protection Biotics Database 2015).

3.2 Paulins Kill
The Paulins Kill flows through a rural, agricultural area, which may expose it to fertilizer and pesticide runoff. In 1895, H. A. Pilsbry and S. N. Rhoads collected a relict shell of *A. varicosa* in the Paulins Kill (ANSP 68209) and a second relict shell was found in circa 1907; but surveys in 2002 (two sites), 2004 (three sites), 2005, 2007 and 2008 failed to find *A. varicosa* (New Jersey Dept. of Environmental Protection Biotics Database 2015). Although the Paulins Kill harbors a high diversity of mussels, *A. varicosa* appears to be extirpated.

3.3 Flat Brook
Flat Brook drains a rural, wooded area of NJ including state protected land (NJ Division of Fish and Wildlife 2016). In 2001, one *A. varicosa* was found at each of three sites surveyed in Flat Brook – a total of six mussel species were observed in Flat Brook (Cole 2001, unpublished data).

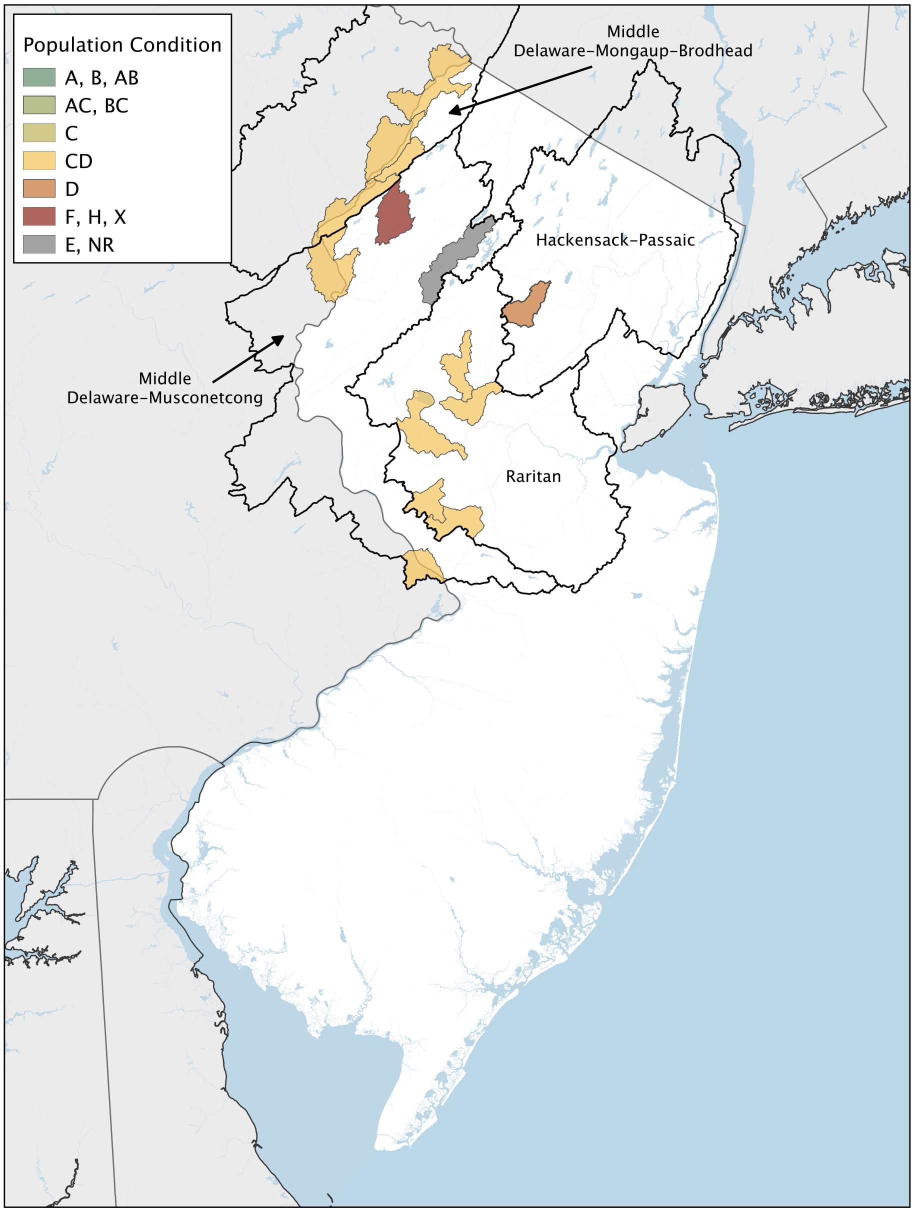


Figure 12. State-level condition map for New Jersey showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

Pennsylvania

Summary. The range of *A. varicosa* in Pennsylvania has contracted. Once widespread in the Susquehanna and Delaware River Basins this species is now considered as one of the least common mussels in the state. Ortmann considered *A. varicosa* "locally common in smaller streams" within the Susquehanna and Delaware River basins (Ortmann 1919, p. 193). However, surveys between 2007 and 2012 failed to find *A. varicosa* in locations where it was documented during the 20th century. Impairment of water quality is a concern in Pennsylvania. Because of pollution, the conservation group American Rivers listed the Susquehanna as among the most endangered American rivers in 2016. Major threats to water quality in Pennsylvania include: increased suspended solids due to land disturbance activities associated with drilling in the Marcellus Shale formation, acid mine drainage, agricultural and urban runoff and siltation (Meyer et al. 2013, p. 21).

The Susquehanna Basin comprises six subbasins: the Chemung (where small numbers of *A. varicosa* have been reported in New York), Upper, mostly in New York (where *A. varicosa* appears to be disappearing), the Middle, West Branch, Lower and Juniata. The only records of *A. varicosa* in the Middle Branch of the Susquehanna are from the 1850s. Approximately 1,939 kilometers (1,205 mi) of streams in the West Branch of the Susquehanna have been impacted by abandoned mine drainage. West Branch populations in Sinnemahoning Creek and Cush Cushion Creek appear to be gone, however surveys from 2000 to 2012 show that Pine Creek and its tributaries Marsh Creek and Little Pine Creek support an important population of *A. varicosa.* This population appears to be the largest in the entire Susquehanna Basin and perhaps the state. Pine Creek is the second largest tributary of the West Branch and is heavily forested; however extensive land disturbances associated with natural gas extraction in the Marcellus Shale formation in the Pine Creek watershed is cause for concern. An insular population of *A. varicosa* is also present in Kettle Creek but additional surveys are needed to determine the size and spatial extent of the population. The Pine Creek and Kettle Creek populations appear to be the only remaining populations in the West Branch.

The Lower Susquehanna is intensely agricultural and the most urbanized subbasin. Lower Susquehanna surveys between 2008 and 2012 failed to find *A. varicosa* at historical locations in Conestoga Creek, Conodoguinet Creek, Stony Creek and Swatara Creek; only two live animals were found in Conewago Creek. During 2010-2011 surveys, live *A. varicosa* were found in three locations in Penns Creek and one location on the northern segment of the Lower Susquehanna main stem. Surveys between 2008 and 2012 failed to find *A. varicosa* where they were historically present in the Juniata subbasin: Juniata River mainstem, Frankstown Branch, Raystown Branch and Aughwick Creek. One live animal was found in Tuscarora Creek. Despite surveys in the 1990s and early 2000s, there have been no records of *A. varicosa* in tributaries of the Potomac River Basin since the early 20th century.

Nineteenth and early 20th century records show that *A. varicosa* was once widespread in the Delaware River Basin (including the lower Schuylkill in Philadelphia, the type locality for the species); however there are no recent records for most locations. Exceptions include Jordan Creek and Lizard Creek, both tributaries of the Lehigh River. Six *A. varicosa* were found in Lizard Creek and larger numbers were found in Jordan Creek between 2008 and 2012. Jordan Creek appears to hold the largest remaining *A. varicosa* population in the Delaware River Basin in Pennsylvania. Maiden Creek, a tributary of the upper Schuylkill, supports a small population of *A. varicosa*.

In a survey of mussel biologists, the Pennsylvania respondent reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats. In summary: (1) There are no streams where water quality and habitat have improved enough to consider reintroduction or augmentation. (2) Pine Creek, Penns Creek and Jordan Creek were ranked as conservation priority streams based on their healthy *A. varicosa* populations. (3) The Delaware River, Pine Creek and Penns Creek were named as conservation priorities because of the immediate threat to *A. varicosa*. Penns Creek needs improvement in the size and extent of riparian buffers and in surrounding land use practices.

1.0 Middle Branch of the Susquehanna

The middle subbasin of the Susquehanna is a coal-mining region that has become highly urbanized (Susquehanna River Basin Commission). *Alasmidonta varicosa* was reported in the Middle Branch of the Susquehanna River in the 1850s (attributed to Charles Wheatley). There have been no records since that time (PA Natural Heritage Program, unpublished occurrence data).

2.0 West Branch of the Susquehanna

The West Branch, the largest tributary of the Susquehanna River, drains an extensively forested landscape that includes 1.7 million acres of public land: 83% of the basin is forested, 10% is agricultural land and 7% is developed or disturbed (Susquehanna River Basin Commission 2008). While 2,011 km (1,249 mi) of the stream waters are classified as exceptional value, abandoned mine drainage (AMD) has polluted 1,939 km (1,205 mi) of stream making it the most AMD-impaired subbasin in the Susquehanna River Basin (Susquehanna River Basin Commission 2008, p. 1). Still, this subbasin holds the largest *A. varicosa* population in Pennsylvania (see Pine Creek below). No recent records of *A. varicosa* have been found in the West Branch outside Kettle Creek and Pine Creek (Walsh and Meyer 2012, p. 20).

2.1 Driftwood Branch Sinnemahoning Creek

The only occurrence records of *A. varicosa* in Sinnemahoning Creek were reported pre-1919 (Rhoads, S.N.) (ANSP 79765) and Ortmann, A.E. 1904 (CMNH 61.897) referenced in Ortmann 1919. Because of AMD pollution, Sinnemahoning Creek is considered one of the most impaired tributaries in the West Branch (Susquehanna River Basin Commission 2008). Surveys in 2011 failed to find any individuals (Walsh and Meyer 2012, p. 20).

2.2 Cush Cushion Creek

The only occurrence record of *A. varicosa* in Cush Cushing Creek is from a collection by Atkinson in 1908 (CMNH 61.3719) (Ortmann 1919). In 1909 Ortmann considered Cush

Cushion Creek a "clear tributary" (i.e. unpolluted by mine drainage) and "important" in that it held the westernmost extent of Atlantic slope fauna in the state (Ortmann, 1909, p. 108). Biologists failed to find *A. varicosa* during two surveys in 1994 (Bogan and Proch 1994, unpublished field data) and one survey in 2011 (Walsh and Meyer 2012, p. 20).

2.3 Kettle Creek

Ninety percent of the Kettle Creek Watershed is forested and 90 percent is also classified as having water of exceptional value; however, acid mine drainage has impacted the lower portion of the watershed making Kettle Creek one the largest contributors of acidity and iron to the West Branch (Susquehanna River Basin Commission 2008). Thus, highly impaired waters of the lower drainage isolate the *A. varicosa* population in Kettle Creek. *Alasmidonta varicosa* was found in Kettle Creek in 1993 (PA Natural Heritage Program, unpublished occurrence data) and again 2011 surveys (Walsh and Meyer 2012, p. 20). More surveys are needed to assess the size and spatial extent of this population.

2.4 Pine Creek

Pine Creek, the second largest tributary of the West Branch of the Susquehanna, harbors a substantial population of *A. varicosa*. The watershed is about 80 percent forested with 10 percent agricultural land (Susquehanna River Basin Commission) and supports six species of mussels (Walsh and Meyer 2012, p. 20). For over a century, Babb Creek, a major tributary of Pine Creek, was heavily polluted by AMD: a 1990 status assessment showed the stream to be "dead and sterile" with no fish and scant macroinvertebrates (West Branch Susquehanna Restoration Coalition 2015). Moreover, a plume of pollution extended into Pine Creek for five miles downstream. However, a series of restoration efforts including diversion wells, treatment systems and reclamation of abandoned mine lands has nearly eliminated AMD discharge and the water quality in Babb Creek has greatly improved (West Branch Susquehanna Restoration Coalition 2015). A new cause of concern is the potential impairment of water quality from disturbances associated with extensive gas development in the Marcellus Shale formation (2013 update, Pine Creek Watershed Rivers Conservation Plan). A catastrophic pollution event could jeopardize the high quality mussel habitat of Pine Creek (Walsh and Myer 2012, p. 22). *Alasmidonta varicosa* survey data show that, in different sites: in 1996, one site yielded a CPUE of 9.33 mussels/hr; in 1997, the average CPUE of five sites was 3.73 mussels/hr; in 2006, the CPUE at one site was 1.3 mussels/hr; in 2007, the average CPUE of 13 sites was 3.16 mussels/hr; and in 2008, the average CPUE of 21 sites was 3.02 mussels/hr (J. Cole, USGS Northern Appalachian Research Laboratory, unpublished data). The range of CPUE in 2007 was 0.56 to 17.81 mussels/hr and in 2008, 0.25 to 11.4 mussels/hr. The Pine Creek *A. varicosa* population extends into Marsh Creek and Little Pine Creek – major tributaries of Pine Creek. In 2008 three *A. varicosa* (CPUE 0.02 mussels/hr) were found in Marsh Creek and two (CPUE 0.02 mussels/hr) were found in Little Pine Creek (J. Cole, USGS Northern Appalachian Research Laboratory, unpublished data). Additional surveys are needed in order to understand the size and spatial extent of the population in these tributaries. Pine Creek appears to hold the largest population of *A. varicosa* in the entire Susquehanna Basin.

3.0 Lower Susquehanna

The Lower Susquehanna subbasin is an area of intense agriculture, major hydroelectric dams and is the most developed of all the subbasins (Susquehanna River Basin Commission). Stone collected *A. varicosa* from the Lower Susquehanna River, no date (ANSP 101663) as did Ortmann in 1910 (CMNH 61.4672, 61.5874, UMMZ 62186). *Alasmidonta varicosa* was found at one mainstem site in the northern portion of the subbasin (CPUE of 0.10 mussels/hr) and in three Penns Creek sites. Penns Creek harbors the richest mussel community of the Lower Susquehanna (Meyer et al. 2013).

3.1 Conestoga Creek

Biologists failed to find *A. varicosa* in 1995 (Bogan 1995) at two sites or during surveys in 2011-2012 at one site (surveyed twice) (Meyer et al. 2013). Mussel diversity in Conestoga Creek has declined to one species from seven species historically (Meyer et al. 2013).

3.2 Muddy Creek

E. W. Roper (no date, MCZ 105673) collected *A. varicosa* from Muddy Creek, a tributary of Conestoga Creek.

3.3 Conewago Creek (West side of the Susquehanna River)

Ortmann collected *A. varicosa* from Conewago Creek in 1910 (CMNH 61.4673) as did Clench and van der Schalie in 1933 (MCZ 142493, UMMZ 58312), Athearn in 1952 (OSUM 24568, CMN 013711) and Franz in 1957 (ANSP 214873). However, only shells were collected in 1994 and 1996 (PA Natural Heritage Program, unpublished occurrence data); one live individual was found in 2008 (J. Cole, USGS Northern Appalachian Research Laboratory, unpublished data). Although a high-diversity mussel community is present in Conewago Creek, it is dominated by *Elliptio complanata*. Surveys in 2013 failed to find any live animals or shells of *A. varicosa* (Meyer et al. 2013, p. 21), however, one live individual was found in 2016 (Walsh 2016, PA Natural Heritage Program, unpublished occurrence data). Nutrients and suspended solids from agricultural runoff have impaired the water quality of Conewago Creek.

3.4 Bermudian Creek

Clench and Vander Schalie collected *A. varicosa* from Bermudian Creek – a tributary of western Conewago Creek – in 1933 (UMMZ 58310). It was also found in 1991 (PA Natural Heritage Program, unpublished occurrence data) and live individuals were found at one of two sites surveyed by Bogan and Proch (1995 unpublished field data).

3.5 Conodoguinet Creek

Ortmann collected *A. varicosa* from Conodoguinet Creek in 1909 and 1910 (CMNH 61.5873, UMMZ 101157); it was also found in 1995 at one location of four surveyed by Bogan (Field Sheets 1995). However, in surveys between 2010-2011 only shells were found (Meyer et al. 2013). Conodoguinet Creek mussel diversity has declined to three species in recent surveys from seven species historically (Meyer et al. 2013, p. 12).

3.6 Swatara Creek
Swatara Creek is moderately impaired as a result of acid mine drainage, agricultural and urban runoff and storm sewers (Meyer et al. 2013, p. 21). Although *A. varicosa* was found in Swatara Creek in 2003, surveys between 2010-2012 in three locations failed to find any animals (Meyer et al. 2013, p. 42).

3.7 Quittapahilla Creek
Quittapahilla Creek is a tributary of Swatara Creek. Designated as an impaired watershed, it carries a heavy load of agricultural and urban pollutants (Quittapahilla Watershed Association). Bogan and Proch (1995, unpublished field data) found one weathered *A. varicosa* shell in 1995.

3.8 Stony Creek
Alasmidonta varicosa was not found during surveys in 2010 (Meyer et al. 2013, p. 15).

3.9 Penns Creek
Alasmidonta varicosa was found in Penns Creek in 1990, 2006 (relic shells) and in 2010-2011 surveys when live individuals were found at three locations with CPUEs of 3.8, 0.6 and 2.0 (Meyer et al. 2013, p. 15). Whereas instream habitat appears to be excellent, water quality, as documented by Meyer et al. (2013, p. 21), shows elevated nitrate and total nitrogen – potential stressors that the authors attributed to agricultural and urban runoff (Meyer et al. 2013, p. 21). Although modest in size, Penn's Creek appears to harbor the largest *A. varicosa* population in the Lower Susquehanna basin.

4.0 Juniata Subbasin
The Juniata River is the second largest tributary of the Susquehanna River. Koenig collected *A. varicosa* in the mainstem of the Juniata River in 1906 (CMNH 61.1823), Nordgren in 1908 (CMNH 61.3718), and one collection from 1940 (MCZ 113836) and 1966 (OSUM 22782) but none were found in surveys at 4 sites on Juniata River in 2008 (Walsh and Meyer 2010, p. 16). Elevated levels of total nitrogen and nitrate from agricultural runoff are widespread within the basin (Campbell 2011). Major pollution sources include acid mine drainage, agricultural, residential and urban runoff, combined sewage outfalls and industrial point sources; the percent of impaired benthic communities has increased from 1995 to 2010 (Campbell 2011, p. 12).

4.1 Raystown Branch
Koenig, 1906 (CMNH), Nordgren, 1908 (CMNH 61.5871) and Ortmann, 1908 (UMMZ 101160) and 1909 (CMNH 61.4265, 61.4266) collected *A. varicosa* from the Raystown Branch; it was also reported in 1966 and 1994 (one fresh shell) (PA Natural Heritage Program, unpublished occurrence data). However, surveys at 10 locations in 2008 failed to find any animals (Walsh and Meyer 2010). Results of water quality sampling on the Raystown Branch show most sites to be non-impaired. Areas of impaired water quality were attributed to agricultural activities and, to a lesser degree, acid mine drainage (Campbell 2011, p. 12).

4.2 Frankstown Branch

Most testing sites on the Frankstown Branch show impaired water quality resulting from acid mine drainage and agricultural and urban runoff (Susquehanna River Basin Commission). During an assessment of 11 sites in the Frankstown Branch in 2010, only one site had a non-impaired benthic community; nitrogen and phosphorus exceeded background levels at most of sites surveyed (Campbell 2011). There is one undated historical record of *A. varicosa* from the Frankstown Branch (listed in Carke 1981) but three surveys in 2008 failed to find any individuals (Walsh and Meyer 2010, p. 20).

4.3 Aughwick Creek

One relict shell was collected in 2002 (PA Natural Heritage Program, unpublished occurrence data). Surveys in 2008 at four sites failed to find any animals (Walsh and Meyer 2010, p. 20).

4.4 Tuscarora Creek

One *A. varicosa* (CPUE 0.72 mussels/hr) was found in Tuscarora Creek in 2008 (J. Cole, USGS Northern Appalachian Research Laboratory, unpublished data).

5.0 Delaware River

Caffery found *A. varicosa* in the Delaware River in 1894 (CMNH 61.6951) and in 1911 (Ortmann 1919). Ortmann collected it in 1908 and 1914 (CMNH 61.3725, 61.7335), Rhoads, prior to 1919 (Ortmann 1919) as well as Thomas in 1961 and 1991 (ANSP A17938). Few *A. varicosa* have been found in the Delaware River recently. For example, in a 2000-2001 survey of 201 continuous kilometers (125 mi) of the Delaware River in NY, only 24 widely scattered *A. varicosa* were found out of over 307,000 total mussels discovered (W. Lellis, USGS Ecosystems Mission Area, unpublished data). All the *A. varicosa* were old animals. Lellis listed siltation, nutrient enrichment, invasive species and altered hydrology as potential threats to mussel populations. Walsh (2015, p. 14-15) failed to find *A. varicosa* during surveys at 32 sites on the Delaware River in Pennsylvania in 2011.

5.1 Frankfort Creek

There are only two reports of *A. varicosa* in Frankfort Creek – one undated historical record collected by Tyrone and the second made in 1997 (PA Natural Heritage Program, unpublished occurrence data); neither record includes any other information.

5.2 Pennypack Creek

There are three historical records of *A. varicosa* in Pennypack Creek: specimens collected by J. Lippincott (no date) (ANSP 179757), B. Long (no date) and Fowler in 1912 (ANSP 105142). Biologist's survey records in 1994 state: "searched for and not found in Pennypack Creek below Bethayres and Lorimer Co. Park, N. of Council Rock. No bivalve shells found. Bethayres area degraded." (PA Natural Heritage Program, unpublished occurrence data).

5.3 Neshaminy Creek
Schick in 1895 (Ortmann 1919) and H. W. Fowl in 1909 (ANSP 130685) found *A. varicosa* in Neshaminy Creek. However, in 1996 Bogan and Proch did not find *A. varicosa* at one site and in 2007 Walsh did not find *A. varicosa* while surveying three sites in Neshaminy Creek nor at one site in Little Neshaminy Creek (PA Natural Heritage Program, unpublished occurrence data).

5.4 Pine Run
Walsh found one weathered *A. varicosa* shell in 2007 but no live animals were found (PA Natural Heritage Program, unpublished occurrence data).

5.5 Ridley Creek
C. H. Conner collected *A. varicosa* from Ridley Creek in 1910 (CMNH 61.4680). R. Hartenstine observed *A. varicosa* in Ridley Creek in the 1970s (R. Hartenstine, Independent Mussel Biologist, pers. comm.). In 1996 Bogan and Proch found one weathered shell but no live animals at one site surveyed. Walsh failed to find *A. varicosa* at two sites surveyed in 2007 (PA Natural Heritage Program, unpublished occurrence data).

5.6 Cobbs Creek
Griffith collected *A. varicosa* (undated historical) from Cobbs Creek, a tributary of Darby Creek (NMNS 86185). There are no recent records.

5.7 Crum Creek
Lea recorded *A. varicosa* in Crum Creek in 1838 (Ortmann 1919). There are no recent records.

5.8 Marshalls Creek
Two *A. varicosa* were found in Marshalls Creek in 2001 (J. Cole, USGS Northern Appalachian Research Laboratory, unpublished data).

5.9 Tohickon Creek
Schick found *A. varicosa* in Tohickon Creek in 1895 (Ortmann 1919, p. 190).

5.10 Munckinipattus Creek
Schick found *A. varicosa* in Munckinipattus Creek in 1895 (Ortmann 1919, p. 190).

5.11 Lehigh River
W. J. Holland collected *A. varicosa* from the Lehigh River (CMNH 61.1987). There is no date or data for this historical record.

5.12 Mahoning Creek
Ortmann collected *A. varicosa* from Mahoning Creek, a tributary of the Lehigh River in 1909 (CMNH 61.4252). One survey in 2003 and two by Walsh in 2014 failed to find any animals (PA Natural Heritage Program, unpublished occurrence data).

5.13 Princess Run (Creek)
Ortmann collected *A. varicosa* from Princess Run, a tributary of the Buckwha Creek, in 1910 (CMNH 61.4671). Buckwha Creek flows into Aquashicola Creek, which then joins the Lehigh River. Surveys in 2007 failed to find *A. varicosa* in Princess Run or Buckwha Creek (J. Cole, USGS Northern Appalachian Research Laboratory, unpublished data).

5.14 Lizard Creek
Six *A. varicosa* were found in Lizard Creek, a tributary of the Lehigh River, in 2008 (J. Cole, USGS Northern Appalachian Research Laboratory, unpublished data).

5.15 Jordan Creek
Jordan Creek joins Little Lehigh Creek just before its confluence with the Lehigh River. In 2011, 38 *A. varicosa* were found (CPUE of 19) in Jordan Creek (Walsh 2015, unpublished data). This is the largest known population in the Delaware River Basin in Pennsylvania.

5.16 Schuylkill River
The Schuylkill River in Philadelphia is the type locality of Lamarck's 1819 species description of *A. varicosa* (Ortmann, 1919). There is also one historic collection from the Schuylkill River with no data (MCZ 150628). Heavy coal mining in Northeastern Pennsylvania has left a legacy of acid mine drainage that greatly impacted area streams including the Little Schuylkill, the West Branch of the Schuylkill and the Schuylkill Mainstem (Sadak 2008). While many sections of the river are now orange from iron hydroxide precipitate due to acid mine drainage, for those growing up near some parts of the Schuylkill the water "was like bad smelling India ink" (J. Feick, Professor Emeritus, Saint Anselm College, pers. comm.).

5.17 Manatawny Creek (Schuylkill Watershed)
H. A. Pilsbry collected *A. varicosa* from Manatawny Creek in 1902 (ANSP 88150), as did J. B. Sessions in 1966 (ANSP 373831) and Sessions and Stanton in 1967 (ANSP 373834). There are no recent records.

5.18 Swamp Creek (Schuylkill Watershed)
The 1912 collection by B. Long (ANSP 106022) is the only record for *A. varicosa* in Swamp Creek.

5.19 Maiden Creek (Schuylkill Watershed)
There is one historic record of *A. varicosa* in Maiden Creek with no data (ANSP 101552). At one site, one fresh *A. varicosa* shell was found in 2014 and, at a second location, one live

animal was found in 2015 (Walsh 2015, PA Natural Heritage Program, unpublished occurrence data).

5.20 Sacony Creek (Schuylkill Watershed)

J. H. Matter, Jr. collected *A. varicosa* in Sacony Creek (a tributary of Maiden Creek) in 1910 (ANSP 101555). Surveys in 2014 at two locations in Sacony Creek failed to find *A. varicosa* or any other mussel species in a stream that historically held six mussel species (Walsh 2015). The lack of mussels was attributed to changes in water quality due to industrial effluents and the increase of impervious surfaces (Walsh 2015, PA Natural Heritage Program, unpublished occurrence data).

5.21 Pickering Creek (Schuylkill Watershed)

C. M. Wheatley (no date) collected *A. varicosa* from Pickering Creek (MCZ 118277); there is also a second historical collection record without date or data from Pickering Creek (ANSP 126762). Surveys in 2007 at two locations in Pickering Creek, failed to find *A. varicosa* (Walsh, PA Natural Heritage Program, unpublished occurrence data).

5.22 Pine Creek (Schuylkill Watershed)

One *A. varicosa* was found during two surveys in Pine Creek (Walsh 2015, PA Natural Heritage Program, unpublished occurrence data).

5.23 Perkiomen Creek (Schuylkill Watershed)

One highly eroded *A. varicosa* was found during surveys in Perkiomen Creek in 2016. (J. Snively, Normandeau Associates, pers. comm.).

5.24 White Clay Creek

Ortmann collected *A. varicosa* from White Clay Creek in 1909 (CMNH 61.4251). There are no recent records: Bogan found no *A. varicosa* during a survey in 1996 and surveys at four sites in 2014 failed to find *A. varicosa* (Walsh 2015, PA Natural Heritage Program, unpublished occurrence data).

5.25 Birch Run

Birch Run is a tributary of Brandywine Creek, which flows directly into the Delaware River. There is one record of *A. varicosa* in Birch Run collected by A. F. M., University of Pennsylvania, in 1861 (ANSP 126770).

6.0 Potomac River Basin

6.1 Conococheague Creek

Ortmann collected *A. varicosa* in Conococheague Creek in 1909 and 1910 (CMNH 61.4264, 61.4674, 61.4675, 61.4676). There is also one 1919 record from the West Branch of Conococheague Creek reported by Ortmann (1919). In a survey of two sites in 1986, Wilkinson, Master, Albright, and Cifelli found only spent shells; then in 1996, Bogan and Proch failed to find *A. varicosa* at seven survey sites in Conococheague Creek and the West

Branch of Conococheague Creek (PA Natural Heritage Program, unpublished occurrence data).

6.2 Tonoloway Creek

Ortmann collected *A. varicosa* in Tonoloway Creek in 1909 (CMNH 61.4263). However, no *A. varicosa* were found during two surveys in Tonoloway Creek in 2004 (PA Natural Heritage Program, unpublished occurrence data).

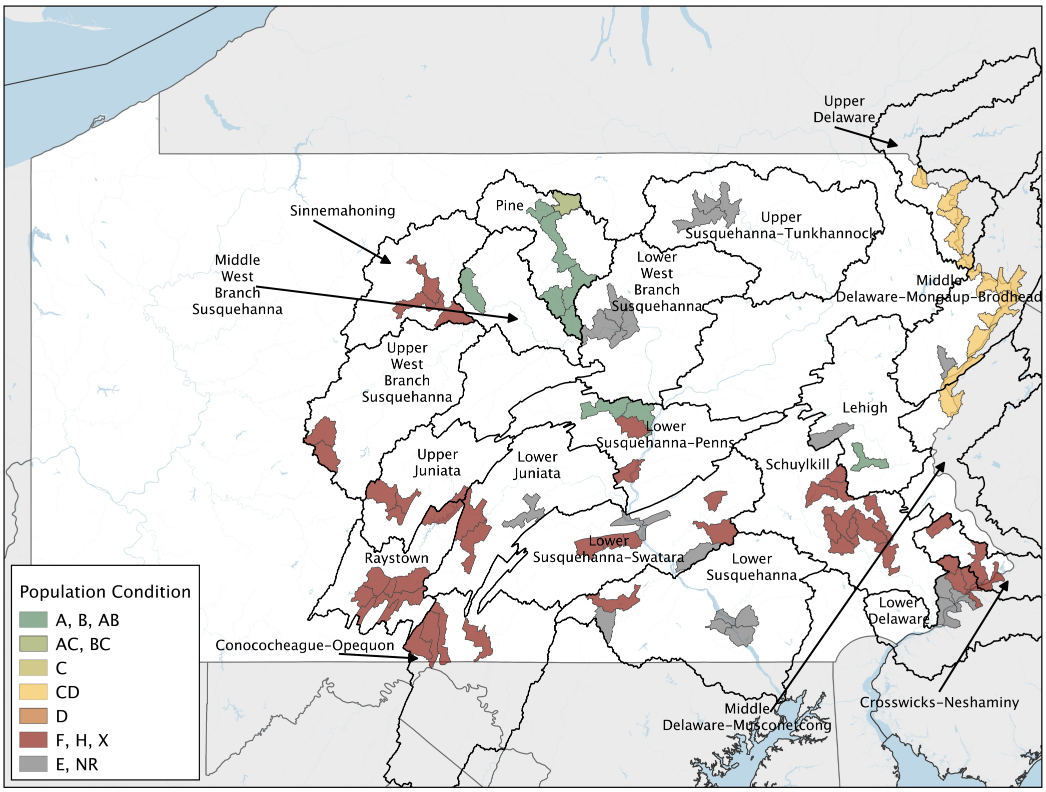


Figure 14. State-level condition map for Pennsylvania showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled

Delaware

Summary. *Alasmidonta varicosa* has been extirpated from Delaware. A collection by S. N. Rhoades from Red Clay Creek in 1903 is the only record of *A. varicosa* in the state. Despite survey efforts it has not been found since that time.

Delaware River Basin

Red Clay Creek

Red Clay Creek watershed is 39% agricultural, 33% forested/wetland and 27% urban/suburban (University of Delaware, Delaware Watersheds). The creek has elevated concentrations of phosphorus, nitrogen and zinc as well as contaminants including volatile organic compounds, semi-volatile organic compounds, pesticides, polychlorinated biphenyls and metals (University of Delaware, Delaware Watersheds 2016). S. N. Rhoades collected *A. varicosa* from Red Clay Creek in 1903 (ANSP 85227). No other occurrences have been reported.

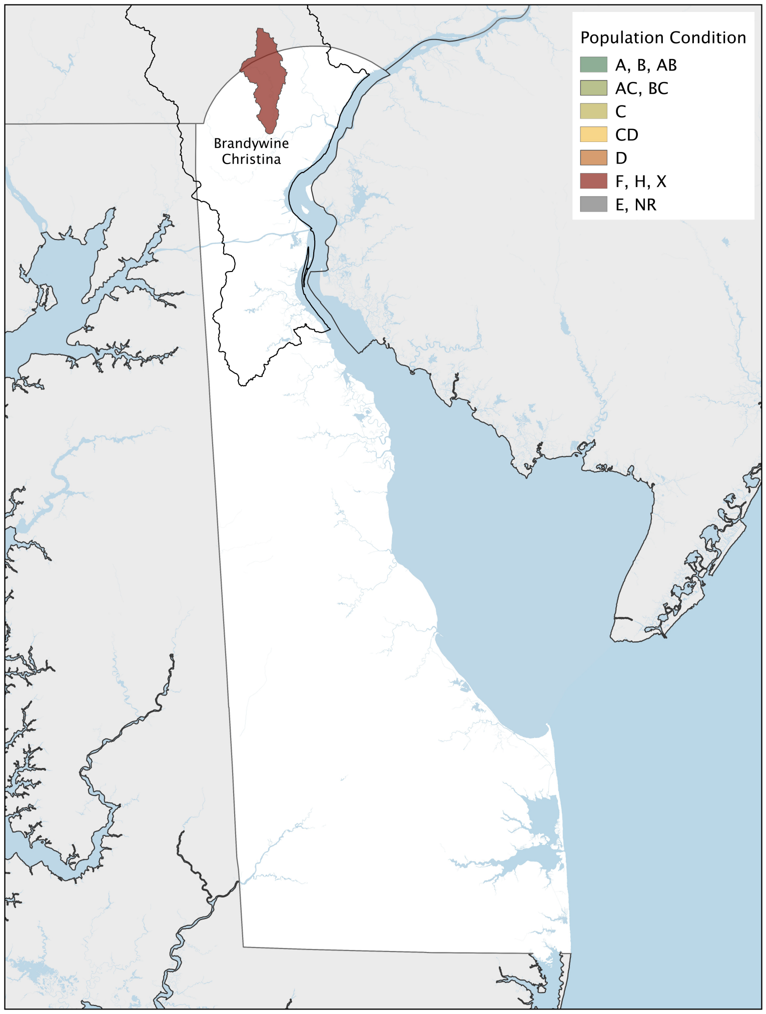


Figure 5. State-level condition map for Delaware showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

Maryland

Summary. It is uncertain whether any viable *A. varicosa* populations remain in Maryland. Deforestation, inadequate riparian buffers, agricultural, suburban and urban runoff (including periodic releases of untreated sewage) are major threats to *A. varicosa* in Maryland. Emerging threats to the basin include rapid population growth, unsustainable sprawl, continued forest loss and the concomitant increase in impervious surfaces. The conservation group American Rivers named the Potomac River as America's most endangered river in 2012. Despite intensive survey efforts, very few *A. varicosa* have been found in streams where they were historically present and little or no recruitment has been detected. In the Potomac River Basin they appear to be gone from Wills

Creek, Town Creek, Sideling Hill Creek, Conococheague Creek (in both Pennsylvania and Maryland), Antietam Creek, Toms Creek, Little Pipe Creek and Linganore Creek. Scattered individuals survive in the Potomac River mainstem and small, sparse populations are found in the Upper Monocacy River and Licking Creek. However, it is possible that a viable population may still persist in the upper Potomac River. Abundance appears to be greatest, although still relatively low, in the upper Potomac especially in the section that flows through eastern Allegany and western Washington Counties in the Ridge and Valley physiographic region. Because water and habitat quality are higher in the upper Potomac, additional surveys may reveal a larger *A. varicosa* population. Both the upper Potomac River and Licking Creek appear to be the best hope for viable *A. varicosa* populations in Maryland. The species is likely extirpated from Gwynns Falls in the Gunpowder-Patapsco River Basin – no unionids of any species were found during surveys of 1996 and 1997. In a survey of mussel biologists, Maryland respondents reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats. In summary: (1) Water quality and habitat have improved enough to consider reintroduction or augmentation of *A. varicosa* in Town Creek and possibly Sideling Hill Creek. (2) Licking Creek and the upper Potomac mainstem were considered conservation priorities because of their extant populations of *A. varicosa*. (3) Licking Creek, the Monocacy River and the upper Potomac mainstem were named as a conservation priorities because of immediate threats to *A. varicosa*.

1.0 Potomac River Basin

1.1 Potomac River

In addition to concerns of impairment from agricultural runoff and inadequate riparian vegetation, the water quality of the 612 km (380 mi) Potomac River is facing emerging threats from rapid population growth, unsustainable sprawl, increased impervious surfaces, loss of forests and, in urban areas, the decline of aging sewer and stormwater infrastructure (Potomac Conservancy 2014). The conservation group American Rivers named the Potomac River as America's most endangered river in 2012 (American Rivers 2012). However, *A. varicosa* persists in the Potomac River. There are historical records of *A. varicosa* in the Potomac River mainstem from the 19th and 20th centuries: Pilsbry and Ives in 1892 (ANSP 64819), Sterki in 1889 (CMNH 61.10695), Ortmann in 1909 (CMNH 61.4262), two undated records from Great Falls (MCZ 142169, 142170), Morrison, no date (MCZ 120543), Calls, no date (MCZ 137733), Bartsch, no date (NMNS 590590), Stein in 1973 (OSUM 34754) and M. Johnson in 2013 found one animal in the Piedmont Plateau region of the Potomac (unpublished data). There are also records from the 1950s, 1960s, and 1980s but surveys in 1994, 2001, and 2007 found very few or no *A. varicosa* – although more animals were found in the upper Potomac River (MD Natural Heritage Program, unpublished data). In a 2013 survey that included excavation of 72 sites within two reaches of the Potomac River, five *A. varicosa* were found among the 247 mussels encountered – all *A. varicosa* were over 60 mm long suggesting older individuals (Cummins 2013, p. 4 and unpublished data). Additionally, surveys show that, although few *A. varicosa* were encountered, they are scattered widely within the mainstem of the Potomac River from the vicinity of Great Falls in the Piedmont to as far upriver as the confluence of Sideling Hill Creek in the Ridge and Valley physiographic region (Cummins, Interstate Commission on the Potomac River Basin unpublished data; MD Natural Heritage Program, unpublished data). The upper Potomac (Ridge and Valley section), where water quality is less impaired, appears to support the greatest abundance although CPUE is overall quite low even here. Additional surveys are needed to better assess the size, distribution and long-term viability of populations in the Potomac.

1.2 Wills Creek

In 1905, Ortmann collected *A. varicosa* from Wills Creek, a 62 km (39mi) tributary of North Branch of the Potomac River (CMNS 61.1612), the lower 11 km of which flows through Maryland. Ortmann (1909, p 108) reported that Wills Creek became polluted by mine drainage at Savage Junction, Maryland. Surveys during 1992-96 failed to find *A. varicosa* and very few mussels of any other species were found (MD Natural Heritage Program, unpublished data). In 2004, Wills Creek was cited for water impairment due to fecal coliform, nutrients, low pH, and cyanide (MD Department of the Environment 2006, p. 36).

1.3 Town Creek

The Town Creek watershed drains both Pennsylvania and Maryland (about 45% of the watershed) where the land use is dominated by forestry and agriculture. Although water quality has improved, Town Creek has a history of water quality impairment due to high nutrient loads, suspended sediments and altered hydrology resulting in a "poor biological community" listing (MD Department of the Environment 2006, p. 25). There are two pre-1960s records for *A. varicosa* in Town Creek (Gerberich 1984): one near the confluence of Maple Run, the other in or near the confluence of Murley Branch. Despite intensive surveys throughout Town Creek during the early-mid 1990's, no *A. varicosa* were found (MD Natural Heritage Program, unpublished data). Because of its improved water quality, Town Creek has been identified in the MD State Wildlife Action Plan as a high priority reintroduction site.

1.4 Sideling Hill Creek

Clarke (1981, p.81) lists an undated historical collection of *A. varicosa* from Sideling Hill Creek (however in a review of ANSP records, no Sideling Hill Creek specimens were found). This stream has been frequently surveyed since the late 1980's and as recently as 2006-7 when surveys were conducted throughout the stream's Maryland portion as part of a statewide status reassessment of *A. varicosa* and *Lasmigona subviridis* (Bartgis and MacIvor 1994; Maryland Natural Heritage Program, unpublished data). While the mussel fauna in Sideling Hill Creek appears to be relatively intact with seven species present, including a regionally significant population of *L. subviridis*, no *A. varicosa* has been found here. However, the species does occur in the Potomac River, immediately downstream from the confluence with Sideling Hill Creek. As many as 14 individuals (in 1994) have been found there (the most ever recorded at a single location in MD) and it has been observed near the confluence as recently as 2007 (Bartgis and MacIvor 1994; Maryland Natural Heritage Program, unpublished data). No *A. varicosa* have been recorded upstream from this point in the Potomac River. Perhaps *A. varicosa* has persisted undetected in low numbers in Sideling Hill Creek near its confluence with the Potomac.

1.5 Licking Creek

Licking Creek is a 91 km (57 mi) river that drains a 48,433 ha (119,680 acre) watershed in Pennsylvania and Maryland (15% in MD); it is 83% forested, 12% agricultural and 5% urban (MD Department of the Environment 2014, pp.2-4). About 43% of stream miles in the drainage have "fish and/or benthic indices of biological impairment in the poor to very poor categories (MD Department of the Environment 2014, p. 7). A small, sparse population of *A. varicosa* was first recorded in Licking Creek in 1993 and it continued to be found in low numbers during the most recent surveys in 2007 (MD Natural Heritage Program, unpublished data).

1.6 Conococheague Creek

Located in Pennsylvania and Maryland, Conococheague Creek drains and area of 1,471 km^2 (568 mi^2) of which 11.6% lies in Maryland; the watershed is 54% agricultural, 30% urban and 16% forested (MD Department of the Environment 2013, pp. 2-4). Conococheague Creek once harbored eight species of mussels (Bartgis and MacIvor 1994, Maryland Natural Heritage Program, unpublished data). Although present in the early 20th century, extensive surveys in 1986 and 1996 failed to find live *A. varicosa* in Conococheague Creek in Pennsylvania (PA Natural Heritage Program, unpublished occurrence data). In 1959, W. F. Grimm and G.F. Grimm collected 12 *A. varicosa* from the Maryland portion of Conococheague Creek (CMN 34754); Morris collected it (CMN 059581); H. D. Athearn also collected it in 1971 (NCSM 5584), as did C. B. Stein in 1973 (OSUM 34496). However, intense and repeated surveys since 1990 failed to find any live animals (MD Natural Heritage Program, unpublished data). Conococheague Creek is listed as having 85% of stream miles impaired with fish and/or benthic indices in the poor to very poor categories (MD Department of the Environment 2013, p. 7).

1.7 Antietam Creek

Antietam Creek drains a total of 751 km^2 (290 mi^2) of which 64% lies in Maryland; the watershed is 45% agricultural, 30% forested and 25% urban (MD Department of the Environment 2012, pp. 2-4). There is a record for *A. varicosa* in Antietam Creek from 1997, based on a single relict shell (MD Natural Heritage Program, unpublished data). Much of the watershed shows biological impairment in the poor and very poor categories resulting from inadequate riparian buffers and urban and agricultural runoff containing high levels of suspended sediments, phosphorus, nitrogen and sulfate (MD Department of the Environment 2012a, pp. iv-v).

1.8 Monocacy River

The 94 km (59 mi) long Monocacy River drains 2,500 km^2 (966 mi^2) of an agricultural, forested and urban landscape in Pennsylvania and Maryland; 77% of the watershed lies in Maryland (MD Department of the Environment 2012b, p. 2). Lee collected *A. varicosa* from the Monocacy River in 1968 (OSUM 23463) and Stein collected it in 1973 (OSUM 34521). A small, sparse population persists in an upper, 10 km section of the river near the MD-PA border. *Alasmidonta varicosa* was first observed there in 1992 and as recently as 2007 during

2006-7 surveys aimed at reassessing the species' status in Maryland. This survey effort failed to find it at previously documented sites in the 1990's and few unionids of any species were found anywhere indicating an overall decline in the mussel fauna (MD Natural Heritage Program, unpublished data). Much of the river is listed as impaired with fish and/or benthic indices in the very poor to poor categories. The middle and upper river sections in Maryland are heavily impacted by agricultural runoff, livestock grazing in riparian areas, inadequate riparian buffers, expanding residential development, and overall lack of forest cover throughout most of the watershed. In particular, water quality and river habitat has been impaired by highly altered natural flow regimes (i.e., very "flashy"), increased channel erosion and high suspended sediment and nutrient levels (esp. nitrogen and phosphorus) (MD Department of the Environment 2012b, pp. iv-v). The lower section, as it approaches and flows through the city of Frederick and eventually joins the Potomac River, is even more degraded.

1.9 Toms Creek

In 1960, when G. B. Morris visited Toms Creek – a tributary of the Monocacy River – the *A. varicosa* population appears to have been significant: at four locations he collected 12, 10, 4, and 10 animals (CMN 057542, 057544, 057550, 057557). However *A. varicosa* was last observed in 1993 and despite extensive survey efforts in 2006-7 no animals were detected (MD Natural Heritage Program, unpublished data). The stream appears to have been impacted by decades of inadequate riparian buffers, agricultural runoff and episodic releases of untreated sewage (MD Natural Heritage Program, unpublished data).

1.10 Little Pipe Creek

Little Pipe Creek joins Big Pipe Creek to form Double Pipe Creek, a tributary of the Monocacy River. The Double Creek watershed is 68% agricultural, 20% forest/herbaceous and 12% urban with much of the stream impaired: 65% of stream miles show fish and/or benthic indices in the poor to very poor category as a result of agricultural and urban runoff (MD Department of the Environment 2012c, pp. 4-7). Although *A. varicosa* was observed in the 1960s, surveys in 2007 failed to find any animals (MD Natural Heritage Program, unpublished data).

1.11 Linganore Creek

Linganore Creek, a tributary of the Monocacy River, has been heavily impacted by agricultural uses. Although observed in 1960, surveys in 2006 failed to find *A. varicosa* and mussels of any species were rarely detected (MD Natural Heritage Program, unpublished data).

2.0 Gunpowder-Patapsco

2.1 Gwynns Falls

W. F. Grimm collected *A. varicosa* in Gwynns Falls in 1955 (CMN 090085). However, during surveys in 1996 and 1997, no unionid species were found (MD Natural Heritage Program, unpublished data).

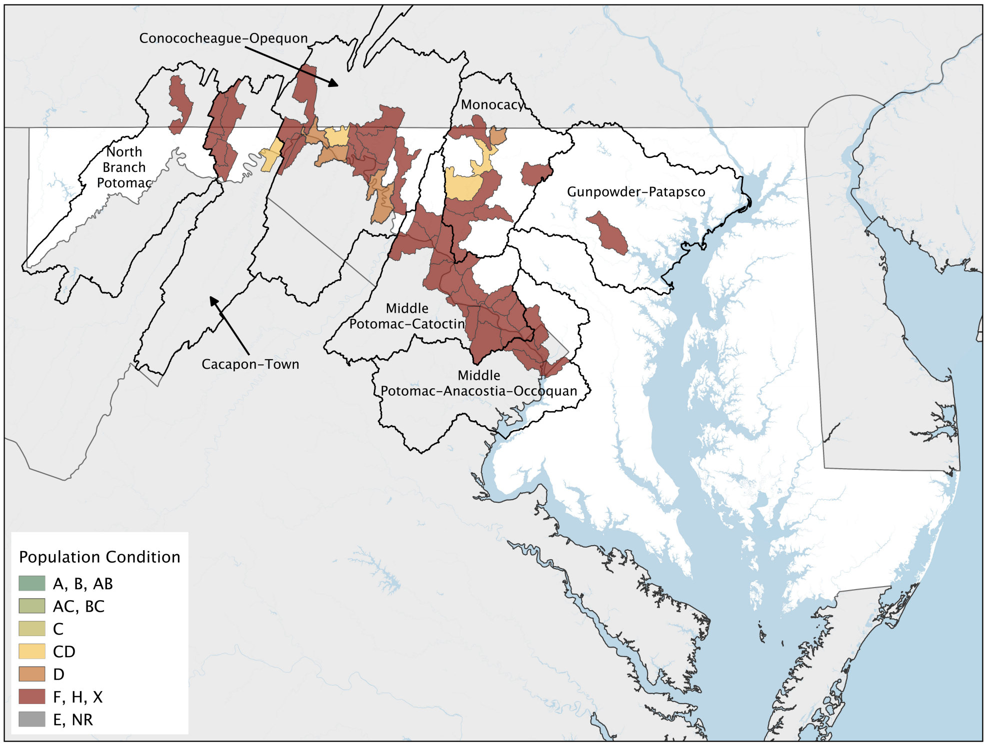


Figure 8. State-level condition map for Maryland showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

Virginia

Summary. It is unlikely that any *A. varicosa* populations remain in Virginia. The last confirmed living *A. varicosa* observed in Virginia was in 1998 (see Broad Run below). In 2006, Campbell reported a possible *A. varicosa* from the South Fork of Quantico Creek but there was no positive confirmation of that record (Campbell 2006, p. 5). *Alasmidonta varicosa* was historically present in Christians Creek, the Middle River, South River, the South Fork of the Shenandoah, Cedar Creek, Smith Creek, the North Fork of the Shenandoah and the Shenandoah mainstem. It now appears to be gone from the entire Shenandoah River basin. There appears to have been a significant die-off of *A. varicosa* in the North Fork of the Shenandoah River just prior to 1990 when dozens of fresh dead *A. varicosa* of all age classes were found. There has been no evidence of recovery. The Shenandoah River and its tributaries once harbored nine species of unionids but water quality has

been severely impacted by agricultural and urban runoff containing nutrients, sediment and toxic chemicals. Mercury released into the South River from the now defunct Waynesboro DuPont facility between 1929 and 1959 has contaminated the South River, South Fork and the mainstem of the Shenandoah River. Only three mussel species are now known to persist in the basin – all found upstream of Waynesboro. It appears that *A. varicosa* is also gone from the Occaquan River and its tributaries Bull Run and Broad Run. Heavy urban development has impacted the water and habitat quality of Broad Run. Despite intensive survey efforts in 2002, 2004, 2005 and 2007, no *A. varicosa* have been detected in Broad Run since 1998. In 1997, during 248 survey hours, Arthur Clarke found nine *A. varicosa* in the James River; the only other known occurrence in this basin is an 1846 record from the Calfpasture River. In 2006 two mussels from the James River, tentatively identified as *A. varicosa,* were proven, through DNA analysis, to be *Alasmidonta undulata*. *Alasmidonta varicosa* is ranked Tier one in Virginia, Critical Conservation Need: "Faces an extremely high risk of extinction or extirpation". Populations of these species are at critically low levels, face immediate threat(s), or occur within an extremely limited range. Intense and immediate management action is needed." (VA State Wildlife Action Plan 2015, p. 2-1).
In a survey of mussel biologists, Virginia respondents reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats. In summary: (1) Virginia respondents reported that water quality and habitat have improved enough to consider reintroduction or augmentation of *A. varicosa* in Smith Creek, Christians Creek and possibly Broad Run. (2) No streams were considered conservation priorities because of their healthy populations of *A. varicosa.*

1.0 Potomac River Basin
In addition to concerns of impairment from agricultural runoff and inadequate riparian vegetation, the water quality of the 612 km (380 mi) Potomac River is facing emerging threats from rapid population growth, unsustainable sprawl, increased impervious surfaces, loss of forests and, in urban areas, the decline of aging sewer and stormwater infrastructure (Potomac Conservancy 2014).

1.1 Occaquan River
The Occaquan River is a 40 km (25 mi) tributary of the Potomac River. A collection made by Bartsch in 1938 (NMNS 530799) is the only record of *A. varicosa* in the Occaquan River.

1.2 Bull Run
Bull Run is a 53 km (33 mi) tributary of the Occaquan River. In 1949 Jeffries collected *A. varicosa* in Bull Run (NMNS 597546). Bull Run was cited in a 2004 Water Quality Assessment as having impaired quality due to exceeding standards for fecal coliform bacteria and polychlorinated biphenyls as well as benthic impairment (Louis Berger Group, Inc. 2006).

1.3 Broad Run
Broad Run is a 61 km (38 mi) tributary of the Occaquan River that is facing intense urban development (VA Wildlife Action Plan 2015, p. 26-24). H. D. Athearn collected *A. varicosa* from Broad Run (MCZ 160549, no date). During surveys in 1991, one *A. varicosa* (which was unable to close its valves) was collected, one shell was collected in 1993 and two live *A.*

varicosa were found in 1998 (VA Department of Game and Inland Fisheries, unpublished data). These two animals were the last live *A. varicosa* observed in Virginia. Roble (1998, p. 7) concluded that there was no evidence of a viable *A. varicosa* population in Broad Run. Despite multiple surveys in 2002, 2004, 2005 and 2007 no live *A. varicosa* have been found in Broad Run (VA Department of Game and Inland Fisheries, unpublished data).

2.0 Shenandoah River Basin

The North Fork and South Fork of the Shenandoah converge to form the Shenandoah River mainstem, which flows through Virginia and West Virginia to empty into the Potomac River. In 2006 the conservation organization American Rivers ranked the Shenandoah River as the fifth most endangered river in the United States (American Rivers 2006). Forty-six percent of Virginia's dairy farms and 75 percent of the states poultry farms are located within the Shenandoah River basin (The Virginia Chesapeake Bay Showcase Watershed: Smith Creek, USDA Natural Resource Conservation Service 2013, p. 1). Agricultural and urban runoff (including nutrients, sediments and toxins), and the release of industrial toxic wastes (including mercury and PCBs) have impaired the water quality of the Shenandoah River (see below). Moreover, since 2004 there have been chronic spring fish kills in the Shenandoah River. Although the fish kills are the focus of intense research, no definitive cause has been identified (VA Department of Game and Inland Fisheries 2014a). Historical records show that G. W. Tryon, no date (ANSP 41054) and George Washington University in 1934 (NMNS 515741) collected *A. varicosa* from the Shenandoah River. Only relict shells were found during surveys of 2008 and 2009 (Chazel 2009, p. 13). It is unlikely that *A. varicosa* still lives in the Shenandoah River.

2.1 North Fork Shenandoah River

Excessive nutrients threaten the water quality of the North Fork of the Shenandoah (VA Department of Game and Inland Fisheries 2014b) and benthic macroinvertebrate bioassessments show that portions of the river have impaired water quality requiring cleanup plans (VA Department of Environmental Quality 2014, p. 1a-10). Nine species of mussels were recorded in the North Fork from the early 20th century to the 1970s but surveys from the 1990s to 2009 show that only three species in low numbers still exist (Garst et al. 2014, p. 2). Historical records of *A. varicosa* in the North Fork of the Shenandoah River include collections by: J. Morrison and J. Rosewater in 1957 (MCZ 216721), Morrison in 1963 (NMNH 791515), W. Clench and D. Stansbury in 1968 (MCZ 266346), E. Surber in 1970 (NMNS 756713), Johnson in 1979 (OSUM 45513), D. Wolfe in 1980 (NCSM 48663) and A. Gerberich in 1983 (NCSM 34912). However a significant die-off appears to have occurred prior to 1990: during 1990 surveys at several locations in the North Fork (including sites where numerous *A. varicosa* were found in 1983), dozens of fresh dead *A. varicosa* including all age classes were found – but no live animals (Master 1990, unpublished data). There has been no evidence of recovery. During multiple surveys in 1992, 1995, 2003, 2004, 2007 and 2008 only relict shells were found (VA Department of Game and Inland Fisheries, unpublished data). It appears that *A. varicosa* has been extirpated from the North Fork of the Shenandoah.

2.2 Smith Creek
Smith Creek, a tributary of the North Fork, has been included on Virginia's list of impaired waters since 1996 for problems that include violating state standards for *E. coli* levels and violating the general standard for aquatic life use (Smith Creek Water Quality Improvement Plan 2009). Despite surveys in 1990, 1991, 1995, 1996, 1999, 2005, 2008 and 2011, relict shells are the only evidence of *A. varicosa* in Smith Creek (VA Department of Game and Inland Fisheries, unpublished data; Chazel 2009, p. 14). It appears that *A. varicosa* has been eliminated from Smith Creek.

2.3 Cedar Creek
Portions of Cedar Creek, a tributary of the North Fork, have been listed as having impaired water quality due to poor benthic macroinvertebrate bioassessment and high *E. coli* levels (VA Department of Environmental Quality 2014, p. 1a-11). In 2003, only one relict shell of *A. varicosa* was found in Cedar Creek. It is likely that *A. varicosa* no longer lives in Cedar Creek (VA Department of Game and Inland Fisheries, unpublished data).

2.4 South Fork Shenandoah River
Historical records show that eight mussel species inhabited the South Fork of the Shenandoah from the early 20th century to the 1970s but surveys from the 1990s to 2009 show that only three species in low numbers still persist in the South River watershed upstream of Waynesboro; no mussel species were detected downstream of Waynesboro in the South River or the South Fork during surveys in 2013 (Garst et al. 2014 pp. 2, 7). W. Clench collected *A. varicosa* from the South Fork in 1934 (MCZ 103869). However only relict shells of *A. varicosa* were found during surveys in 1993 (VA Department of Game and Inland Fisheries, unpublished data) and during surveys of 2008 and 2009 (Chazel 2009, p. 13). Water quality of the South Fork is impaired due to mercury released into its tributary the South River from the Waynesboro DuPont manufacturing facility (see below) (VA Department of Environmental Quality 2009, p. 1) as well as high levels of polychlorinated biphenyls (VA Department of Environmental Quality 2014, p. 1a-9). *Alasmidonta varicosa* appears to have been extirpated from the South Fork of the Shenandoah.

2.5 South River
The South River joins the North River to form the South Fork of the Shenandoah. In 1912 Ortmann collected *A. varicosa* from the South River in Waynesboro (CMNH 61.5934). However, between 1929 and 1950 an estimated 45,360 kg (100,000 lb) of mercury was released from the Waynesboro DuPont manufacturing facility into the water and onto the flood plain of the South River leading to the downstream contamination of the South River, South Fork and the Shenandoah Rivers (VA Department of Environmental Quality 2009, p. 1). Additionally, water quality of the South River has been impaired from many decades of agricultural runoff (Chazel and Roble 2011). Survey records from 2013 show three mussel species present in the South River upstream of Waynesboro but no mussels were found downstream of Waynesboro (Garst et al. 2014 p. 7). Just a shell of *A. varicosa* was found in

the South River in 2003 (VA Department of Game and Inland Fisheries, unpublished data). *Alasmidonta varicosa* appears to be extirpated from the South River. The habitat of the South River upstream of Waynesboro was assessed for the potential reintroduction of nine species of mussels including *A. varicosa* (Garst et al. 2014 p. 11).

2.6 Middle River

During surveys in 2008 and 2009 only relict shells were found in the Middle River, a tributary of the South Fork (Chazel 2009, p. 13).

2.6 Christians Creek

Christians Creek is a tributary of the Middle River of the South Fork Shenandoah. During surveys in 2008 and 2009 no live mussel species were encountered; only relict shells were found including shells of *A. varicosa* (Chazel 2009, p. 14).

3.0 James River Basin

3.1 James River

After more than 150 years after *A. varicosa* was first found in the James River Basin (see below), Arthur Clarke discovered a small, sparse population of *A. varicosa* in the James River in 1997 (Clarke 1997 in Roble 1998, p. 6). Nine specimens were found scattered within a 1 km (0.62 mi) section of the river during 248 survey hours yielding a CPUE of 0.036 mussels/hr (Roble 1998, p. 7). However, during surveys in the James River in 2006 two mussels were tentatively identified as *A. varicosa* due to the presence of corrugations on the posterior slope of the shell, slightly concave ventral shell margins and reduced pseudocardinal teeth (The Catena Group 2007). But genetic analysis at the North Carolina Museum of Natural Sciences showed the tentative *A. varicosa* was actually *Alasmidonta undulata* (The Catena Group 2007, p. 11). In the Northeast, *A. undulata* will sometimes, especially in younger animals, show corrugations on the posterior slope of the shell (B. Wicklow, Saint Anselm College, pers. observation, see section one). Biologists failed to find *A. varicosa* during surveys in the James River in 2010-2011 (A. Chazal et al. 2012). Additional surveys are needed to confirm the presence of *A. varicosa* in the James River.

3.2 Calfpasture River

The Calfpasture River is a 66 km (41 mi) tributary if the James River. In 1846 T. A. Conrad discovered *A. varicosa* (misidentified as *A. marginata*) in the Calfpasture River (Johnson 1970, p. 355).

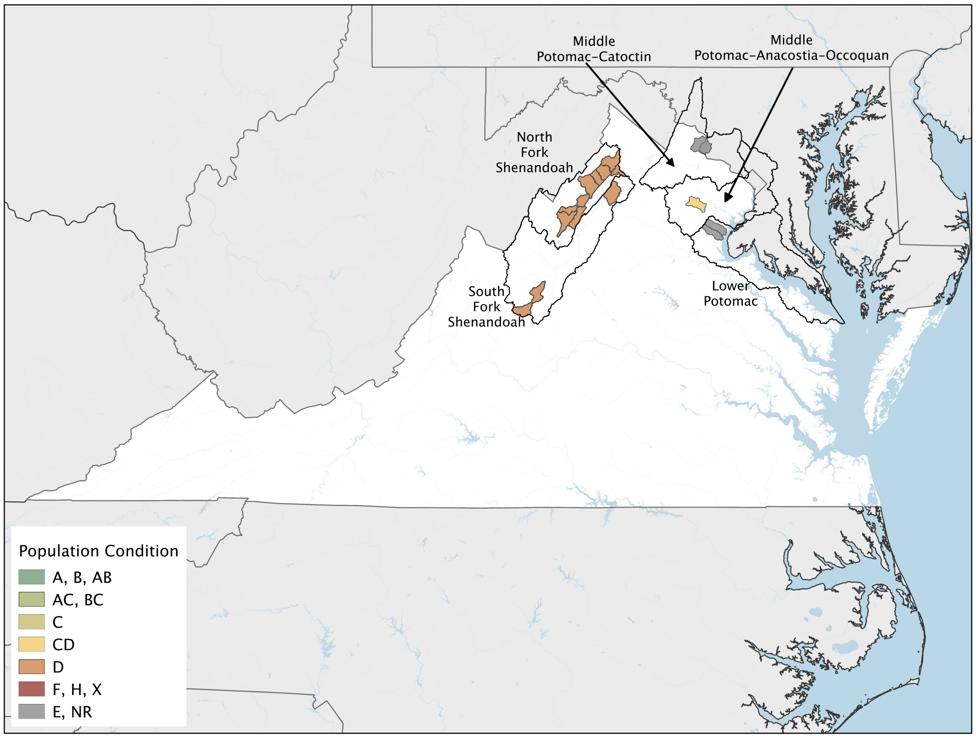


Figure 16. State-level condition map for Virginia showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

West Virginia

Summary. The range of *A. varicosa* in West Virginia has contracted. Once widespread in streams of the eastern panhandle of West Virginia it now appears to be restricted to the Cacapon River and Patterson Creek where important populations remain. Both the Cacapon River and Patterson Creek are located with the ridge and valley physiographic of West Virginia. Multiple stressors including agricultural runoff (nutrients, sediment, and toxins), urban and industrial runoff and sewage have impaired the water quality in streams where *A. varicosa* was once present. Surveys from the late 1990s to 2010 show it is unlikely that populations of *A. varicosa* remain in the South Branch of the Potomac, the Lost River, Back Creek, the Opequon River and the Shenandoah River. The Cacapon River is known for its high diversity of invertebrates and with at least eight mussel species, supporting one of the most diverse mussel communities in the upper Potomac River basin. However, the Cacapon Watershed is also facing increasing threats to water quality from residential development along major streams as well as agricultural expansion. In the upper and middle Cacapon River, a total of 26 *A. varicosa* were found during surveys of 36 sites in 1994, 1995 and1999, however a larger and recruiting *A. varicosa* population that extends for several miles was found during surveys in 2013 in the lower Cacapon River. Patterson Creek also

supports a highly diverse mussel community. A large population of *A. varicosa* was found in Patterson Creek during surveys in 1993-1994. However surveys in 2010 show that the mussel population – while still substantial – was reduced in numbers and the spatial extent of the *A. varicosa* population appears to have become more restricted. Riparian restoration and livestock fencing are needed to help stem agricultural runoff and to prevent livestock from entering the river. The Cacapon and Patterson Creek are within the state designated Cacapon River/Patterson Creek Conservation Focus Area. In a survey of mussel biologists, the Virginia respondent reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats. In summary: (1) Water quality and habitat have improved enough to consider reintroduction or augmentation of *A. varicosa* in Patterson Creek, Cacapon River, Back Creek and possibly Opequon Creek. (2) The Cacapon River and Patterson Creek were considered conservation priorities because of their healthy populations of *A. varicosa*. (3) Patterson Creek was named as a conservation priority because of the immediate threat to *A. varicosa*.

1.0 Upper Potomac River Basin

In the ridge and valley physiographic region of the eastern panhandle of West Virginia, about 36% of stream kilometers are rated as good, 60% rated as fair and 4% rated as poor (WV Integrated Water Quality and Monitoring Assessment Report 2012, p. 25). H. A. Pilsbry and J. E. Ives collected *A. varicosa* from the Potomac River in 1892 (ANSP 64818) and Richmond in 1937 (CMNH 88537), King in 1962 (UMMZ 227361, 227362), C. Stein in 1962-1965 (OSUM 23236, 25652) and Taylor in 1985 (Taylor 1985, p. 86).

1.1 North Branch of the Potomac River

The North Branch has been impacted by abandoned mine drainage for approximately 150 years. In 1985 about half of the 160 km (100 mi) stream length of the North Branch and a total of 1,120 km (700) mi of its tributaries were considered unsuited for aquatic life (Rasin, Interstate Commission on the Potomac River Basin 1985, p. 7). In a 2006 survey no mussels were found (WV Division of Natural Resources, unpublished data). However Patterson Creek, a tributary of the North Branch, harbors a substantial *A. varicosa* population (see below).

1.2 Patterson Creek

A tributary of the North Branch, Patterson Creek harbored seven species of unionids including *A. varicosa* in 1985 (Taylor 1985, p. 86). It is considered one of the most diverse mussel assemblages in the upper Potomac River basin (Clayton et al. 2001, p. 187; Villella and Nelson 2010, p. 20). During surveys between 1993 and 1994 six live species were found (plus one shell of *Lasmigona subviridis*). After *Elliptio complanata*, *A. varicosa* was the second most abundant mussel detected (Clayton et al. 2001, pp. 182-183). A total of 221 *A. varicosa*, including all age classes, were found in 12 of 21 sites surveyed in Patterson Creek – with six sites having ten or more individuals (at one site 128 individuals were found) (Clayton et al. 2001, p. 183). However, Clayton et al. (2001) warned of habitat and water quality threats from agricultural uses. During surveys of eight sites in 2004, a total of 55 *A. varicosa* were located at four sites (43 were found at one site) in Patterson Creek and 12 animals were found at one site surveyed in the North Fork of Patterson Creek (WV Division of Natural Resources, unpublished data). In 2010, 13 sites on Patterson Creek were surveyed including five of the 1993-1994 survey sites where *A. varicosa* numbers were highest (Villella and Nelson 2010, p. 1). Seven species were still present including a fresh shells of *Lasmigona subviridis*, however the mussel community was reduced in numbers. *Alasmidonta varicosa* was found at four of the 13 sites surveyed: of a total of 56 *A. varicosa* detected, 52 animals were located at one site near the location of the highest numbers (128) of *A. varicosa* found in 1993-1994; the remaining four animals observed were spread among three sites (Villella and Nelson 2010, p. 19). The *A. varicosa* population in Patterson Creek appears to have become more spatially restricted in the 17 years since the first surveys. Agricultural runoff and development threaten the water quality of Patterson Creek (WV State Wildlife Action Plan 2015, p.411). Livestock also have access to the stream. Riparian restoration and livestock fences are need to protect the *A. varicosa* population in Patterson Creek (J. Clayton, WV Division of Natural Resources 2016, pers. comm.).

1.3 South Branch of the Potomac River

The South Branch Watershed is 68% forested and 24% agricultural and is one of West Virginia's most productive agricultural areas for cattle and poultry (Blazer et al. 2010, p. 194; USDA Natural Resource Conservation Service, West Virginia Bay Headwaters). Water quality of the South Branch has been impacted by agricultural and industrial runoff. Multiple stressors that impair water quality have led to fish kills beginning in 2002 (Blazer et al. 2010, p. 204). In 1911 Ortmann collected *A. varicosa* from the South Branch (CMNS 61.5386), as did N. Richmond in 1935 (UMMZ 64036). Taylor located it at two sites in the South Branch in 1985 (Taylor 1985, p. 86). However no *A. varicosa* were found during surveys in 1997, 2005 and 2006 (WV Division of Natural Resources, unpublished data).

1.4 Cacapon River

Seventy-nine percent of the 1,230 km^2 (475 mi^2) Lost River/Cacapon River Watershed is forested and 19 percent is agricultural land (Constantz et al. 2005, p. 7). The Cacapon River Watershed is notable for its high diversity of aquatic invertebrates. It is also facing increasing residential development including second home development along major streams as well as agricultural expansion of corn, livestock and poultry production (WV State Wildlife Action Plan 2015, p.411). In 2005, the Cacapon River ecosystem was considered healthy and meeting state water quality standards (Constantz et al. 2005, p. 9). More recently it was cited as having excessive algal growth (WV Integrated Water Quality and Monitoring Assessment Report 2012, p. 17). The Cacapon River holds a diverse mussel community comprising at least eight species including *A. varicosa* (Garst et al. 2014 p. 24). Tubbs and Troutman collected *A. varicosa* in the Cacapon River in 1932 (UMMZ 63936), Chappman collected it, no date (NMNS 466946), Morgan collected it in 1973 (OSUM 34531) and Taylor collected it in 1985 (Taylor 1985, p. 86). In 1994 two *A. varicosa* were found during surveys of ten sites; in 1995 25 *A. varicosa* were found during surveys of 14 sites; but in 1999 no *A. varicosa* were found during surveys of 12 sites (WV Division of Natural Resources, unpublished data). However, a substantial population of *A. varicosa* was located in the lower Cacapon River in 2013 (Garst et al. 2014 p. 24). This population extends for several miles and shows evidence of recruitment (Jess Jones, USFWS 2015, unpublished data). In 2017, 64 *A. varicosa* (CPUE 14/ hr) were found in the lower Cacapon River (J. Mays, USFWS, pers. comm.).

1.5 Lost River

The Lost River is a 50 km (31 mi) headwater stream that is continuous with the Cacapon River. For about 40 km (2.5 mi) the Lost River flows underground as its water percolates through cracks and channels in the limestone streambed only to reemerge as the Cacapon River (Constantz et al. 2005, p. 6). The only record of *A. varicosa* in the Lost River is a collection by J. Morrison in 1939 (NMNH 539013). Surveys in 2003 and 2005 failed to find *A. varicosa* in the Lost River (WV Division of Natural Resources, unpublished data).

1.6 Back Creek

The mussel community of Back Creek includes seven species – making it one of the most diverse unionid streams in the upper Potomac River basin (Villella and Nelson 2008 in Back Creek Watershed Protection Plan 2014). Back Creek is within the state designated Sleepy Creek/Back Creek Conservation Focus Area. N. D. Richmond collected *A. varicosa* from Back Creek in 1937 (CMNH 70780); nine animals were also collected in 1953 (WV Division of Natural Resources, unpublished data). However, there are no recent records of *A. varicosa* in Back Creek despite extensive surveys of twenty sites on the stream in 2008 (WV Division of Natural Resources, unpublished data).

1.7 Opequon Creek

Opequon Creek was cited for biological impairment along its entire length (WV Integrated Water Quality and Monitoring Assessment Report 2012, p. B-74). Opequon Creek is within the state designated Greater Shenandoah Valley Conservation Focus Area. Surveys in Opequon Creek in 1994 (three sites), 2006 (one site) and 2008 (one site) failed to find *A. varicosa* but Villella found a total of three relict shells during a survey of 13 sites in 2009 (WV Division of Natural Resources, unpublished data).

1.8 Shenandoah River

The North Fork and South Fork of the Shenandoah converge to form the Shenandoah River mainstem, which flows through Virginia and West Virginia to empty into the Potomac River. In 2006 a conservation organization ranked the Shenandoah River as the fifth most endangered river in the United States (American Rivers 2006). Agricultural and urban runoff (including nutrients, sediments and toxins), and the release of industrial toxic wastes (including mercury and PCBs) have impaired the water quality of the Shenandoah River. Moreover, since 2004 there have been chronic spring fish kills in the Shenandoah River. Although the fish kills are the focus of intense research, no definitive cause has been identified (VA Department of Game and Inland Fisheries 2014a). Ortmann collected *A. varicosa* from the Shenandoah River in 1911 (CMNS 61.5387) as did Fowler in 1916 (ANSP 115149), Goodrich in 1930 (UMMZ ?), Morrison in 1936 (NMNH 466913) and Richmond in 1937 (UMMZ 70784). In 2010 a single live *A. varicosa* was found in the lower Shenandoah River near its confluence with the Potomac River – no other mussels were found (WV Division of Natural Resources, unpublished data).

Greenbrier River (Ohio River Basin)
A misidentified specimen collected by K. Borror in 1974 was the only record of *A. varicosa* from the Greenbrier River (OSUM 35254) and there are no records of *A. varicosa* from the New River drainage in Virginia or North Carolina (VA Department of Game and Inland Fisheries, unpublished data; NC Wildlife Resources Commission, unpublished data). After reviewing the 1974 specimen, G. Thomas Watters concluded it is likely an *Alasmidonta marginata* not *A. varicosa* (G. Thomas Watters, Curator of Molluscs, The Ohio State University 2016, pers. comm.).

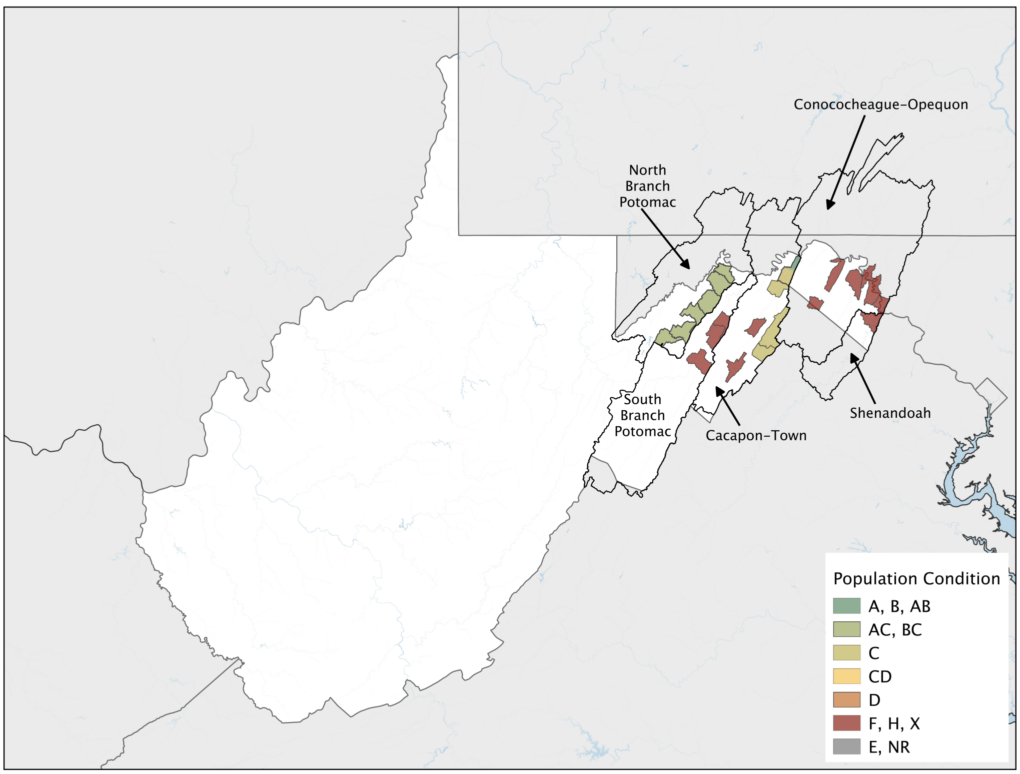


Figure 18. State-level condition map for West Virginia showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

North Carolina

Summary. The distribution of *A. varicosa* in North Carolina is limited to the upper regions of three large river basins: the Catawba, Yadkin-Pee Dee and Cape Fear. There are apparently healthy *A. varicosa* populations in the Catawba and Yadkin-Pee Dee basins but populations appear to be in jeopardy in the Cape Fear basin. Impaired water quality and habitat loss are concerns in North Carolina where population growth is one of the highest in the nation. The Catawba River is highly fragmented by impoundments and hydropower facilities; threats to water quality include sedimentation, agricultural and urban runoff, nutrient loading, loss of riparian forests, sewage and industrial discharges, development and increased impervious surfaces (NC Wildlife Action Plan 2015, p. 123). The conservation group American Rivers listed the Catawba-Wateree River (in North and South Carolina) as among the most endangered rivers in America (American Rivers 2008, 2013). Small numbers of *A. varicosa* persist in the upper Catawba River (only one was found in 2015 and six during surveys in 2016) and it is considered extirpated from the North Fork of the Catawba River where several industries are located and a fish kill occurred in 2015. However, important *A. varicosa* populations remain in the headwaters of the upper Catawba basin, which drains the eastern slopes of the Blue Ridge Mountains in western North Carolina. These include the Linville River, Upper Creek, Mulberry Creek and Wilson Creek.

The Linville River, a state designated Natural and Scenic River, flows through Pisgah National Forest before emptying into Lake James. It supports a small, sparse and insular population of *A. varicosa* that was impacted by extensive flooding from three September hurricanes in 2004. The floods caused changes in channel morphology and the distribution of bed load. Comparisons between pre- and post-flood population surveys showed an 89% decrease in mussels found per person hour. Additional numbers of *A. varicosa* were found in 2015 and 2016 (see below). *Alasmidonta varicosa* occurs in Upper Creek and Wilson Creek. Wilson Creek is a designated National Wild and Scenic River, which originates in the Blue Ridge Mountains and flows through Pisgah National Forest before joining the Johns River. Much of Wilson Creek is classified as Outstanding Resource Waters. Fewer numbers of *A. varicosa* have been located in the Johns River – none were found in 2015 surveys and only one was found in 2016. However, in 2015 and 2016 a dense population of *A. varicosa* was discovered within a 3.2 km (2 mi) reach of Mulberry Creek (a tributary of the Johns River). Due to water quality impairment, *A. varicosa* may no longer live in Long Creek, a tributary of the South Fork of the Catawba River; but the South Fork headwaters, Henry and Jacob Rivers, are under consideration as sites for *A. varicosa* introduction.

The northwest part of the Yadkin-Pee Dee River basin drains the Blue Ridge physiographic region then flows south through the northwest and southern piedmont regions. The Yadkin River joins the Uwharrie River to form the Pee Dee River. Mainstem impoundments and numerous dams on its tributaries have fragmented the Yadkin-Pee Dee River system (due to the large number of dams the Pee Dee River was listed by the conservation group, American Rivers as among the most endangered American rivers in 2016). Other major threats include sedimentation from urban development, agriculture, and instream mining as well as water quality impairment due to nutrient, chemical and waste water effluents from agriculture (including large-scale cattle and hog farms) and industrial sources (NC Wildlife Action plan 2015, p 642). Small populations of *A. varicosa* persist in the Yadkin River but larger populations are present in both the Roaring River and the Mitchell

River, which originate in the Blue Ridge escarpment and empty into the Yadkin River. The Roaring River may hold the largest population of *A. varicosa* in North Carolina. Additional surveys are needed to assess the size and spatial extent of populations in the East and Middle Prong of the Roaring River, the Mitchell River and the Fisher River.

The headwaters of the Uwharrie River originate in the Uwharrie Mountains and flow through Uwharrie National Forest. The pattern of ownership of land along streams in the Uwharrie National Forest is fragmented among individual landowners and the national forest making water quality dependent on each landowner. Threats to water and habitat quality include increased development, agricultural runoff and timber activities. There may be two species of *Alasmidonta* in Uwharrie headwater streams. Genetic sequencing of the *A. varicosa* collected in the Uwharrie headwaters show it represents a clade separate from that of *A. varicosa* sampled from other watersheds (Bogan et al. 2008). This clade may end up being identified as the presumed extinct *A. robusta* or as a separate species of *Alasmidonta*. Moreover, both the newly identified clade of *Alasmidonta* and *A. varicosa* may be present – but both rare – in the Uwharrie headwaters. Bogan et al. plan to submit the genetic research to a refereed journal (A. Bogan 2017, NC Science Museum, pers. comm.). Additional surveys are needed to assess the size and spatial extent of *Alasmidonta* populations in Uwharrie headwater streams: South Fork of Second Creek, Caraway Creek, Toms Creek, Barnes Creek, Rocky Creek, Poison Creek and Reed Creek. Small numbers of *A. varicosa* have been found in tributaries of the Pee Dee River including Dumas Creek, the Little River, the West Fork of the Little River, and Densons Creek. The only record from Brown Creek is a shell collected in 1987.

The Cape Fear River basin, the largest basin within North Carolina, faces multiple threats to water and habitat quality including runoff from agricultural and urban areas as well as forestry and construction practices that have had severe and long-term effects in the basin. The conservation group American Rivers listed the Cape Fear River as among America's most endangered Rivers (American Rivers 2017). The only records of *A. varicosa* in the Cape Fear River basin are from the headwaters region: the Haw River, Deep River and their tributaries. A large portion of the headwaters pass through densely populated highly urbanized areas. The conservation group American Rivers listed the Haw River as among America's most endangered Rivers (American Rivers 2014). One *A. varicosa* was found in Collins Creek (a tributary of the Haw River) in 2002, however the creek is listed as impaired for aquatic life including fish, shellfish and wildlife protection and propagation. New Hope Creek flows into Jordan Lake Reservoir before joining the Haw River. A total of four *A. varicosa* were found in New Hope Creek during 37 hours of search time in 2003, 2004 and 2005. Deep River flows through a rural and agricultural area. The river is fragmented by 13 dams and has been impacted by excessive nutrients from agricultural waste, faulty septic systems, wastewater, and lack of riparian forests. About 29 km (18 mi) of the river are listed as impaired for aquatic life including fish, shellfish and wildlife protection and propagation due to excessive algal growth and mercury and copper contamination. Few *A. varicosa* have been reported from Deep River and its tributaries Richland Creek, Brush Creek and Bear Creek. Despite extensive surveys in 2009 and 2010, no *A. varicosa* have been recovered from the Rocky River since 1990.

In a survey of mussel biologists, North Carolina respondents reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations that face immediate threats. In summary: (1) Water quality and habitat have improved enough to consider reintroduction or augmentation of *A. varicosa* in the upper Catawba River where point source industrial discharge has decreased and nonpoint source pollution has lessened resulting in improved water quality. However, habitat restoration is needed to mitigate for past gravel mining operations. Henry and Jacob Forks, major tributaries of the South Fork of the Catawba River, appear to have recovered enough from pass perturbations to allow introduction of *A. varicosa*. (2) In the Johns River system, Mulberry Creek and Wilson Creek and in the Yadkin River headwaters, the Roaring River and Mitchell River are considered conservation priorities because of their healthy populations of *A. varicosa*. (3) The Warrior Fork (because of increased erosion and siltation), the Yadkin River, Little River, Deep River and Rocky River were named as conservation priorities because of immediate threats to *A. varicosa* (see below).

1.0 Catawba River Basin

The headwaters of the upper Catawba River basin drain the eastern slopes of the Blue Ridge Mountains in western North Carolina. Water quality in the upper Catawba River tributaries is good (although some sections of the Catawba and Johns River are listed as impaired for fish consumption due to mercury contamination) and contrasts with the remaining Catawba-Wateree basin in North and South Carolina which was listed as the most endangered river in America in 2008 and the fifth most endangered river in America in 2013 by the conservation group American Rivers (EPA Water Quality Assessment Report for the Upper Catawba Watershed 2014; American Rivers 2008 and 2013). Impoundments and hydropower facilities have fragmented much of the Catawba River and nearly the entire river from Lake James south is a series of impoundments (NC Wildlife Action Plan 2015, p. 123). Although there are 14 major dams on the Catawba River, its upper tributaries including the Linville River, Johns River and Wilson Creek are free flowing. Still, impacts from mining, logging, agricultural and silvacultural runoff, steep slope development, and septic effluents threaten water quality in the Upper Catawba Watershed (Riverkeeper Foundation 2016).

1.1 Catawba River

Industrial discharge, nonpoint source pollution and gravel mining have impacted the upper Catawba River (S. Fraley, NC Wildlife Resources Commission 2015, pers. comm.) Ortmann collected *A. varicosa* from the upper Catawba River in 1914 (CMNH 61.7132) – prior to the commissioning of the Duke hydroelectric dams that formed Lake James. Additionally, a total of 19 *A. varicosa* were found during surveys of four sites (CPUE 0.75, 2.17, 0.5 and 0 mussels/hr) on the Catawba River in 2011 (NC Wildlife Resources Commission, unpublished data). However, surveys in 2015 failed to find *A. varicosa* in locations they were previously located – just one juvenile was found in a site downstream from previously documented occurrences; six *A. varicosa* were found during surveys in 2016 (CPUE 0.67 and 2.0) (NC Wildlife Resources Commission, unpublished data).

1.2 Linville River

The 48 km (30 mi) Linville River – 21 km (13 mi) of which is designated as a state Natural and Scenic River – begins in the eastern Blue Ridge Mountains and flows through Pisgah National Forest before emptying into Lake James. Sections of the river are listed as having high quality water (North Carolina Division of Water Quality). The lower Linville River is listed as a tier 1, highest priority watershed for freshwater conservation and the upper

Linville River is listed as a tier two, high priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J2). The Linville River supports a small, sparse and isolated population of *A. varicosa* (Fraley and Simmons 2006, p. 2). *Alasmidonta varicosa* were located during surveys of the Linville River in 1987 (NCSM 34915), 1989 (ANSP 377896), 1991, 1993 (CPUE 20.6), 1997 (CPUE 4.7 mussels/hr), and 1998 (CPUE 31.5 mussels/hr) (NC Wildlife Resources Commission, unpublished data). Lenat and Mormon also collected the species in 2002 (NCSM 27207). However in September of 2004, Hurricane Frances (followed closely by Hurricanes Ivan and Jeanne) passed over western North Carolina releasing heavy rains of up to 58 cm (23 in) in some areas and causing extensive flooding and record flows in the Linville River (Goddard Earth Sciences Data and Information Services Center, NASA; Fraley and Simmons 2006, p. 3). During post-hurricane surveys in 2005 a total of 12 *A. varicosa* were found during surveys of five sites (CPUE 1.7, 0, 0, 0 and 1.0 mussels/hr) and in 2011 a total of eight *A. varicosa* were found during surveys of three sites (CPUE 0.5, 0.83 and 0 mussels/hr). At one site where 63 *A. varicosa* were found in 1998 only six were located in 2005 (NC Wildlife Resources Commission, unpublished data). There was evidence of significant changes in channel morphology and the distribution of bed load. The CPUE for *A. varicosa* decreased by 32 mussels per person hour – an 89% change (Fraley and Simmons 2006, pp. iii, 10). During a 2015 survey, five *A. varicosa* (CPUE 5.0 mussels/hr) were found in an upstream reach of the gorge section of the Linville River extending the known range by 8 km (5 mi) (M. Perkins 2015, NC Wildlife Resources Commission, unpublished data). During 2016 surveys, biologists found 32 *A. varicosa* (CPUE 1.5 and 14.5 mussels/hour) in the Linville River (NC Wildlife Resources Commission, unpublished data).

1.3 Warrior Fork

Warrior Fork is threatened by increased erosion and siltation (S. Fraley, NC Wildlife Resources Commission 2015, pers. comm.). Lower Warrior Fork is listed as a tier one, highest priority watershed for freshwater conservation and upper Warrior Fork is listed as a tier two, high priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J2-J3). Four *A. varicosa* were found in the Warrior Fork in 2015 (CPUE 2.0 mussels/hr) and one was found in 2016 (CPUE 1.0 mussels/hr).

1.5 Upper Creek

Although a smaller population than in the Linville River, upper Creek harbors a substantial population of *A. varicosa*. Upper Creek joins the Warrior Fork and flows into the Catawba River downstream of Lake James. *Alasmidonta varicosa* was found during surveys in Upper Creek in 1989 (CPUE 4.3 mussels/hr), 1993 (CPUE 23.75 mussels/hr), 2003 (CPUE 8.7 mussels/hr), 2005 three sites (CPUE 4.8, 1.8 and 0.93 mussels/hr), 2008 two sites (CPUE 0.93 and 0.75 mussels/hr), 2009 two sites (CPUE 4.5 and 0.5 mussels/hr), 2010 two sites (CPUE 12.75 and 4.0 mussels/hr) and three sites in 2016 (CPUE 4.0, 4.0 and 0.5 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

1.6 Johns River

Although sections of the Johns River are listed as impaired due to mercury in fish tissue (EPA Water Quality Assessment Report for the Upper Catawba Watershed 2014), other sections are considered high quality waters (North Carolina Division of Water Quality). The lower and middle segments of the Johns River are listed as a tier 1, highest priority watersheds for freshwater conservation and the upper Johns River is listed as a tier two, high priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J3). A combined total of 100 *A. varicosa* were found during 17 surveys in the Johns River: 1989 (CPUE 2.5 mussels/hr), 1993 (CPUE 7.0 mussels/hr), 1998 two sites (CPUE 3.3 mussels/hr and no data), 2000 two sites (CPUE 1.3 and 1.5 mussels/hr), 2003 two sites (CPUE 0.17 and 2.5 mussels/hr) (NCSM 29409), 2004 (CPUE 0.5 mussels/hr), 2005 two sites (CPUE 0.42 and 1.0 mussels/hr), 2008 (CPUE 2.5 mussels/hr), 2009 (CPUE 2.5 mussels/hr), and 2011 four sites (CPUE 0, 1.8, 0.17 and 0 mussels/hr) (NC Wildlife Resources Commission, unpublished data). Fridell collected *A. varicosa* from the Johns River in 1999 (NCSM 27445). In an assessment of flood damage from 2004 hurricanes Frances, Ivan and Jeanne, two sites that had been surveyed in 2003 were resurveyed in 2005. Results show a total reduction of stream CPUE of -1.7 mussels/hr (Fraley and Simmons 2006, pp. iii, 10). Biologists failed to find *A. varicosa* during 2015 surveys in the Johns River, however one *A. varicosa* was found in 2016 (CPUE 0.5 mussels/hr) (M. Perkins 2016, NC Wildlife Resources Commission, unpublished data).

1.7 Mulberry Creek

Mulberry Creek is a 150 km^2 (58 mi^2) watershed that empties into the Johns River. It is as a tier two, high priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J3). An important population (235 individuals in eight sites surveyed) of *A. varicosa* was discovered in 3.2 km (2 mi) reach of Mulberry Creek in 2015: at six sites where *A. varicosa* was present, CPUEs were 0.67, 4, 19, 48, 24, and 22.5 mussels/hr (M. Perkins 2015, NC Wildlife Resources Commission, unpublished data). During three surveys in 2016, biologists found a total of 194 *A. varicosa* (CPUE 41, 15.33, 0.67 and 8.0 mussels/hr). A mark-recapture study (n = 193) was begun in 2016.

1.8 Wilson Creek

Wilson Creek, a designated National Wild and Scenic River, originates in the Blue Ridge Mountains and flows through Pisgah National Forest before joining the Johns River. Much of Wilson Creek is classified as Outstanding Resource Waters (North Carolina Division of Water Quality). Lower Wilson Creek is listed as a tier one, highest priority watershed for freshwater conservation, and upper Wilson Creek is listed as a tier two, high priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J3). Ten *A. varicosa* were found in Wilson Creek in 2004 two sites (CPUE 2.25 and 0.19 mussels/hr), 14 in 2005 two sites (CPUE 2.0 and 0.33 mussels/hr), and 49 in 2011 two sites (CPUE of 1.8 and 6.7 mussels/hr) (NC Wildlife Resources Commission, unpublished data). In 2015, biologists located 5 *A. varicosa* upstream of previously documented sites in Wilson Creek (M. Perkins 2015, NC Wildlife Resources Commission, unpublished data). During four surveys in 2016, biologists found a total of 162 *A. varicosa* (CPUE 32.89, 18.67, 4.0 and 10.0 mussels/hr). A mark-recapture study (n = 160) began in 2016.

1.9 South Fork of the Catawba River

The South Fork begins at the confluence of Henry Fork and Jacob Fork Rivers. The basin is 47% forested, 30% agricultural and 18% urban (North Carolina Department of Water Quality, Catawba River Basin Plan 2010). Both Henry and Jacob Fork Rivers are listed as priority watersheds for freshwater conservation (NC Wildlife Action Plan 2015, p. J3) and – although *A. varicosa* has not been found in these rivers – the NC Wildlife Resources Commission considers both rivers as possible *A. varicosa* introduction sites (W. Russ, NC Wildlife Resources Commission, pers. comm.).

1.10 Long Creek

Long Creek is a tributary of the South Fork of the Catawba River. There is one undated record of a University of Pennsylvania collection of *A. varicosa* from Long Creek (ANSP 126755). Long Creek's water quality status is listed as impaired for aquatic life (including fish, shellfish and wildlife) for about 24 km (15 mi) (EPA Water Quality Assessment Report for Long Creek 2012) making it less likely that any *A. varicosa* still remain.

2.0 Yadkin-Pee Dee River Basin

The 18,682 km^2 (7,200 mi^2) Yadkin-Pee Dee River basin is the second largest basin in the state with the northwest portion draining the Blue Ridge physiographic region and much of the remainder draining the northwest and southern piedmont regions (NC Wildlife Action plan 2015, p 638). The Yadkin and Uwharrie Rivers join to form the Pee Dee River. The Yadkin-Pee Dee River basin is about 55% forested, 24% agricultural, 6% grassland and 13% developed but will be facing more intense development pressure as the population increases by an expected 36% by 2020 (NC Wildlife Action plan 2015, p 638). Thirty-nine percent of rivers and streams in the Yadkin River basin are listed as impaired by the North Carolina Division of Water Quality (Yadkin-Pee Dee River Basin Priority Watershed Atlas 2010, p.1). Eight impoundments on the mainstem and numerous dams on its tributaries have fragmented the Yadkin-Pee Dee River system; other major threats include sedimentation from urban development, agriculture, and instream mining as well as water quality impairment due to nutrient, chemical and waste water effluents from agriculture (including large-scale cattle and hog farms) and industrial sources (NC Wildlife Action plan 2015, p 642; Carpenter et al. 2002, p. 8). Twenty-three streams in the Yadkin-Pee Dee River basin are listed as conservation priority watersheds (Yadkin-Pee Dee River Basin Priority Watershed Atlas 2010, p.8).

2.1 Yadkin River

Small numbers of *A. varicosa* persist in the Yadkin River and only downstream of the Kerr Scott Reservoir. Biologists located *A. varicosa* during surveys of the Yadkin River in 2000, 2007, 2009 and 2010. In 2000, a total of 15 *A. varicosa* were found during four surveys (CPUE 3.33, 2.0, 15.0 and 4.0 mussels/hr); in 2007, a total of 39 *A. varicosa* were found during five surveys (CPUE 0, 0.3, 0.34, 1.67 and 0.56 mussels/hr); in 2009, two *A. varicosa* were found (CPUE 2.0 mussels/hr); and 10 *A. varicosa* were found during a survey in 2013 (CPUE 1.02 mussels/hr) (NC Wildlife Resources Commission, unpublished data). Upstream of Kerr Scott Reservoir the best populations of native mussels exist in Buffalo and Elk Creeks, although *A. varicosa* have not been found in those streams, the NC Wildlife Resources Commission considers them as a possible *A. varicosa* introduction sites (W. Russ, NC Wildlife Resources Commission, pers. comm.). Biologists surveyed nine sites in the Yadkin River in 2016, yielding 80 *A. varicosa* (CPUE 1.0, 6.33, 2.0, 5.67, o.62, 5.54, 0.2, 2.60, 0.25 mussels/hour) (NC Wildlife Resources Commission, pers. comm.).

2.2 Roaring River

The Roaring River, a tributary of the Yadkin River, holds an important population of *A. varicosa* and is considered a NC Wildlife Action Plan tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J11). Its headwaters – the East, Middle and West Prongs – originate in the Blue Ridge physiographic region. Fifty-one *A. varicosa* were found during a survey in 2009 (CPUE 25.5 mussels/hr) (NCSM 43978) and in 2011, 66 *A. varicosa* were found during two surveys (CPUE 4 and 15.15 mussels/hr). Smaller numbers were found in 2012 (CPUE 2.63 mussels/hr) and 2014 (CPUE 1.25 mussels/hr) but a total of 255 individuals were found at multiple sites in 2016 (CPUE 9.3, 13.33, 11.67, 14.75 and 32 mussels/hr); 96 *A. varicosa* were tagged (NC Wildlife Resources Commission, unpublished data).

2.3 East Prong Roaring River

The East Prong of the Roaring River is listed as a high conservation priority river in the Yadkin-Pee Dee Basin Atlas; it is about 80% forested and 14% agricultural land (Yadkin-Pee Dee Basin Atlas 2010, p. 72). The East Prong River is also listed as tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J11). Few *A. varicosa* have been found in the East Prong of the Roaring River. Four were found during a 2011 survey (CPUE 2.35 mussels/hr), one was found at each of two survey sites in 2014 (CPUE 0.5 and 0.5 mussels/hr) and three were found in two survey sites in 2016 (CPUE 0.85 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

2.4 Middle Prong Roaring River

The Middle Fork of the Roaring River is listed as a high conservation priority river in the Yadkin-Pee Dee Basin Atlas; it is about 75% forested and 18% agricultural land (Yadkin-Pee Dee Basin Atlas 2010, p. 74). The Middle Prong River is also listed as tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J11). Five surveys have been conducted in the Middle Prong of the Roaring River: 2011 when four *A. varicosa* were found (CPUE 0.25 mussels/hr), 2012 when 13 *A. varicosa* were located (CPUE 5.65 mussels/hr) and 2016 when 37 *A. varicosa* were found during three surveys (CPUE 8.0, 0.5, 3.5 mussels/hour) (NC Wildlife Resources Commission, unpublished data). Additional surveys are needed to assess the size and spatial extent of this population.

2.5 Mitchell River
Mitchell River originates in the Blue Ridge escarpment and flows south through the piedmont region to join the Yadkin River. The upper Mitchell River is 93% forested and is listed as a high priority watershed for freshwater conservation in both the NC Wildlife Action Plan and the Yadkin-Pee Dee Basin Atlas; however, sedimentation, erosion and forest loss are cause for concern (Yadkin-Pee Dee Basin Atlas 2010, p. 104; NC Wildlife Action Plan 2015, p. J11). *Alasmidonta varicosa* was collected from the Mitchell River in 1967 and 1969 (OSUM 23218, 23219). Biologists conducted surveys in the Mitchell River from 1997 to 2012: during two surveys in 1997, eight *A. varicosa* were found (CPUE 1.25 and 1.07 mussels/hr); during five surveys in 2004, 77 *A. varicosa* were located (CPUE 0.5, 13.85, 6.0, 2.75 and 3.0 mussels/hr) (NCSM 44130); during four surveys in 2005, 15 *A. varicosa* were found (CPUE 0, 0.57, 0.86 and 0.71 mussels/hr); during a survey of one site in 2006, 16 *A. varicosa* were found (CPUE 4.21 mussels/hr); two *A. varicosa* were found (CPUE 0.65 mussels/hr) during a survey of one site in 2012; and 60 *A. varicosa* were found (CPUE 0.5, 6.0, 12.44, and 6.33 mussels/hr) during a survey of four sites in 2016 (NC Wildlife Resources Commission, unpublished data).

2.6 Fisher River
The Fisher River originates in the Blue Ridge Mountains flowing south to its confluence with the Yadkin River. A portion of the river was listed as impaired for aquatic life due to turbidity (EPA water quality assessment report 2012). There is collection record of *A. varicosa* from the Fisher River in 1970 (OSUM 26155). C. B. King collected one *A. varicosa* from the Fisher River (NCSM 33125) and one *A. varicosa* was found (CPUE 0.5 mussels/hr) during one survey in 2004, however, no *A. varicosa* were found during 25 survey hours in 2016 (NC Wildlife Resources Commission, unpublished data).

2.7 Uwharrie River Watershed
The headwaters of the 100 km (60 mi) Uwharrie River originate in the Uwharrie Mountains and flow through the 20,639 hectare (51,000 acre) Uwharrie National Forest (USDA Forest Service). The Uwharrie River flows southwest to its confluence with the Yadkin River forming the Pee Dee River. The pattern of ownership along the 258 km (160 mi) of streams in the Uwharrie National Forest is fragmented among private landowners and the national forest thus making the quality of the water and habitat of the stream dependent on the landowner (USDA Forest Service). The Uwharrie headwaters are listed as high conservation priority watersheds in the Yadkin-Pee Dee Basin Atlas with 65% of the headwaters forested and 25% agricultural lands; threats to water and habitat quality include increased development, agricultural runoff and timber activities (Yadkin-Pee Dee Basin Atlas 2010, p. 95). There is one undated record of a University of Pennsylvania collection of *A. varicosa* from the Uwharrie River (ANSP 126757).

A second species of *Alasmidonta* may live in the Uwharrie headwaters including Poison Creek, Rocky Creek and Caraway Creek (J. Alderman 2016, pers. comm.). Genetic sequencing shows that specimens initially identified as *A. varicosa* from the upper Uwharrie basin form a clade separate from that of *A. varicosa* from the Savanna, Santee, upper Catawba, Cape Fear and Potomac basins (Bogan et al. 2008). With additional data the Uwharrie *Alasmidonta* species may end up being identified as the presumed extinct *A. robusta* or as a separate species (Bogan et al. 2008; A. Bogan 2016, pers. comm.). Moreover, the upper Uwharrie headwaters may harbor both *A. varicosa* and the separate *Alasmidonta* species – both of which are rare in those headwaters (J. Alderman 2016, pers. comm.). Due to taxonomic uncertainty we refer to the specimens of *Alasmidonta* found in the Uwharrie headwater streams as *Alasmidonta* sp. Bogan et al. plan to submit the genetic research to a refereed journal (A. Bogan 2017, NC Science Museum, pers. comm.).

2.8 South Fork Second Creek (Uwharrie River Watershed)
Eight *Alasmidonta* sp. were found during a survey of the South Fork Second Creek in 2002 (CPUE 2.29 mussels/hr); none were located during a survey at a different site in 2004 (NC Wildlife Resources Commission, unpublished data).

2.9 Caraway Creek (Uwharrie River Watershed)
During a survey in 1993 one *Alasmidonta* sp. was found (CPUE 0.9 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

2.10 Toms Creek (Uwharrie River Watershed)
One *Alasmidonta* sp. shell was found in Toms Creek in 2000. Biologists found a total of six live and one shell of *Alasmidonta* sp. during surveys of two sites in 2002 (CPUE 0.48 and 1.25 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

2.11 Barnes Creek (Uwharrie River Watershed)
Barnes Creek is listed as a high conservation priority river in the Yadkin-Pee Dee Basin Atlas; it is about 82% forested and 8% agricultural land but is considered at risk of development (Yadkin-Pee Dee Basin Atlas 2010, p. 66). Barnes Creek is listed as tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J12). First recorded during a survey in 1993, *Alasmidonta* sp. has been found in small numbers in Barnes Creek through 2010: 1993 (CPUE 1.0 mussels/hr), 2000 (CPUE 1.1 mussels/hr), 2002 (CPUE 1.5 mussels/hr), 2004 (CPUE 0.17 mussels/hr) (NCSM 29538), 2006 (CPUE 0.25 mussels/hr), 2007 (CPUE 0 mussels/hr), 2008 (CPUE 1.74 mussels/hr), 2010, two sites (CPUE 15 and 1.43 mussels/hr) (NC Wildlife Resources Commission, unpublished data). In 2017, J. Mays found four *A. varicosa* during one hour of searching in Barnes Creek; he noted that the creek has potential to hold a larger population of *A. varicosa* relative to other creeks in the Uwharrie River watershed (J. Mays, USFWS, pers. comm.).

2.12 Poison Fork (Uwharrie River Watershed)
One *Alasmidonta* sp. shell was found in Poison Creek, a tributary of Barnes Creek, in 2002 (NC Wildlife Resources Commission, unpublished data).

2.13 Rocky Creek (Uwharrie River Watershed)

Rocky Creek is listed as a high conservation priority river in the Yadkin-Pee Dee Basin Atlas. It is about 75.5% forested and 6.3% agricultural land but of the 23 high conservation priority rivers listed in the Yadkin-Pee Dee Basin Atlas, it is considered the most at risk of development (Yadkin-Pee Dee Basin Atlas 2010, p. 68). It is also listed as tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J12). One *Alasmidonta* sp. was found in Rocky Creek in 1993 (CPUE 0.5 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

2.14 Reed Creek (Uwharrie River Watershed)

In Reed Creek, a tributary of Rocky Creek, two *Alasmidonta* sp. were found in 2002 (CPUE 0.22 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

2.15 Big Bear Creek

Big Bear Creek flows into the Rocky River that empties into the Yadkin River. In 1987, Keferl collected *A. varicosa* from Big Bear Creek (NCSM 33170).

2.16 Dumas Creek (Pee Dee River Watershed)

Dumas Creek is a tributary of Clarks Creek that flows into the Pee Dee River. Clarks Creek is listed as tier two, high priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J12). Six *A. varicosa* were found in Dumas Creek during surveys of two sites in 2002 (CPUE 0.64 and 0.08 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

2.17 Little River (Pee Dee River Watershed)

The Little River flows directly into the Pee Dee River. The Little River is listed as a high conservation priority river in the Yadkin-Pee Dee Basin Atlas; it is about 64% forested and 15% agricultural land with most development concentrated in its headwater region (Yadkin-Pee Dee Basin Atlas 2010, p. 86). It is also listed as tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J12). As part of a river restoration project in the Little River basin (which harbors three endangered unionids including *A. varicosa*), the removal of four dams near the confluence of the Densons Creek and the Little River will reconnect 58 km (36 mi) of the Little River and 16 km (10 mi) of Densons Creek as well as 232 km (144 mi) of tributaries – three of the dams were removed in 2012 and 2013 and one is in process (USFWS 2013). The *A. varicosa* population in the Little River appears small and sparse. Dawley collected *A. varicosa* from the Little River in 1962 (NCSM 410, 33126). Survey results from 1999 to 2010 show: in 1999 five shells were found, in 2000 two live animals (CPUE 2.0 mussels/hr), 2003 one live animal (CPUE 0.67 mussels/hr), 2004 a total of four live animals at two sites (CPUE 0.2 and 0.25 mussels/hr) (NCSM 29435, 29536, 29537 mussels/hr), 2008 two live animals (CPUE 1.0 mussels/hr) and in 2010 a total of 13 live animals at three sites (CPUE 0.08, 0.33 and 0.49 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

2.18 West Fork Little River (Pee Dee River Watershed)
The West Fork of the Little River is listed as a high conservation priority river in the Yadkin-Pee Dee Basin Atlas; it is about 69% forested and 17% agricultural land but remains unprotected from development and large-scale livestock operations (Yadkin-Pee Dee Basin Atlas 2010, p. 76). It is also listed as tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J12). Two *A. varicosa* shells were found in the West Fork of the Little River in 1993 and one live *A. varicosa* was found during each of surveys in 1997 (CPUE 0.67 mussels/hr) and 2005 (CPUE 0.17 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

2.19 Densons Creek (Little River Watershed)
A tributary of the Little River, Densons Creek is listed as a high conservation priority river in the Yadkin-Pee Dee Basin Atlas; it is about 73% forested and 9% agricultural land (Yadkin-Pee Dee Basin Atlas 2010, p. 80). It is also listed as tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J12). Survey results for *A. varicosa* in Densons Creek show that: in 1987 one shell was found (NCSM 33169), in 1991 three animals were found (CPUE 1.0), in 1995 two animals were found (CPUE 1.25 mussels/hr), in 1997 no animals were found and in 2000 three animals were found (CPUE 1.2 mussels/hr) (NC Wildlife Resources Commission, unpublished data). In a 2017 survey of two sites, J. Mays found a total of nine *A. varicosa* (CPUE 2 and 3 mussels/hr) (J. Mays, USFWS, pers. comm.).

2.20 South Deep Creek
South Deep Creek flows into Deep Creek that empties into the Yadkin River. Fraley et al. collected *A. varicosa* from South Deep River in 2004 (NCSM 29991).

2.21 Brown Creek (Pee Dee River Watershed)
Brown Creek is a tributary of the Pee Dee River. The only record for *A. varicosa* in Brown Creek is a shell found in 1987 (NC Wildlife Resources Commission, unpublished data).

3.0 Cape Fear River Basin
The Cape Fear River basin is the largest river basin within the state with 10,277 km (6,386 mi) of streams draining an area of 23,696 km^2 (9,149 mi^2) (Cape Fear Basin Water Quality Plan 2005, p. xxii). There are three regions of the Cape Fear River basin: headwater, middle and lower. The cumulative effects of multiple stressors including runoff from agricultural and urban areas as well as forestry and construction practices have had severe and long-term effects in the basin (NC Wildlife Action plan 2015, p 519). A large percentage of headwaters flow through densely populated highly urbanized areas that negatively impact stream water and habitat quality (NC Wildlife Action Plan 2015, p. 515). At 2005 rates, an additional 405,000 hectares (1,000,000 acres) will be developed by 2020 (Cape Fear Basin Water Quality Plan 2005, p. xxii). The conservation group American Rivers listed the Cape Fear River as among America's most endangered Rivers (American Rivers 2017). The only reports of A. varicosa in the Cape Fear basin are from the headwaters region including the Haw River, Deep River and their tributaries (NC Wildlife Resources Commission, unpublished data).

3.1 Deep River

The Deep River subbasin is mostly rural and agricultural. The water quality of the 200 km (125 mi) Deep River has been impacted by excessive nutrients from agricultural waste, faulty septic systems, waste water, and lack of riparian forests; additionally, 13 small dams have fragmented much of the river (Upper Cape Fear River Basin Conservation and Restoration Analysis and Strategy 2012, p. 42). About 29 km (18 mi) of the river are listed as impaired for aquatic life including fish, shellfish and wildlife protection and propagation due to excessive algal growth and mercury and copper contamination (EPA Water Quality Assessment Report 2012). Few *A. varicosa* have been found in the Deep River. Only a combined total of nine *A. varicosa* were found in the Deep River during surveys from 1993 to 2011. During 1993 and 1997 surveys only shells were found and just one animal was found in 1997. The average CPUE during four timed searches in 2005 was 0.47 mussels/hr and during 12 hours search time in 2011 just one *A. varicosa* was found (CPUE 0.08 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

3.2 Richland Creek (Deep River)

The only record of *A. varicosa* in Richland Creek (a tributary of the Deep River) is one animal found in 2002 (NC Wildlife Resources Commission, unpublished data). Lower Richland Creek is listed as tier one, highest priority watershed for freshwater conservation and upper Richland Creek is listed as tier two, high priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J12).

3.3 Brush Creek (Deep River)

The large area of impervious surface – such as roads, residential development and the Piedmont Triad International Airport – within the Brush Creek watershed has impacted water and habitat quality; additional stressors include storm sewers, altered hydrology, lack of riparian forest, erosion and sedimentation (Cape Fear Basin Water Quality Plan 2005, p. 21). Brush Creek is listed as tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J1). Eleven *A. varicosa* were found during two surveys in Brush Creek in 2002 and one animal was located in 2003 (NCSM 28110) (no search times available); a total of 12 *A. varicosa* were found during three surveys in 2010 (CPUE 0.31, 0.31 and 0.75 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

3.4 Rocky River (Deep River Tributary)

The 60 km (37 mi) Rocky River is a major tributary of the Deep River. Rocky River is listed as tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J12). Henry van der Schalie collected nine *A. varicosa* from the Rocky River in 1932 (UMMZ). L. Hubricht also collected it in 1951(UMMZ), as did R. Shelly in 1972 (NCSM 33167) (Shelly 1987, p. 71) and Biggins in 1984 (ANSP 364900). Biologists failed to find *A. varicosa* during surveys in 1991, 1995, 2005, 2009 and 2010 (NC Wildlife Resources Commission, unpublished data; Alderman and Alderman 2010, p. 8). The last live *A. varicosa* found in the Rocky River was in 1990. In 2000, one shell was found in the Rocky River tributary, Richardson Creek (NCSM 45060). Of the 16 species of unionids historically present in the Rocky River, only nine species were located during extensive surveys in 2009 and 2010 (Alderman and Alderman 2010, p. 8).

3.5 Bear Creek (Rocky River)

The headwaters of Bear Creek are listed as a tier two, high priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J12). R. M. Shelly collected *A. varicosa* from Bear Creek in 1971 (NCSM 33166, 33168, 36370, OSUM 33736) (Shelly 1987, p. 71). One *A. varicosa* shell was found in 1990, one live *A. varicosa* was found during a survey in 1992 and another was found during a survey in 1998 (NC Wildlife Resources Commission, unpublished data).

3.6 Haw River

The 177 km (110 mi) Haw River drains the northern-central piedmont region of the Cape Fear basin. In 2014 the conservation group American Rivers listed the Haw River as one of the most endangered rivers in America: the Haw River faces multiple threats including urban runoff from expanding impervious surfaces and raw sewage spills due to overwhelmed sewage treatment infrastructure (American Rivers 2014). The river is considered as having nutrient sensitive waters (NC Wildlife Action Plan 2015, p. 516). In 1972, R. Shelly and C. Liebrandt found *A. varicosa* in the Haw River (NCSM 30140; OSUM 33693).

3.7 Collins Creek (Haw River)

About 11 km (7 mi) of the Collins Creek are listed as impaired for aquatic life including fish, shellfish and wildlife protection and propagation due to impaired biota – cause unknown (EPA Water Quality Assessment Report 2014). One *A. varicosa* was found in Collins Creek in 2002 (NC Wildlife Resources Commission, unpublished data).

3.8 New Hope Creek (Haw River)

New Hope Creek flows into Jordan Lake Reservoir before joining the Haw River. The headwaters of New Hope Creek are listed as tier one, highest priority watershed for freshwater conservation (NC Wildlife Action Plan 2015, p. J1). However, about 13 km (8 mi) of the New Hope Creek are listed as impaired for aquatic life including fish, shellfish and wildlife protection and propagation due to impaired biota (EPA Water Quality Assessment Report 2014). A combined total of four *A. varicosa* was found during 37 hours of surveys in 2003 (CPUE 0.07 mussels/hr), 2004 (CPUE 0.14 mussels/hr) (NCSM 30074) and 2005 (CPUE 0.02 mussels/hr) (NC Wildlife Resources Commission, unpublished data).

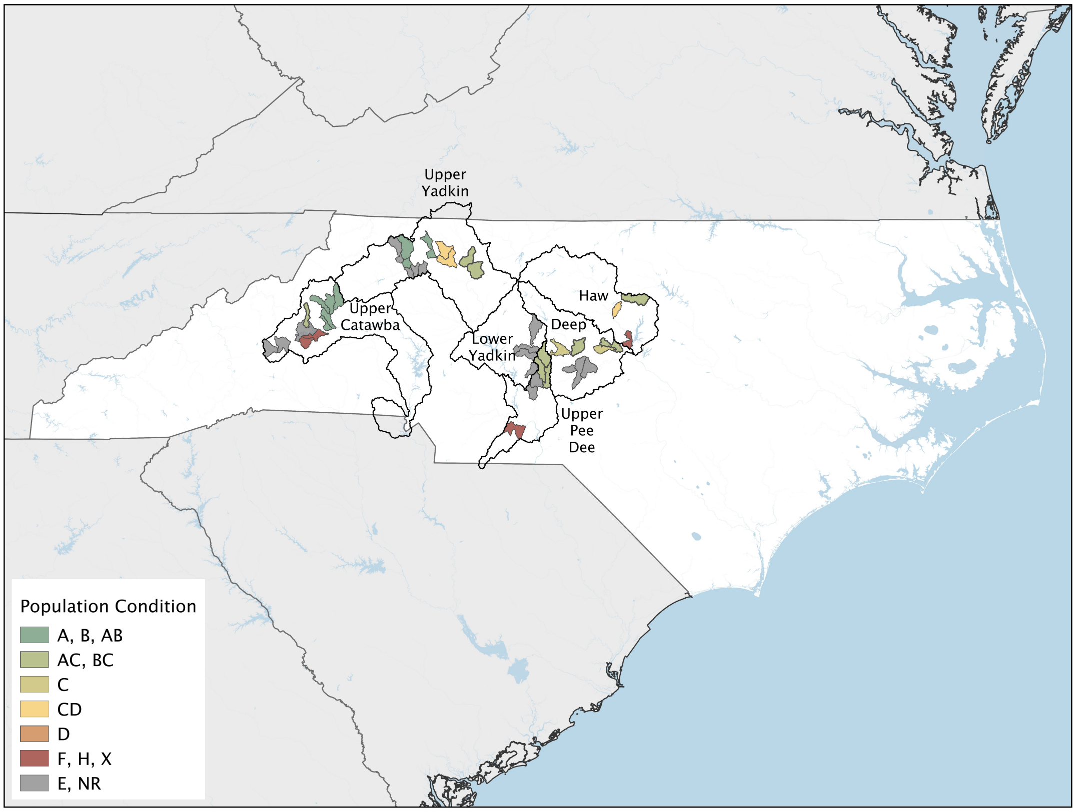


Figure 10. State-level condition map for North Carolina showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

South Carolina and Georgia

Summary.

The distribution *A. varicosa* in South Carolina is limited to the upper Lynches River subbasin of the Pee Dee River basin, the Stevens Creek subbasin of the Savannah River basin and the Chattooga River, a major tributary of the Tugaloo River in the Savannah River basin. In 2009, the conservation group American Rivers listed the Saluda River (Santee River basin) as among the most endangered rivers in America and, due to the large number of dams, the Pee Dee River was listed as among the most endangered American rivers in 2016. Although *A. varicosa* was once present in the Saluda River its occurrence is considered historic. Since the 1970s, a rapid increase in human population growth accompanied by a rapid economic expansion in South Carolina has led to one of the fastest rates of land conversion to urban from rural in the nation (SC Wildlife Action Plan 2014, p. 1-1). Additionally, climate change is projected to have profound effects on mussel communities in South Carolina and Georgia. In the Lynches River *A. varicosa* occurs in small numbers within scattered patches of suitable habitat. Small numbers of *A. varicosa* have also been found in Flat Creek, a tributary of the Lynches River. The legacy of past pollution events and severe bank erosion has impacted habitat quality and mussel diversity in the Lynches River. For example, in 1990 a breeched dam released mine wastewater containing cyanide, copper and mercury into Little Fork Creek, a tributary of the Lynches River, causing a fish kill in 79 km (49 mi) of the Lynches River. In Stevens Creek mussel diversity is low and *A. varicosa* is scarce. However, Turkey Creek, a tributary of Stevens Creek, harbors is a diverse mussel community with at least 12 unionid species including the federally endangered Carolina heelsplitter, *Lasmigona decorata*, and a small and sparse population of *A. varicosa*. Much of Turkey Creek lies within the Sumter National Forest including designated Critical Habitat for the *L. decorata*. Several *A. varicosa* shells and live individuals have been recovered from Turkey Creek tributaries: Rocky Creek, Beaverdam Creek and Log Creek. The Chattooga River flows through the Blue Ridge physiographic region forming the northern border between South Carolina and Georgia. Ninety-two km (57 mi) of the Chattooga River are designated as a Wild and Scenic. Sixty-eight percent of the Chattooga River basin lies within the Frances-Marion Sumter National Forest in South Carolina and in the Chattahoochee-Oconee National Forest in Georgia. The Chattooga River supports a healthy *A. varicosa* population that is considered one of the most viable populations in the Southeast from Maryland to Georgia. In a survey of mussel biologists from South Carolina and Georgia, respondents reported on current habitat conditions, potential reintroduction/augmentation recommendations and conservation priorities based on healthy *A. varicosa* populations as well as populations face immediate threats. In summary: (1) Water quality and habitat have improved enough to consider reintroduction or augmentation of *A. varicosa* in the Pee Dee River basin, Lynches River headwaters, Stevens Creek subbasin and the Savannah River basin. (2) The Chattooga River was considered a conservation priority because of its healthy population of *A. varicosa*. (3) The Chattooga River was also named as a conservation priority because of the immediate threat of climate change to *A. varicosa* populations.

1.0 Pee Dee River Basin

1.1 Lynches River

Although originating in North Carolina, most of the Lynches River flows through the piedmont region of South Carolina before joining the Pee Dee River. *Alasmidonta varicosa* appears to be limited to the upper Lynches River subbasin. The upper subbasin is about 62% forested, 31% agricultural, 5% urban, 1.4% scrub/shrub and 1.3% forested wetland (SC Department of Health and Environmental Control, Pee Dee River Basin 2007, p. 44). Data from a monitoring station on the upper Lynches River showed that aquatic life was not supported because copper concentrations exceeded acute aquatic life standards; additionally, high cadmium concentrations were recorded in 2003 and 2004 (SC Department of Health and Environmental Control, Pee Dee River Basin 2007, p. 45). Other stations showed aquatic life to be fully supported but sections of the river have been impacted by past contamination events. In 1990, a breeched holding pond at Brewer Gold Mine released a solution containing cyanide, copper and mercury into Little Fork Creek, a tributary of the Lynches River, causing a fish kill in 79 km (49 mi) of the Lynches River (US Environmental Protection Agency *in* The Catena Group 2007, p. 1). The legacy of this spill is still apparent in the reduced diversity and relative abundance of mussels downstream of the Fork Creek watershed (The Catena Group 2014, p. 21). Populations of *A. varicosa* in the Lynches River appear to be small and sparse. Keferl collected *A. varicosa* from the Lynches River in 1987

(NCSM 33173, 34911, GMNH 8581) and 1990 (GMNH 9294), as did Dillon in 1988 (GMNH 9396), Porter and Mehenick in 1992 (NCSM 21804) and Savidge in 2005 (NCSM 30365). There are additional records from 1997 (no additional data) (SC Department of Natural Resources, unpublished data). Surveys of the same river segments in 2006, 2011 and 2014 showed small numbers of *A. varicosa* distributed in small patches of suitable habitat in four of the 12 river segments surveyed, yielding CPUEs of 0.24 mussels/hr in 2006, 1.01 mussels/hr in 2011 and 1.06 mussels/hr in 2014 (The Catena Group 2014, p. 24). Severe erosion of stream banks and the small numbers and patchy distribution of mussels suggests that habitat quality may be declining in the Lynches River (The Catena Group 2014, p. 25).

1.2 Flat Creek

Flat Creek is a tributary of the Lynches River. At an upstream monitoring site on Flat Brook, macroinvertebrate community data show that aquatic life is partially supported while at the downstream monitoring site, aquatic life is not supported because copper concentrations exceed the aquatic life acute standards; additionally a significant trend in increasing total nitrogen is evident in the creek (SC Department of Health and Environmental Control, Pee Dee River Basin 2007, p. 45). Keferl collected *A. varicosa* from Flat Creek in 1987 (NCSM 33171), as did Fridell in 2002 (NCSM 27304). Keferl also collected one individual from the Lynches River at the mouth of Flat Creek in 1990 (GMNH 9342). There are additional records from 1997 (no additional data) (SC Department of Natural Resources, unpublished data).

2.0 Santee Basin

2.1 Saluda River

The Saluda River flows from Blue Ridge through Piedmont and Sand Hills physiographic regions; the basin is about 53.7% forested, 26.1% agricultural, 12.9% urban, and 2.1% forested wetland (SC Department of Health and Environmental Control, Saluda River Basin 2011, p. 38). The confluence of the Saluda River with the Broad River forms the Congaree River, which then joins the Wateree River to form the Santee River. In 2009, the conservation group American Rivers listed the Saluda River as among the most endangered rivers in America, citing excessive amounts of phosphorous released from wastewater treatment facilities (American Rivers 2009). Athearn collected *A. varicosa* from the Saluda River in 1960 (NCSM 58257) but there are no recent records and the occurrence is considered historic (Bogan and Alderman 2008, p. 14)

3.0 Savanna River Basin

3.1 Stevens Creek

The Stevens Creek watershed flows from the piedmont to the upper coastal plain physiographic region of South Carolina. The middle portion of the Stevens Creek Watershed lies within Sumter National Forest. The upper Stevens Creek watershed, from the headwaters to Turkey Creek, is 69.3% forested, 21% agricultural, 7.2% urban and 1.9%forested wetland whereas the lower watershed, from Turkey Creek to the confluence with the Savannah River is 75.8% forested, 17% agricultural, 3.8% urban and 1.9% forested wetland (SC Department of Health and Environmental Control, Savannah River Basin 2010, p. 141). (We discuss Turkey Creek, a tributary of Stevens Creek below.) Data from monitoring stations on the upper Stevens Creek and the upper reaches of lower Stevens Creek show that aquatic life is fully supported; however, data from the downstream station of lower Stevens Creek show aquatic life is not supported because copper concentrations exceed standards and there is a rising trend in biochemical oxygen demand (SC Department of Health and Environmental Control, Savannah River Basin 2010, pp. 145). Except for Turkey Creek, Alderman considered the Stevens Creek subbasin as "relatively poor in mussel diversity and abundance" (Alderman 1995, p. 3). During surveys in 2009, one *A. varicosa* was found in Stevens Creek downstream of its confluence with Turkey Creek (Alderman 2009, p.46).

3.2 Turkey Creek (Stevens Creek Tributary)

The Turkey Creek watershed occupies piedmont and upper coastal physiographic regions in South Carolina; it is about 76.3% forested, 16.4% agricultural, 4.4% urban and 1.9% forested wetland (SC Department of Health and Environmental Control, Savannah River Basin 2010, pp. 145). Twenty-one km (13 mi) of the Turkey Creek mainstem lies within the Sumter National Forest including 14.3 km (8.9 mi) of designated Critical Habitat for the federally endangered Carolina heelsplitter, *Lasmigona decorata* (The Catena Group 2013, p. 2). Since the 1990s, Turkey Creek has been recognized for its diverse mussel community with at least 12 species present including *L. decorata* and *A. varicosa* (Alderman 1995, p. 3; The Catena Group 2013, p. 14). Macroinvertebrate community data from both upstream and downstream monitoring stations on Turkey Creek show the stream fully supports aquatic life, however biochemical oxygen demand and pH show significant increasing trends (SC Department of Health and Environmental Control, Savannah River Basin 2010, p. 145). Johnson collected *A. varicosa* from Turkey Creek in prior to 1970 (MUMZ 58024) (Johnson 1970). There are additional records from 1993 and 1994 (no additional data) (SC Department of Natural Resources, unpublished data). Alderman collected *A. varicosa* from Turkey Creek in 2004 (NCSM 30312, 30313). Twelve *A. varicosa* were found in Turkey during a 2013 survey of nine sites (CPUE 0.34 mussels/hr) compared to six *A. varicosa* located in previous surveys (CPUE 0.44 mussels/hr) (The Catena Group 2013, p. 21). Additionally, nine *A. varicosa* including three juveniles were found in Turkey Creek in 2015 (Mhatre et al. 2015). In 2017, M. Wolf and J. Mays found ten *A. varicosa* in a 40 m section of the creek suggesting the presence of a larger population; smaller numbers of *A. varicosa* were found in Mountain Creek and Sleepy Creek (J. Mays, USFWS, pers. comm.).

3.3 Rocky Creek (Turkey Creek Tributary)

Macroinvertebrate community data on Rocky Creek show the stream fully supports aquatic life (SC Department of Health and Environmental Control, Savannah River Basin 2010, p. 146). There is one record of *A. varicosa* in Rocky Creek from 1994 (no additional data) (SC Department of Natural Resources, unpublished data), however, Alderman failed to find *A. varicosa* during surveys in 1995 (Alderman 1995).

3.4 Beaverdam Creek (Turkey Creek Tributary)

Based on macroinvertebrate community data on Beaverdam Creek, the stream partially supports aquatic life – both biochemical oxygen demand and pH show significant increasing trends (SC Department of Health and Environmental Control, Savannah River Basin 2010, p. 146). During 1995 surveys of Beaverdam Creek one *A. varicosa* shell was found (Alderman 1995, p. 12) and in 2010 two shells and three live *A. varicosa* were located (Alderman et al. 2010). There is an additional record from 1997 with no additional data (SC Department of Natural Resources, unpublished data).

3.5 Log Creek (Turkey Creek Tributary)

Macroinvertebrate community data on Log Creek show the stream fully supports aquatic life (SC Department of Health and Environmental Control, Savannah River Basin 2010, p. 146). There is one record of *A. varicosa* in Log Creek 1995 (no additional data) (SC Department of Natural Resources, unpublished data), however, Alderman failed to find *A. varicosa* during surveys in 1995 (Alderman 1995).

3.6 Chattooga River (South Carolina/Georgia)

The Chattooga River Watershed begins in North Carolina and extends into South Carolina and Georgia within the Blue Ridge physiographic region. It drains about 73,000 ha (180,000 acres), of which 49,000 ha (122,000 acres) are located within the Frances-Marion Sumter National Forest in South Carolina and the Chattahoochee-Oconee National Forest in Georgia; 92 km (57 mi) of the Chattooga River are designated as a Wild and Scenic River (Krause and Roghair 2013, p.3). Data from two monitoring stations on the Chattooga River show aquatic life to be fully supported although there are significant increasing trends in biochemical oxygen demand (SC Department of Health and Environmental Control, Savannah River Basin 2010, p. 40). The *A. varicosa* population in the Chattooga River extends from the Adeline Branch downstream to the Tugaloo Reservoir (Alderman 2008, p. 5; Krause and Roghair 2013, p. 12). Atkins (1995) found 11 *A. varicosa* in four of 19 sites surveyed in the Chattooga River in 1995. Four of the surveyed sites were Chattooga River tributaries: Warwomen Creek, the West Fork, Reed Creek and Stekoa Creek where much of the substrate consisted of fine silt (Atkins 1995). No *A. varicosa* were found in the tributaries – apparently *A. varicosa* is only found on the Chattooga River mainstem (J. Alderman 2016, Alderman Environmental Services, Inc., pers. comm.) where there is sufficient current velocity to remove silt. Alderman found *A. varicosa* in the Chattooga River during surveys in 2003 and 2008 (Alderman 2004, 2008) and considers it the most viable population in the Southeast from Maryland to Georgia (Alderman 2008, p. 5). Although restricted in spatial extent, *A. varicosa* is possibly abundant in the Chattooga River (J. Wisniewski, 2016, Georgia Department of Natural Resources, person. comm.). The *A. varicosa* population in the Chattooga River is protected by the surrounding national forest however climate change is projected to have profound effects on wildlife communities in Georgia (Georgia Department of Natural Resources 2015).

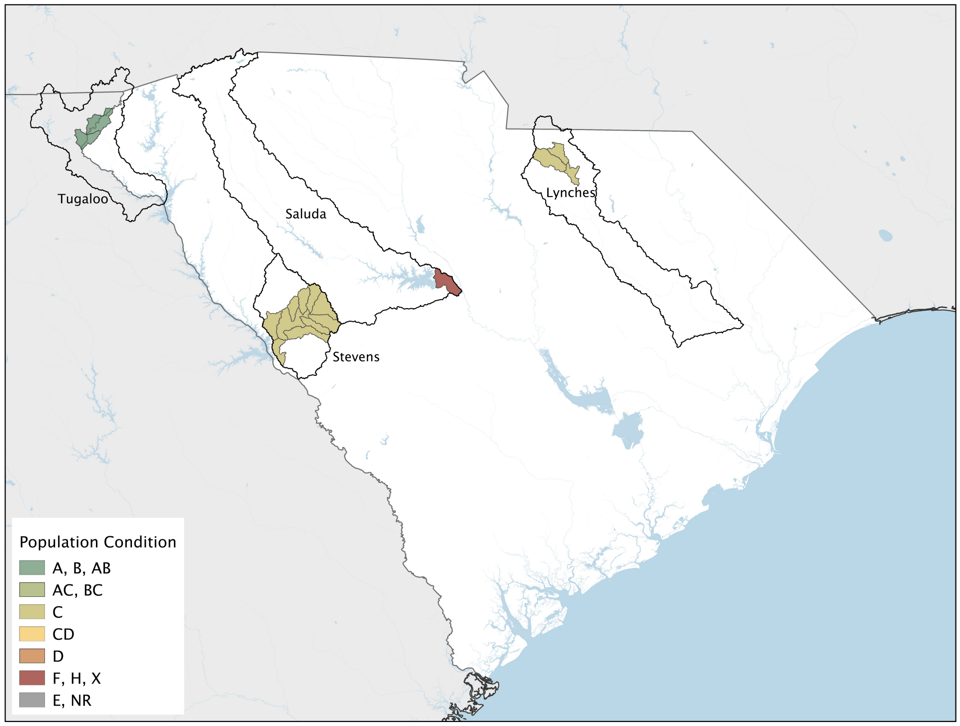


Figure 15. State-level condition map for South Carolina showing EO condition generalized from specific locations to the HUC12 watershed scale. In watersheds where multiple conditions exist, the color gradient approximates a linear transition between EO locations. The larger, black boundaries correspond to the HUC8 watersheds, which are also labeled.

Part 3. Human Impacts to Brook Floater Populations

Introduction

According to the literature and biologists across the species' range, there are a number of threats to *A. varicosa* populations. These threats can be parsed into two categories, those caused by direct assaults to streams and rivers and those caused indirectly by land use and climate change. Direct assaults on rivers primarily stem from harnessing waterways and using them as dumping grounds during almost 300 years of industrialization. Dams, toxins and sewage dumped into rivers, habitat destruction, and habitat fragmentation have added up to leave few intact stream segments with good water quality along the Atlantic slope. On the other hand, near 100% deforestation, multiple generations of agricultural land use and increased impervious surface through development have changed flow regimes, increased sediment and introduced pollutants across the range of *A. varicosa*. Finally, a changing climate brings challenges such as increasing temperatures and increased frequency of droughts and flooding not typical to Atlantic coastal watersheds. While not all of these threats have a simple solution, several of them can be ameliorated to increase the resiliency of *A. varicosa* populations into the future.

A. The legacy of 19th and 20th century industrialization and dams: toxic effluents, untreated sewage, habitat destruction and fragmentation.

Dams – The destruction of riverine mussel habitat by dams and impoundments in the United States began in the 17th century, increased in the late 18th century, but burgeoned in the 19th and early 20th centuries. Haag (2012, p. 331-332) considers the elimination of free-flowing rivers from 1924 to 1984 to be largely responsible for the first wave of mussel extinctions in the United States (see also Neves et al. 1997, p. 63). Data from the Army Corps of Engineers, National Inventory of Dams show that over 90,000 dams exist in the United States (USACE NID 2016). An estimated average of one dam for every 48 km (30 mi) of river channel of third to seventh order streams in the United States has resulted in a homogenization of flow dynamics that may affect 50% of river channel length (Poff et al. 2007, p. 5734). Dams and impoundments fragment rivers and streams and transform fluvial processes, putting riverine species at risk of extinction (Newton et al. 2008, p. 429). Habitat transformation may lead to large extinction debts (Tilman et al. 1994, pp. 65-66), which because of the long life spans of mussels may take decades to be realized (Strayer 2008, p. 31). As an Atlantic slope species, the range of *A. varicosa* coincides with the greatest density of dams in the United States with the highest density found in New England (Graf 1999, p. 1306). Over 14,000 dams are scattered throughout New England waterways (Magilligan et al. 2016, p. 3). Because *A. varicosa* is a strictly riverine species, the rapid increase in dam construction and resulting impoundments would have been especially devastating to this species.

The Blackstone River, which flows from central Massachusetts through Rhode Island to Narragansett Bay, had one dam for every river mile (Rhode Island Department of Environmental Management 2013, p. 24). The construction of a milldam on the Blackstone River in 1793 was followed by a century of extensive dam construction, industrial expansion and population growth that rendered the river badly fragmented and heavily polluted. By the end of the 19th century it was considered the most polluted river in the nation because of contaminated stream sediments (Blackstone River Valley National Heritage Corridor Commission 1998, p. 6). The only reports of *A. varicosa* in the Blackstone River are from the 19th century (see section 2). It is likely that *A. varicosa* was extirpated from the Blackstone River shortly after that period. Similar events unfolded in many other river basins.

By 1850 the construction of milldams on the Merrimack River in New Hampshire and Massachusetts blocked fish migration, transformed lotic habitat and degraded water quality. As water-powered industries flourished and adjacent urban population centers expanded, industrial effluents and sewage poured into the river. After his 1839 boat trip on the Merrimack River, Thoreau wrote: "Salmon, shad, and alewives were formerly abundant...until the dam...and the factories put an end to their migrations...Perchance, after a few thousand years, nature will have leveled...the dam...and the factories and the Grass-ground River will run clear again" (Thoreau 1849, pp. 28-29).

In the 1950s the Merrimack River was deemed the most polluted river in the United States (Robinson et al. 2003, p 2). The degradation resulted in the absence of pollution sensitive benthic species in the Merrimack River from Manchester, New Hampshire through northeastern Massachusetts to the Atlantic Ocean (Oldaker 1966, part 3, p. 35). McLain found just six *A. varicosa* during a SCUBA survey of the Merrimack River in Manchester (McLain 2004). However, surveys show that *A. varicosa* populations persist in the more rural, forested upper Merrimack River north of Concord, New Hampshire (see Part 2).

Although dam construction has abated, dams continue to disrupt metapopulation processes of mussel populations by blocking genetic exchange, preventing the rescue of declining or extirpated populations and barring the colonization of unoccupied but suitable habitat (Strayer 2008, p. 31). Scour at high flows and emersion during low flows also jeopardize mussel populations (Vaughn and Taylor 1999, p. 917) while discharge of cold hypolimnetic water can suppress the reproductive cycle of mussels causing recruitment failure (Heinricher and Layzer 1999, p. 146).

Figure 1. The dramatic fluctuation in flow from a peak hydroelectric dam appears to have contributed to the rapid decline of an insular *A. varicosa* population in the Piscataquog River (left). Over 190 *A. varicosa* were stranded or lost to opportunistic predators during a two-week period of extreme low flows in July 1997. Rapid dewatering and the presence of cobble and boulder prevent mussels from moving to deeper water. Photos © Barry J. Wicklow

Two hydroelectric dams and their impoundments have isolated a rapidly declining *A. varicosa* population in the Piscataquog River, a fourth order tributary of the Merrimack River in New Hampshire (Figure 1). Further, extreme low water related to stream flow regulation of a peak hydroelectric dam appears to have hastened the population decline: over 190 *A. varicosa* were stranded or lost to opportunistic predators during a two-week period of extreme low flows in July 1997 (Figures 1 and 2) (B. Wicklow, Saint Anselm College 2008, unpublished data). This reduced population may now be non-viable and vulnerable to extirpation through stochastic processes.

In the upper Catawba River basin in North Carolina, four dams form Lake James at a site where Arnold Ortmann collected *A. varicosa* in 1914. Lake James has also isolated *A. varicosa* populations in the upper Catawba River and the Linville River – both of which empty into the lake (see section 2). The remainder of the Catawba River south of Lake James is fragmented by a nearly continuous series of dams and impoundments (NC Wildlife Action Plan 2015, p. 123).

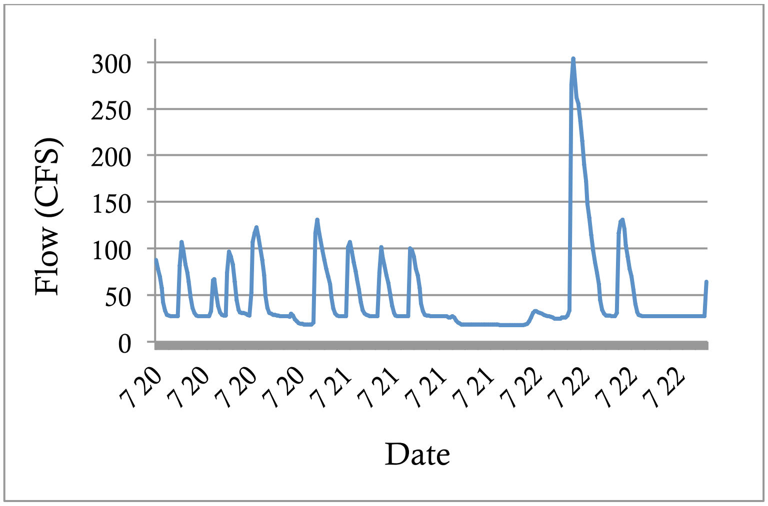


Figure 2. The dramatic fluctuation in flow from a peak hydroelectric dam appears to have contributed to the rapid decline of an *A. varicosa* population in the Piscataquog River. The hydrograph shows fluctuating flow during three days in July 1997. Over 190 *A. varicosa* were stranded or preyed on by opportunistic predators during a two-week period of low water (< 20 CFS) in July 1997 (Wicklow unpublished data).

Dam removal is an important component of river restoration projects but post-removal ecosystem effects vary with dam and reservoir size, river gradient, water temperature, removal methods as well as the available species pool (see Claeson and Coffin 2016; Gillette et al. 2016). Uncontrolled dam removal during restoration projects and flood-breeched dams may put downstream unionid populations at risk and can jeopardize lentic mussel species established within impoundments (Nedeau et al. 2000, p. 27; Sethi et al. 2004, p. 162). In Wisconsin, a 3.3 m (10.8 ft) dam originally built in 1848 was abruptly breeched in 2000 causing downstream mussels to be smothered by the sudden release of trapped sediment while mussels in the former impoundment were quickly stranded (Sethi et al. 2004, p. 162). In order to reduce the ecological cost of dam removal, Sethi et al. (2004, p. 163) recommend quantitative pre-removal assessments of the mussel populations, slow drawdowns and upstream relocation of mussels. These recommendations were implemented in North Carolina, where a before-after-control-impact study was used to assess the impacts to mussels of a dam removal on the Deep River. The results show little evidence of negative short-term effects on the downstream mussel assemblage – most likely due to the slow drawdown during dam removal (Heise et al. 2013, p. 48).

Although the impact of small dams (<4 m) on *A. varicosa* populations is less clear, fragmentation and isolation still contribute to the extinction risk that populations face from stochastic events (see Haag 2012, pp. 336-338). Dams also affect the distribution of mussels within river networks (Watters 1996, p. 80). From the 18th century on, small dams on tributaries increased exponentially. For example, over 120 dams were built on the Piscataquog River. Many of these small, low head dams were transitory – washed out by floods or destroyed by fire – but many others persisted. Parker's Mill (formerly White's

Mill), built circa 1800, lasted until it was breeched by a flood in 1997 (newbostonhistoricalsociety.com; B. Wicklow, Saint Anselm College, 1997, pers. observation). We speculate that this barrier to dispersal may be responsible for the paucity of *A. varicosa* in what appears to be suitable riverine habitat upstream of the dam (only one individual has been found), whereas *A. varicosa* populations occur downstream of the dam (B. Wicklow, Saint Anselm College 1994, unpublished data).

In New York, the 1.8 m (6 ft) Cuddebackville Dam on the Neversink River restricted upstream dispersal of the federally endangered dwarf wedgemussel *Alasmidonta heterodon*, which was only found downstream of the dam (Baldago 2003-2004, p. 3). The Cuddebackville Dam was removed in 2004. In New England 127 dams have been removed (45% being small dams) during the last several decades (Magilligan et al. 2016, p. 4).

Often, mussel communities that may include rare species become established immediately downstream of small low head dams or within their impoundments. For example, nearly 500 *Alasmidonta heterodon* were relocated during a dam removal project on the Ashuelot River, New Hampshire (Nedeau 2010, p. 6). Dams may protect mussel populations and warmer reservoir water that is higher in food resources may increase mussel density, richness and growth rates of mussel populations (Gangloff et al. 2011, p. 1113; Singer et al. 2011, p. 1911; Hornback 2014, p. 289). The short- and long-term ecological effects of dam removal on *A. varicosa* populations should be cautiously analyzed before proceeding with river restoration projects that require dam removal. Each project should be reviewed on a case-by-case basis (Gangloff et al. 2011, p. 1114). For example, a well-established *A. varicosa* population that has been monitored since 2006 is located just upstream of a one-meter dam on the Suncook River, New Hampshire (Wicklow 2008, p. 11). Dam removal at this site would need to be carefully managed and require an extensive relocation effort with long-term post-removal monitoring.

Figure 3. The Winterport Dam on Marsh Stream, Penobscot River basin, Maine showing the dam removal from downstream (left) and the upstream drawdown of the reservoir in 2010. The removal

of the small non-operating dam allows Atlantic salmon passage and opens 128 km (80 mi) of upstream riverine habitat to a once isolated *A. varicosa* population. Photos © Barry J. Wicklow

Still, river restoration projects that involve dam removal can provide long-term benefits to riverine mussel assemblages by reconnecting insular mussel populations, opening suitable upstream habitat to dispersal and rescuing declining mussel populations (Strayer 2008, p. 25; Haag 2012, p. 401). A reproducing but isolated population of *A. varicosa* in Marsh Stream (Penobscot River basin, Maine) is located downstream of the Winterport Dam built in 1800 then converted to hydropower in the 1980s. The small dam was removed in 2010 (Figure 3), thereby opening 128 km (80 mi) of upstream riverine habitat to this population (NOAA Habitat Conservation 2010). In Massachusetts the Millie Turner Dam on the Nissitissit River that separated two *A. varicosa* populations since 1750 was removed in 2015. During a slow drawdown volunteers collected 200 target species including 75 *A. varicosa* for temporary relocation; a study of the effects of this dam removal is ongoing (Hazelton 2016, pers. comm.).

Sewage and Industrial Discharge – For most of the last 300 years, untreated sewage and industrial wastes were discharged directly into rivers and streams. For example, between 1929 and 1950 an estimated 45,360 kg (100,000 lb) of mercury was released from the Waynesboro DuPont manufacturing facility into the water and onto the flood plain of the South River leading to the downstream contamination of the South River, South Fork and the Shenandoah Rivers in Virginia (VA Department of Environmental Quality 2009, p. 1). The 1972 Clean Water Act halted much of this pollution by requiring municipalities to develop sewage treatment facilities and industries to remove toxins from industrial wastewater prior to its discharge into rivers (see Brosnan and O'Shea 1996, p. 34; Hundal et al. 2014, pp. 134-135). The Clean Water Act is considered the most important water quality legislation in the US; its aim is to protect the "chemical, physical and biological integrity of the Nation's waterways" (Hawkins 2015, p. 1585). Still, leaky septic systems, aging infrastructure and the reliance on combined sewage outflow systems continue to allow untreated sewage to flow into rivers and streams impact mussels populations (Gillis 2012; Gillis et al. 2017, p. 678). Sewage effluents have been reported as threats to *A. varicosa* populations in nearly every state within its range (see section 2).

Acid Mine Drainage (AMD) – Rivers continue to be plagued by discharge from abandoned coal mines (Neves 1997, p. 69; Zipper 2016, p. 612). In Pennsylvania, 6,400 km (4,000 mi) of rivers are polluted with AMD with expected remediation costs of $15 billion (Chesapeake Bay Foundation, p. 7). For example, approximately 1,939 km (1,205 mi) of the West Branch of the Susquehanna River have been impacted by AMD causing mussel extirpations and isolating *A. varicosa* populations in Kettle Creek and Pine Creek, tributaries of the West Branch. In the Delaware River basin, the Schuylkill River (the type locality of Lamarck's 1819 species description of *A. varicosa*) has also suffered from ADM (see section 2). Ortmann (1909, p. 97) described streams polluted by AMD as having a "bluish-green color of the water and rusty-red deposit on the bottom" (Figure. 4). Taskine et al (2011, p. 1774) show that both low pH and the presence of aluminum harm glochidia of the eastern pearl shell *Margaritifera margaritifera*. Recent research shows that the presence of coal particles in the sediment can damage major organs and compromise physiological and reproductive function in mussels (Henley et al. 2015, p. 1023).

Figure 4. Acid mine drainage forms as pyrite, iron sulfide, in abandoned coal mines is oxidized to sulfuric acid and iron hydroxide, a red precipitate; low pH dissolves heavy metals like aluminum that in high concentrations turns water turquoise blue and precipitates as white aluminum hydroxide (see Sadak 2008, p. 8). Clearfield Creek, a tributary of the West Branch of the Susquehanna River (left) and the West Branch of the Susquehanna River. Photos © Courtesy of Mary Walsh

Restoration efforts including diversion wells, treatment systems and reclamation of abandoned mine lands has nearly eliminated AMD discharge and greatly improved the water quality in Babb Creek, a tributary of Pine Creek (West Branch Susquehanna Restoration Coalition 2015). Limestone treatments have also been used to neutralize AMD in West Virginia streams (Clayton et al, 2015, p. 270).

B. The late 20th and early 21st century: continued loss of stream habitat, loss of riparian forests, increased development, impervious surfaces, agriculture and associated pollutants.

Even as extinction debts that accrued during the period of industrialization and dam construction are realized, human impacts continue to threaten mussel populations. While fragmentation of river networks has had devastating effects on mussel populations, smaller unimpounded tributaries are thought to have retained their characteristic mussel assemblages well into the twentieth century (Haag and Williams 2013, p. 48). This appears to be true for *A. varicosa* throughout much of its range (see section 2). However, beginning in the 1960s many mussel populations, including those of *A. varicosa*, began to inexplicably crash ("enigmatic declines") while the remainder of the stream community – fish and macroinvertebrates – appeared healthy (Haag 2012, p. 341). Enigmatic declines appear to be related to recruitment failure, and all mussels within the stream are affected similarly (Haag 2012, p. 342).

However, *A. varicosa* populations have declined sharply or have disappeared from streams that have otherwise intact, high diversity mussel assemblages. *Alasmidonta varicosa* favors low productivity streams with low levels of calcium; high levels of calcium are considered an indicator of eutrophication (Strayer 1993, pp. 241, 243).

Eutrophication and Nitrogen Loading – The rapid rise in synthetic fertilizer use is coincident with the rapid decline of freshwater mussels beginning in the 1960s (Haag 2012, p. 379). Eutrophication can cause recruitment failure in mussels (Strayer 2014, p. 280) and nitrogen loading to streams can be especially harmful to aquatic life including mussels (Strayer 2008, p. 57; Haag 2012, p. 379). Reactive forms of nitrogen – such as nitrate, nitrite and ammonia – are widespread pollutants that are carried into streams as runoff from agricultural fertilizers, through bacterial decomposition of organic matter such as manure and through atmospheric deposition (Fowler et al. 2013, p. 4). By the late 1990s the amount of global nitrogen had doubled and has since continued to increase sharply due primarily to the industrial production of synthetic fertilizers (Vitousek et al. 1997, p. 738; Fowler et al. 2013, p. 5; Winiwarter et al. 2013, p. 899). Pinkney et al. (2015, p. 364) note that climate change may adversely affect mussel populations indirectly as runoff from increased precipitation increases nutrient and contaminate loading to streams. Nutrient loading in streams exposed to agricultural runoff diminishes or renders irrelevant key ecological pathways involving nutrient cycling (Spooner et al, p. 1122-1123).

Alasmidonta varicosa appears to be among the least tolerant freshwater mussels to agricultural runoff and eutrophication. In the agricultural upper Susquehanna River basin, New York, a 1996-1997 resurvey of sites that had been surveyed for mussels between 1955 and 1965 show that average species richness and the range of most species remained the same with one species expanding its range. However the range of *A. varicosa* contracted sharply (Strayer and Fetterman 1999, p. 333). In the lower Susquehanna River basin in Pennsylvania, which is intensely agricultural, *A. varicosa* was collected in Conewago Creek from the early through the mid-20th century but despite extensive surveys from 1994-2016, only two individuals have been found in an otherwise diverse mussel assemblage (see section 2). Water quality in Conewago Creek is impaired by agricultural runoff (Meyer et al. 2013, p. 21).

Additionally, in Cumberland County Maine, the lack of recruitment and dramatic decline *A. varicosa* in the Pleasant River appears to be linked to agricultural impacts and the lack of riparian forest buffers (Nedeau 2013, p. 7). Parts of the river are listed as impaired for fish, shellfish and wildlife protection and propagation due to low levels of dissolved oxygen resulting from runoff containing phosphorus, nitrogen, sedimentation and sewage (Maine Department of Environmental Protection 2012).

The early loss of *A. varicosa* from streams may, in some cases, portend a future loss of mussel diversity. In the Housatonic River basin, seven mussel species were found in Webatuck Creek, but no live *A. varicosa*. However, the dozens of *A. varicosa* shells found indicate that *A. varicosa* was once common in the creek (Strayer 2010, p. 5). Webatuck Creek is high in calcium and nutrients (Strayer 1999, p. 496) and the mussel community there shows signs of decline (Strayer 2010, p. 7). Further, in 2012, Strayer and Malcom (2012, p. 1785) found no evidence of *Elliptio complanata* recruitment in Webatuck Creek. Additionally, recent surveys failed to relocate *A. varicosa* in Conodoguinet Creek in the lower Susquehanna River basin, Pennsylvania, where the number of species has declined to three from seven present historically and where water is impaired due to organic enrichment, high suspended solids, urban runoff and low dissolved oxygen levels (Meyer et al. 2013, p. 21).

Biologists have reported reduced recruitment or its absence in numerous *A. varicosa* populations and many *A. varicosa* populations from Maine to South Carolina appear to exist only as declining numbers of aging adults (see section 2). Juvenile mussels spend up to three years burrowing and feeding within sediments where they may be exposed to toxins within pore water and to contaminants bound to sediments or food (Yeager et al. 1994, p. 221; Cope et al. 2008, p. 453).

Fine sediments that block hydrologic exchange between oxygenated surface water and interstitial water can reduce oxygen availability in the sediments and trap toxins (Geist and Auerswald 2007, p. 2311; Scheder et al. 2015, pp. 36-38). Low interstitial oxygen levels (especially during summer low flows) and higher temperatures (due to loss of riparian forests and climate change) favor the conversion of ammonium to toxic unionized ammonia (Strayer and Malcom 2012, p. 1788).

Laboratory studies show unionized ammonia to be highly toxic to unionids (Augspurger et al. 2003, p. 2574; Newton 2003, p, 2543), especially to juveniles (Mummert et al. 2003, p. 2548; Newton and Bartsch 2007, p. 2061; Wang et al. 2007, p. 2041; Wang et al. 2008, p. 1144). In a key field study Strayer and Malcom (2012) showed that interstitial unionized ammonia, in concentrations as low as 0.2 μg/L, were strongly associated with recruitment failure in populations of *Elliptio complanata*. The results showed no evidence of recruitment failure due to fine sediments, low interstitial dissolved oxygen, the presence of the predatory rusty crayfish or the absence of the primary host fish (Strayer and Malcom 2012, pp. 1783-1787). Strayer and Malcom (2012, p. 1788) conclude "toxicity from unionized ammonium has the potential to prevent recruitment of many mussels species over broad regions of the world". In a recent study, the low survival of juvenile mussels exposed to sediments collected from the water column of agriculture-associated streams was correlated with increased ammonia concentrations (Archambault et al. 2017, p. 402).

Pesticides – Pesticides may have acute, chronic and sublethal effects on mussels (Haag 2012, pp. 374-379). Although specific effects on *A. varicosa* are unknown, the extensive use of pesticides, especially in no-till agriculture, also coincides with the decline in mussel populations beginning in the 1960s (Haag 2012, p. 376). Only a small fraction of the many thousands of pesticides in use today have been tested on mussels. We provide three examples of pesticides – each showing harmful effects on mussels. The herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D) is among the most widely used pesticides worldwide (Alves et al. 2014, p. 15). Although 2,4-D has been shown to be less toxic to mussels during acute tests than other contaminants (Milam et al. 2005, p. 171), recent studies show that prolonged exposure to 2,4-D disrupts shell maintenance and growth in mussels (Alves et al. 2014, p. 17).

Atrazine, one of the most extensively used pesticides, is known to persist in aquatic systems during spring and summer (Greymore et al. 2001, p. 493) – coincident with unionid juvenile recruitment peaks (Haag 2012, p. 378). Laboratory tests show mussel glochidia and juveniles were not sensitive to acute atrazine exposure, however chronic exposure was found to be toxic to juveniles (Bringoff et al. 2007a, p. 2092). Jacomi et al. (2006, p. 390) show that during laboratory experiments, mussels rapidly bioaccumulate atrazine. Additionally, atrazine acts as an endocrine disrupter in mussels.

Studies show that environmentally relevant concentrations of atrazine disrupt aggregation behavior in mussels (Flynn and Spellman 2009, p. 1232) and cause feminization of male mussels (Flynn et al. 2013, p. 10). Further, synergistic effects of multiple contaminants are known to harm bivalves. Atrazine and Roundup (see next paragraph) solely or in combination cause harmful genetic and biochemical effects on the Asian clam *Corbicula fluminea* (Kelly et al. 2014, pp. 10-13).

The broad-spectrum herbicide glyphosate is the major ingredient of Monsanto's Roundup. The restricted use of Roundup began in the 1970s however its use soared after genetically engineered herbicide-tolerant crops ("Roundup Ready") became available in 1996 (Benbrook 2016, p. 1). It is now considered one of the most commonly used agricultural herbicides in the world (Annett et al. 2014, p. 458). Glyphosate alone is considered to have low toxicity to aquatic animals including mussels however the associated surfactants – which allow the herbicide to adhere to and pass through the waxy surface of plants – are highly toxic (see review by Annett et al. 2014). The surfactant used in Roundup, MON 0818, is acutely toxic to mussel glochidia and juveniles (Bringoff et al. 2007b, p. 2097). Contaminants can also have indirect trophic effects on aquatic communities (Fleeger et al. 2003, p. 208), for example by limiting microalgal food supplies to growing juvenile mussels (Haag 2012, p. 378).

Residual contaminants within sediments can prevent mussel recruitment to otherwise suitable habitat (Strayer et al. 2004, p. 434). Moreover, estimates show that less than 21% of rivers and streams in the US are in good biological condition and that conditions appear to be getting worse not better (USEPA 2010, 2013 as cited in Hawkins 2015, p. 1586). The legacy of fertilizer production and the profligate use of fertilizers and pesticides may shape ecosystem composition and function far into the future (Perrin et al. 2016, p. 1369).

Loss of Riparian Forests – Riparian forests provide important services to aquatic ecosystems by impeding runoff, retaining nutrients and contaminants, stabilizing banks, shading and cooling streams (Allan 2004, p. 262; Naiman et al. 2005, pp. 274-275). The interception of runoff containing agricultural and residential fertilizers and pesticides appears to be especially important to the reproduction, recruitment and dispersal of mussels (Strayer and Malcom 2012, p. 1788).

Several studies show the importance of riparian forests to mussel assemblages. In a comparative study of grassy versus forested riparian buffers of agricultural basins in Ontario, Morris and Corkum found that rivers with forested buffers had significantly lower mean monthly temperatures and had 17 times less reactive nitrogen concentrations than rivers with grassy buffers (Morris and Corkum 1996, p. 582). Moreover, rivers with grassy buffers became dominated by a single species, *Pyganodon grandis* (Morris and Corkum 1996, p. 584), which is characterized as a "weedy, ubiquitous and opportunist species (Strayer 2008, p. 20; Haag 2012, pp. 275, 391).

The alarming loss of mussel species from streams in Iowa was associated with high agricultural land use and riparian deforestation (Poole and Downing 2004, p. 123). The study compared changes in mussel species richness from 1984-1985 to 1998 in 118 stream reaches that were considered the least degraded mussel habitats across the region. Results show that nearly half of the sites lost all mussels and more than half of the sites lost over 75% of mussel species; further, habitat alteration imposed an extinction debt that may take decades to be paid (Poole and Downing 2004, pp. 118, 122).

Agricultural runoff and development also threaten the water quality of Patterson Creek, West Virginia (WV State Wildlife Action Plan 2015, p.411). In 1993-1994, Clayton et al. (2001) discovered a substantial population of *A. varicosa* in Patterson Creek. However, the authors warned of habitat and water quality threats from agricultural. By 2010, the *A. varicosa* population in Patterson Creek appeared to have declined and become more spatially restricted (Villella and Nelson 2010, p. 19). Riparian restoration and livestock fences are needed to protect the *A. varicosa* population in Patterson Creek (J. Clayton, WV Division of Natural Resources 2016, pers. comm.).

Legacy effects of deforestation and agricultural expansion may explain mussel extirpations in some areas. For example, Popov (2015, p. 90) concludes that historical effects of severe deforestation and agricultural activities are responsible for the elimination of the eastern pearl shell *Margaritifera margaritifera* from rivers in the Baltic region of Russia. At the watershed level Pandolfo et al. (2016, p. 1679) found a negative relationship between mussel occurrence and agricultural land use.

Additionally, Hopkins and Whiles (2011) used multiscale modeling of land use and land cover to prioritize mussel and fish conservation areas in the Ohio River basin. They show that species richness increased with increased percent forest cover and decreased with density of developed or exposed land (Hopkins and While 2011, p. 205). They conclude that, in order to conserve mussel and fish species richness, managers should (1) retain 55% or more riparian forest cover and (2) strictly limit agricultural and urban land uses (Hopkins and Whiles 2011, p. 207). The fixed-width riparian buffers used as regulatory guidelines have not been empirically tested and may be too narrow to maintain riparian and aquatic ecosystem function (Richardson et al. 2012, pp. 235-236).

Many of the best (that is, ranked "excellent" or "good", see section 2) *A. varicosa* populations are found in rivers that flow through heavily forested areas including national and state forests, whereas many of the most imperiled populations are found in areas where riparian forests have been diminished or are now absent and replaced with developed land. Although more definitive studies on the efficacy of riparian forests in protecting mussel populations are needed (Newton et al. 2008, p. 433), we believe that the greatest immediate threat to extant *A. varicosa* populations is the replacement of riparian forests with agricultural, residential or urban development. The reduction of non-point source pollution is necessary to prevent the extirpation of many declining *A. varicosa* populations.

Despite river restoration efforts we continue to face a pervasive transformation of riparian forestlands. For example, a natural reforestation of the New England landscape followed the abandonment of agriculture in the mid-1800s. Although forest cover is now the highest it's been in 150 years, a second wave of deforestation is now occurring in every New England state (Foster et al. 2010, p. 9). In 2009, because of the projected loss of forestland to residential development, the National Forest Service ranked the Merrimack River watershed as the most threatened in the nation: by 2030, a projected 40-60% of forestland in the watershed will be replaced by development and impervious surfaces with associated increased pollution (Stein et el. 2009, part 2, p.14). Citing the replacement of riparian forests with suburban development, the conservation group American Rivers listed the Merrimack River as among the most endangered rivers in America in 2016 (American Rivers 2016). Rapid population growth – especially in the Southeast – is expected to intensify the conversion of forestland to residential, commercial, agricultural and industrial development (Carter et al. 2014, p. 405). Deforestation and both historic and current landuses have negatively impacted diverisity and community structure in streams of the Blue Ridge and Piedmont regions of the southeast (Surasinghe et al. 2014, p. 542).

There is a pressing need to protect riparian forests (Sweeney et al. 2004, p. 14136; Hopkins and While 2011, p. 207). We present a simple results chain (see Foundations of Success 2007) to illustrate a strategy to retain or increase riparian forest cover in order to maintain or increase recruitment in mussel populations (Figure 5). Protecting riparian forests has been an integral part of many river restoration efforts but there are difficult challenges to overcome such as incentivizing landowner cooperation, choosing appropriate buffer widths and acquiring adequate funding. Nevertheless, with federal, regional, state and local coordination, education and outreach this strategy can be successful (Sweeney and Blaine 2016, p. 760). For example, the Natural Resource Conservation Service's Wetlands Reserve Easement Program includes the protection of riparian forests as part of its mission. Land trusts have already protected riparian forests through fee simple transactions and conservation easements. The goal of the Wildlands and Woodlands initiative is to retain 70% of the New England region as forests that are free of development (Foster et al. 2010, p. 4). Moreover, a riparian forest cover strategy along with other potential conservation strategies can be analyzed through a structured decision making process. This approach was used to evaluate the efficacy and cost of various conservation strategies to increase the persistence of the endangered dwarf wedgemussel, *Alasmidonta heterodon* in North Carolina (Smith et al. 2015).

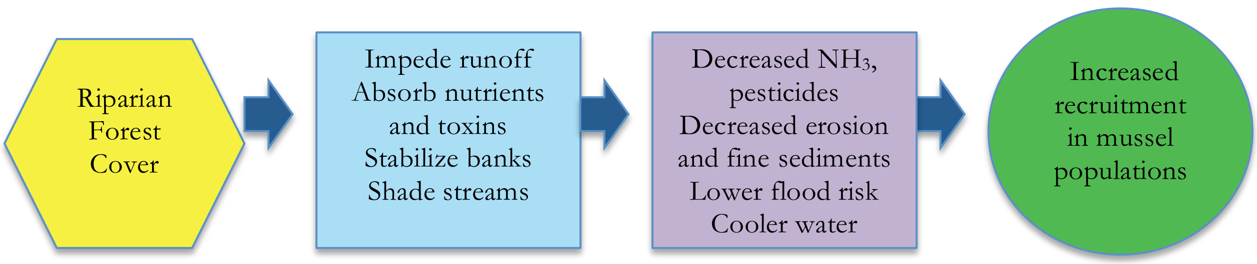


Figure 5. Simple results chain (Foundations of Success 2007) showing a strategy of retaining or increasing riparian forest cover to maintain or increase recruitment in mussel populations. There is an urgent need to protect riparian forests, which continue to be rapidly replaced by development.

We do not expect that riparian forests will protect all *A. varicosa* populations, however, we do believe that preventing deforestation and encouraging reforestation along riparian corridors may be our most practical tool to halt or reverse the decline of many vulnerable *A. varicosa* populations. Unlike the "soft" deforestation where agricultural lands naturally revert to forests, the "hard" deforestation occurring today replaces forests with development that will last far into the future (Foster et al. 2010, p. 9).

Global Climate Change – Climate change looms over all other human impacts to mussel populations. High temperatures and the increased frequency of extreme floods as well as the increased frequency and duration of droughts will continue to have severe impacts on mussel populations (Hastie et al. 2003, p. 45; Newton et al. 2008, p. 432; Haag 2012, p. 419; Pinkney et al. 2015, p. 346; Shea et al. 2013, p. 391; Vaughn et al. 2015, p. 1297). Horton et al. (2014, p. 373) reported climate change in the Northeast: (1) between 1895-2011, temperatures increased by nearly 1.1 ^{0}C; (2) between 1958-2012, rainfall during extreme precipitation events increased by 70% (especially during winter and spring); (3) higher temperatures and droughts are expected during summer months. Recent projections show that hourly extreme precipitation events will significantly increase in North America especially in the Northeast (Prein et al. 2016, pp. 2-3). Additionally, a recent analysis showed that in the Northeast, annual extreme precipitation was 53% higher from 1996-2014 than from 1901-1995 (Huang et al. 2017). Whitman et al. (2013, p. 81) in a review of climate change and biodiversity in Maine, listed *A. varicosa* as one of 14 invertebrate species that have a high vulnerability to climate change. The species is considered at risk to changes in hydrology and low summer water levels. Models of mussel host fish affiliate relationships in 350 U.S. rivers show that climate and water withdrawal disturbances had greater impacts on mussels than fish especially in the Southeast where reductions in flow will be greatest and the mussels impacted per unit flow will be highest (Spooner et al. 2011, pp. 1729-1730). Carter et al. (2014, pp. 397-399) show that for the Southeast: (1) temperatures have increased by nearly 1.1^0 C since 1970; (2) heavy rainfall events and droughts have increased; (3) extreme heat events will become more frequent, more intense and last longer causing a decrease in water availability while increasing competition for water. Consequently, aquatic ecosystems and mussel populations will be increasingly at risk.

Elevated temperatures adversely affect glochidia, juveniles, adult mussels as well as entire mussel communities. Increased temperatures may cause recruitment failure in *A. varicosa* populations. Both glochidia and juveniles of *A. varicosa* have low tolerances to elevated temperatures and may already be exposed to summer temperatures that are near to or exceed their thermal tolerance (Pandolfo et al. 2010, pp. 966-967). Although laboratory studies show that the tolerance of *A. varicosa* adults to higher temperatures increased with higher acclimation temperatures (Galbraith et al. 2012, p. 85), experiments with juveniles (of other mussel species) show acclimation temperature had little effect on thermal sensitivity (Archambault et al. 2014, p. 62). Higher temperatures also affect mussel development and metamorphosis. Taeubert et al. (2016, p. 234) found that metamorphosis success of the thick-shelled river mussel *Unio crassus* was highest at 17 ^{0}C and lowest at 23 ^{0}C. Additionally, increased temperatures have been shown to cause changes in the physiological processes and energy balance in adult mussels (Ganser et al. 2015, p. 1714).

High temperatures and drought can alter riverine species composition. In 2000, a severe drought combined with increased water withdrawal for irrigation led to extreme low flows and extensive mussel mortality in the Flint River basin, Georgia (Galloway 2004, p. 504; Peterson et al. 2011, p. 120). Mussel mortality increased as flow decreased to 0.01 m/s and dissolved oxygen fell to below 5 mg/L (Johnson et al. 2001, p. 11). The drought resulted in a shift toward higher relative abundance of common species and a decrease in rare species or those species inhabiting riffles (Johnson et al. 2001, p. 9). In Alabama and Mississippi, the 2000 drought had little effect on mussel mortality in larger streams with adequate flow but resulted in high mussel mortality in smaller streams with low flow leaving those populations at high risk of extirpation (Haag and Warren 2008, pp.1173-1176). Shea et al. (2013, p. 391) conclude that rare mussel species in small to medium streams are highly susceptible to extirpation due to drought. High temperatures and drought in the Kiamichi River, Oklahoma caused a river-wide shift in the mussel community to a thermally tolerant mussel assemblage from a thermally sensitive mussel assemblage (Galbraith et al. 2010, pp. 1180-1181). Vaughn et al. (2015, p. 1297) report a 60% drought-related decline in Kiamichi River mussel populations, which led to a catastrophic reduction in riverine ecosystem function.

Because *A. varicosa* occurs in small or moderately sized streams, we expect it to be especially vulnerable to high temperatures and drought.

Behavioral responses to dewatering vary among mussel species (Bartsch et al. 2000; Gough 2012, p. 2362). In dewatering experiments, Galbraith et al. (2015, p. 49) found that during low and moderate dewatering *A. varicosa* moved greater distances than most of the other five species tested but suffered high mortality once stranded. The authors report a high frequency of stranded mussels at low and moderate dewatering rates and all mussel species became stranded during fast dewatering rates. Further, of the 21 rivers from Maine to Georgia that were assessed, dewatering rates that were comparable to low and moderate experimental rates occurred regularly and some rivers showed rates similar to fast experimental rates (Galbraith et al. 2015, p. 49). In the wild populations, *A. varicosa* are often found within patches of fine to course sand among cobbles and boulders that can limit movement during dewatering (B. Wicklow, Saint Anselm College, personal observation).

Figure 6. The Suncook River, New Hampshire, experienced extreme low flows during the drought of 2007 (left). Low water levels allowed opportunistic predators access to mussels resulting in the loss of hundreds of *A. varicosa.* Shells seen in a midden along the river; many shells are labeled as part of a mark-recapture study begun in 2006. Photos © Barry J. Wicklow

Predators can stall or reverse the recovery of rare mussels (Neves and Odum 1989, p. 940; Hersey et al. 2013, p. 256; Edelman et al. 2015, p. 480). Low water levels during droughts can compromise rare mussel populations by allowing greater predator access to mussel beds. Higher water levels can serve as deep-water refuges for mussels (Strayer 2008, pp. 107, 130). During a drought in 2007 and a dry period in the summer of 2010, hundreds of *A. varicosa* were lost to opportunistic predators in the Suncook River, New Hampshire (Wicklow 2008, p. 31; B. Wicklow, Saint Anselm College, 2010, unpublished data).

During the 2007 drought, water depth over mussel beds fell to 6-16 cm (2.3-6.3 in) allowing easy access to mussel beds for both swimming and wading predators (Figure 6). Mussel depredation increased as water levels decreased during the drought (Figure 7). The extreme low water in 2007 and 2010 also provided easy All Terrain Vehicle access across sensitive mussel habitat.

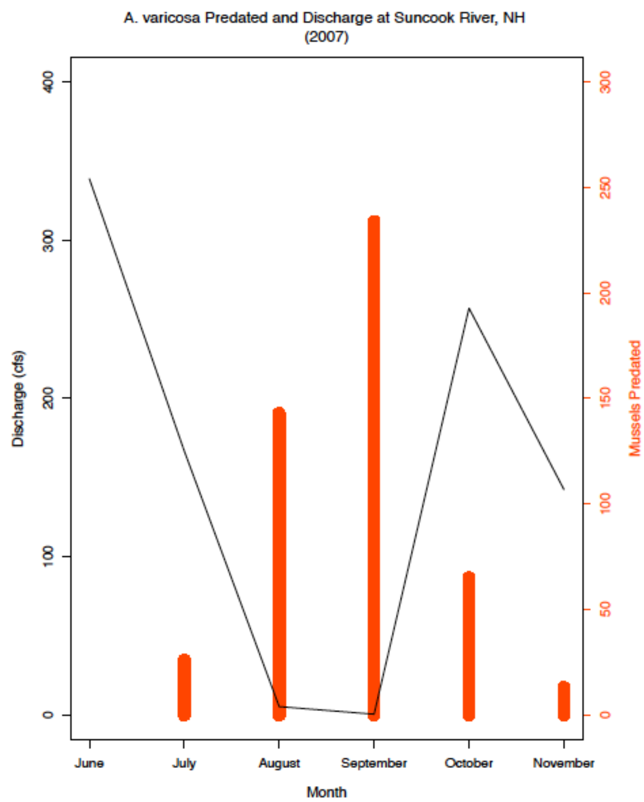


Figure 7. In the Suncook River, New Hampshire large numbers of *A. varicosa* (red) were preyed on during extreme low flow (black) during a 2007 drought (Wicklow, unpublished data).

As extreme rain events increase in frequency and intensity, high magnitude floods will increasingly impact *A. varicosa* populations. Flow refuges help to protect mussel populations (Strayer 1999, p. 472; Allen and Vaughn 2010, p. 392) but even mussel populations within flow refuges suffer high mortality during catastrophic storms and flooding. Hydrodynamic variables such as boundary shear stress help to explain the distribution and density of mussel beds as well as the settlement success of juveniles (Layzer and Madison 1995, pp. 340-344; Strayer 1999, p. 472; Steuer et al. 2008, pp. 75-80; Dario et al. 2010, p. 848; French and Ackerman 2014, p. 50). Because glochidia of *A. varicosa* are released in mucous threads during early spring (April in New Hampshire) and juveniles release from host fish 19-51 days later (B. Wicklow, Saint Anselm College, unpublished data), we expect that higher shear stress following extreme spring rainfall and floods will adversely affect recruitment in *A. varicosa* populations. Moreover, reduced dispersal rates can compromise the ability of mussels to respond to changing ecological conditions and to maintain linkages within metapopulations (Strayer 2008, p. 30).

Large storms in 2005 and 2006 caused extensive flooding of the Neversink River, New York. The floods may have seriously damaged *A. varicosa* populations in the Neversink

River (D. Strayer, Cary Institute of Ecosystem Studies, pers. comm.; J. Cole, USGS, Leetown Science Center, pers. comm.). Up to 13 cm (5 in) of rain fell within a 36-hour period during the 2005 storm and water levels in some areas exceeded 500-year flood elevations (Suro and Firda 2006, p. 21). In post-flood surveys *A. varicosa* populations appeared to have declined sharply (J. Cole, USGS Leetown Science Center, pers. comm.). Additionally, heavy rain from Hurricane Irene followed a week later by rain from Tropical Storm Lee caused extensive flooding in the Neversink in 2011.

Three hurricanes in 2004 caused heavy damage *A. varicosa* populations in the Southeast. Hurricanes Frances, Ivan and Jeanne passed over western North Carolina releasing heavy rains of up to 58 cm (23 in) in some areas and causing extensive flooding and record flows in the Linville River (Goddard Earth Sciences Data and Information Services Center, NASA; Fraley and Simmons 2006, p. 3). Post-hurricane surveys in 2005 show that *A. varicosa* numbers declined in the Lineville River – at one site where 63 *A. varicosa* were found in 1998 only six were located in 2005 (NC Wildlife Resources Commission, unpublished data). The CPUE for *A. varicosa* decreased by 32 mussels per person hour – an 89% change (Fraley and Simmons 2006, pp. iii, 10). Pre- and post-hurricane surveys show *A. varicosa* populations were similarly impacted in the Johns River (Fraley and Simmons 2006, pp. iii, 10).

Figure 8. Catastrophic flooding in 2006 caused an avulsion in the Suncook River. The shorter new channel (left) flows through unstable sediments. The stable, well-armored former channel was dewatered during the avulsion causing the stranding of over 1000 *A. varicosa.* Photos © Barry J. Wicklow

In mid-May 2006, up to 36 cm (14 in) of rain fell in central and southern New Hampshire causing catastrophic flooding equal to or exceeding a 100-year recurrence interval (Olson 2007, p. 1). The floodwaters breeched a glacial ridge and avulsed a new channel in the Suncook River leaving 3.2 km (2mi) of former river channel dewatered (Figure 8). Volunteers rescued over 1000 *A. varicosa* from the dewatered channel; mussels were eventually tagged and translocated to suitable habitat upstream where a resident *A. varicosa* population was already established. However, in April 2007, a second 100-year flood washed large numbers of *A. varicosa* downstream and onto banks causing high mortality (Wicklow 2008, p. 31). The 2007 storm also caused record peak discharges in the Piscataquog River (Flynn 2008, p. 1) where it also damaged *A. varicosa* populations. One mussel bed that had been monitored since 1994 completely disappeared (B. Wicklow, Saint Anselm College unpublished data). The 100-year floods in 2006 and 2007 also caused catastrophic declines in another *A. varicosa* population in the Piscataquog River monitored since 1996 (B. Wicklow, Saint Anselm College unpublished data).

Potential Future Stressors: Invasive Species and Disease – Invasive species have had catastrophic effects on freshwater ecosystems and are projected to have even greater effects in the future (Strayer 2011, pp. 163-167). For example, the huge populations of zebra mussel *Dreissena polymorpha* have caused severe declines in native mussel populations (see Strayer 1999, pp. 75-80). As filter feeders, enormous numbers of *Dreissena* can severely limit food availability to both adult and juvenile native mussels (Strayer 2008, p. 94). The Asian clam, *Corbicula fluminea* can also reach massive densities but its adverse effects on native mussels are equivocal and its large populations may be coincident with mussel declines rather than causal (Haag 2012, pp. 368-370). Although there are areas such as the Susquehanna River basin where the range of *Dreissena* and *A. varicosa* may overlap, we know of no direct linkage between the invasive species and *A. varicosa* population declines.

Disease was thought to be a possible cause for the rapid mussel die-offs in the Tennessee River and the Mississippi River in the 1980s, however, except for the Lea plague virus disease that caused die-off in the Chinese pearl mussel *Hyriopsis cumingii* (Haag 2012, pp. 382-383), there is no definitive evidence linking disease with mussel declines (see review by Carella et al. 2016).

Part 4. Modeling Brook Floater Population Condition with Environmental Variables

Summary

In an effort to (1) better understand the physical and environmental correlates of *A. varicosa* condition and (2) predict potential condition across its full range, we developed models that link geospatial data at known reference sites with population condition at those locations. Geospatial predictors were chosen from among several nationally available data layers and were intended to capture important environmental gradients in the landscape that may be important to *A. varicosa*, including land use, topography, and climate. Using random forests (Breiman, 2001), a nonparametric decision tree ensembling approach to classification, we modeled at two different scales: (1) HUC12 watershed scale, and (2) stream scale, encompassing a 100 m wide buffer of each stream that extends 1 km upstream from each EO. The watershed- and stream-level models explained 86% and 89% of the variation in *A.*

varicosa population condition, respectively. While both models were highly accurate overall, the stream-level model was more consistent and reliable predicting across all classes (87% - 89% accurate), whereas the watershed model predicted the 'poor' class very accurately (96%), but struggled to separate the good (66% accurate) and fair (50%) classes. The stream-level model predicted only 14.7% of all stream segments in the study area (all 15 states including areas outside the Atlantic slope region) to have conditions necessary to support "good" populations of *A. varicosa*. Conversely, well over 2.1 million stream segments (> 65%) were classified as "poor."

Methods

Methods Overview – In an effort to (1) better understand the physical and environmental correlates of *A. varicosa* condition and (2) predict potential condition across its full range, we developed models that link geospatial data at known reference sites with population condition at those locations.

Using the standardized EO layer generated for the distribution mapping task, we conducted a habitat suitability analysis. The goal of the modeling exercise was to identify important environmental correlates of *A. varicosa* occurrences and viability (e.g. riparian cover, distance to impervious surfaces, slope, etc.) using GIS datasets as environmental proxies, as well as to map potential suitable conditions throughout the range. This analysis also aided the identification and reporting of threats to populations.

Using randomForests (Breiman, 2001), a nonparametric decision tree ensembling approach to classification, we modeled at two different scales: 1) HUC12 watershed scale (coarse), and 2) stream scale (fine), encompassing a 100 m wide buffer of each stream that extends 1 km upstream from each EO. Generating models at multiple spatial scales allowed us to describe environmental processes that affect mussel habitat over both large and small areas. We were interested in identifying important factors at the watershed scale, but also within the areas immediately adjacent to mussel populations. We used the resulting models to predict and map potential habitat condition throughout the range.

Data Preparation

HUC 12 Scale Training Data – HUC 12 polygons containing EOs with uniform population conditions were identified as training samples. HUC12 watersheds containing multiple EOs with different conditions were excluded from the training data set to avoid confounding the models.

Stream Buffer Scale Training Data – For each EO, we identified the closest NHD flowline and adjusted the position of the EO to the nearest point on the line. We then calculated a 100-meter wide buffer extending from the identified location on the NHD stream line to the point or points (in the case of branching) located 1 kilometer upstream from the EO (Figure 19).

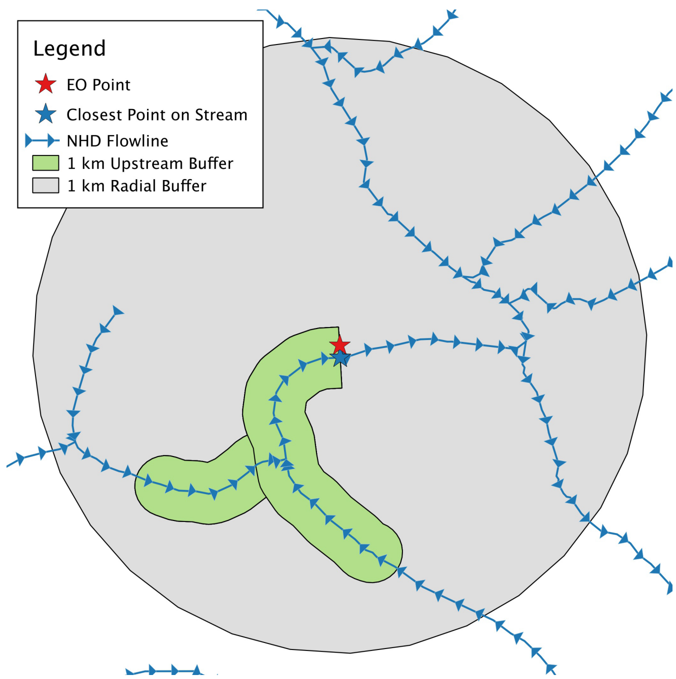


Figure 19. Illustration of methodology for computing 100 m wide, 1 km long upstream buffers (including branches) for each EO. This process was completed for each EO, and the resulting polygons were used to train stream-level RandomForests models. Radial buffer provided for reference to show that a linear kilometer would result in a much different area than winding along the stream.

Classification System – In order to achieve an appropriate number of training samples for each condition, we lumped EO ranks into three classes: good, fair, and poor or extinct (Table 1). EOs ranked as AC (Excellent-Fair), E (verified extant), U (unrankable), NR (not ranked), or F (failed to find) were not included in the modeling data set, as they did not provide clear enough indications of EO condition or quality to link with the environmental data and determine trends. This reclassification of the training data was completed for both the HUC12 scale and the Stream scale.

Original Rank	Model Rank
A, AB, B	Good
BC, C	Fair
CD, D, X, H	Poor/Extinct
AC, E, U, NR, F	Not used

Table 1. NatureServe EO Ranks were lumped into three classes based on a key linking the original rank to a simpler classification for modeling purposes.

Predictor Data – We gathered spatially explicit physical and environmental data to help explain the variation we observed in EO condition (Table 2). Our current understanding of threats to the species includes habitat degradation and pollution due to poor agricultural practices; loss of adequate riparian corridors; climate change; and increased or intensified development, sewage loads, siltation, and impoundments. We chose GIS data layers that were available and consistent across the study area and which best serve as proxies for these known threats. Individual states may be able to improve upon models with more specific, state-level data.

%Developed 1992	**Std dev Slope**	%Herbaceous 2001
%Barren 1992	Max Slope	%Agriculture 2001
%Forest 1992	**Mean PPT Trend 1895-2012**	%Wetland 2001
%Shrub1992	Stdev PPT Trend 1895-2012	Min Canopy Density 2011
%Wetland 1992	**Mean Tmin Trend 1895-2012**	Min Imperviousness 2011
Mean Canopy Density 2011	Std dev Tmin Trend 1895-2012	%Water 2011
Std dev Canopy Density 2011	**Mean Tmax Trend 1895-2012**	%Water Change 2001-2011
Max Canopy Density 2011	Stdev Tmax Trend 1895-2012	Min Slope
Mean Imperviousness 2011	Distance to Roads	Min PPT Trend 1895-2012
Std dev Imperviousness 2011	%Water 1992	Max PPT Trend 1895-2012
Max Imperviousness 2011	%Non-Woody 1992	Min Tmin Trend 1895-2012
%Barren 2011	%Herbaceous 1992	Max Tmin Trend 1895-2012
%Forest 2011	%Agriculture 1992	Min Tmax Trend 1895-2012
%Shrub 2011	Mean Canopy Density 2001	Max Tmax Trend 1895-2012
%Herbacious 2011	Stdev Canopy Density 2001	Mean Elevation
%Agriculture 2011	Min Canopy Density 2001	Stdev Elevation
%Wetland 2011	Max Canopy Density 2001	Min Elevation
%Developed 2011	Mean % Impervious Surface 2001	Max Elevation
%Developed Change 2001-2011	Stdev % Impervious Surface 2001	Mean Topographic Ruggedness Index
%Barren Change 2001-2011	Min % Impervious Surface 2001	Stdev Topographic Ruggedness Index
%Forest Change 2001-2011	Max % Impervious Surface 2001	Min Topographic Ruggedness Index
%Shrub Change 2001-2011	%Water 2001	Max Topographic Ruggedness Index
%Herbaceous Change 2001-2011	%Developed 2001	Mean Roughness
%Agriculture Change 2001-2011	%Barren 2001	Stdev Roughness
%Wetland Change 2001-2011	%Forest 2001	Min Roughness
Mean Slope	%Shrub 2001	Max Roughness

Green = Selected predictor in watershed-level model

Blue = Selected predictor in stream-level model

Orange = Selected predictor in both watershed- and stream-level models

Black = Variable available to, but not selected by either model

Gray = Variable removed prior to modeling (highly correlated to or represented by another variable)

Table 2. Spatially explicit reference and predictor data sources used in modeling *A. varicosa* condition

Using the data from Table 2, we generated 79 potential predictors of *A. varicosa* condition (Table 3). We used the National Elevation dataset to calculate meaningful topographic variables, including slope, Terrain Ruggedness Index (TRI), and roughness. Terrain ruggedness "is defined as the mean difference between a central pixel and its surrounding cells" (GDAL, 2016; see Wilson et al., 2007). "Roughness is the largest inter-cell difference of a central pixel and its surrounding cells," (GDAL, 2016; see Wilson et al., 2007). We calculated minimum, maximum, mean, and standard deviation values for each terrain variable. Terrain, in combination with other variables, like land use, provides insight into the level of runoff a watershed or stream may experience, as well as the degree of development (i.e., development is more difficult on steeper slopes).

Dataset	Data Source	Use
A. varicosa Element Occurrence Data	Individual state biologists	Model reference data
National Hydrography Dataset	United States Geological Survey	Stream buffer network analysis
National Elevation Dataset (and derivatives)	United States Geological Survey	Predictor
PRISM Historical Monthly Climate Grids (and derivatives)	Oregon State University	Predictor
National Land Cover Database - 1992 (and derivatives)	Multi-Resolution Land Characteristics (MRLC) consortium	Predictor
National Land Cover Database - 2001 (and derivatives)	Multi-Resolution Land Characteristics (MRLC) consortium	Predictor
National Land Cover Database - 2011 (and derivatives)	Multi-Resolution Land Characteristics (MRLC) consortium	Predictor
Roads (and derivatives)	Open Street Maps	Predictor

Table 3. Model predictors.

Freshwater mussels and their host fish have been shown to be sensitive to changing temperature and water regimes (see section 3). In an effort to capture large-scale temporal trends in historical climate over the last century, we downloaded modeled monthly climate layers from 1895- 2012 at a 4 km resolution for the entire study area. Variables included minimum and maximum temperature (Tmin, Tmax) as well as precipitation (PPT). We aggregated the monthly grids into annual means and calculated the rate of annual change at the individual cell level using ordinary least squares. To depict decadal changes in climate we multiplied our final predictor layers by 10.

Land use and its effect on water quality are linked to stream health and suitability for freshwater mussels and other fauna (Allan 2004; Strayer 2008; Haag 2012). We calculated the percentage of each land cover type from a simplified version of the hierarchical NLCD 2011 land cover map and land cover change map from 2001- 2011 (Homer et al. 2015).

We extracted each of the 79 predictor layers to the HUC12 watershed and the stream buffers using custom scripts written in Python and Bash.

Modeling Approach – We used RandomForests (Breiman, 2001) to model and predict wall-to-wall maps of habitat condition within states known to have either current or historical *A. varicosa* populations. RandomForests is a nonparametric, machine learning algorithm that performs recursive partitioning of the predictor data using different subsets of bootstrapped samples to estimate a large number of tree-based classifications, resulting in an ensemble model that is generally very accurate and less sensitive to noise within the data. We chose this modeling approach because it has been shown to be robust in a number of ecological applications, including other projects modeling mussel habitat (for example, Cao et al. 2013; Prie et al. 2014; Cao et al., 2015), and it is able to capture complex (i.e., nonlinear) relationships between *in situ* field observations the surrounding physical environment.

Variable Selection – We had 79 variables available to predict *A. varicosa* condition at both scales (Table 3); however, our goal was to generate an ecologically interpretable, accurate model. Removing redundant variables (i.e., variables that overlap in statistical space) was an important step in producing a useful model.

Before modeling, we performed a range of variable selection procedures to reduce the number of variables without sacrificing model performance. First, we removed all of the 2001 land cover variables, as they were implicitly represented by the 2011 National Land Cover Database products, which includes land cover change from 2001 - 2011. We also calculated pairwise Spearman correlation coefficients, a nonparametric measure of correlation, between all predictor variables and removed one member of each pair that had a correlation coefficient greater than 0.75. We performed a second test for multicollinearity using qr-matrix decomposition as implemented in the multi.collinear function in the rfUtils package in R (threshold = 0.05) (Murphy et al., 2010). These preliminary variable selection procedures helped us to reduce the number of predictors from 78 to 35 (Table 3, all records except gray), which is still quite high for an interpretable classification. These 35 predictors were available to both the watershed- and stream-scale models.

To achieve the final set of model predictors at each scale, we used Murphy et al. (2010) model selection approach from the rfUtils package in R. The approach begins with an initial model that includes all 35 predictors and calculates a Model Improvement Ratio (MIR) for each metric. Then it iterates through MIR thresholds from 0-1 in increments of 0.1, retaining all metrics above the specified threshold for competing models (default=0.03). Finally, it selects the minimum set of variables that both minimizes model error, and maximizes the variation explained by the model. For both models, the number of model inputs was further reduced from 35 to a much more manageable and interpretable 9 predictors, though they were not identical sets of variables (Table 3).

With our optimized set of predictor variables for each scale, we developed randomForests models with 1,000 trees (number of bootstrap iterations). In the HUC12 model, we had 265 single-condition watersheds (see Data Preparation section) comprising 29 good, 40 fair, and 196 poor/extinct observations. The stream-scale model had 692 observations, with 224 good, 242 fair, and 226 poor/extinct points.

We experimented with withholding as much as 20% of the training samples to perform an independent model validation on both models. Independent validation results were similar to the bootstrapped results, which gave us confidence in the model's performance. Ultimately, because we had relatively few samples collected under a variety of conditions and by many individuals (i.e., a lot of variability), our models performed better with all samples included, so we proceeded with that approach knowing the independent validation was satisfactory.

Modeling Results

EO Condition – Using the stream-level EO data that we reclassified (for modeling purposes) into good, fair, and poor or extinct observations, we were able to visualize the contributions of data from each state as well as quickly evaluate the overall health of known populations of *A. varicosa* in each state (Figure 20). Maine submitted the most EOs (136), and there was great variation in condition among classes between the states. The same graph could not be accurately generated for the HUC12-level training data, as watershed-level condition was often aggregated from multiple EOs, including EOs from multiple states when watershed boundaries crossed state lines.

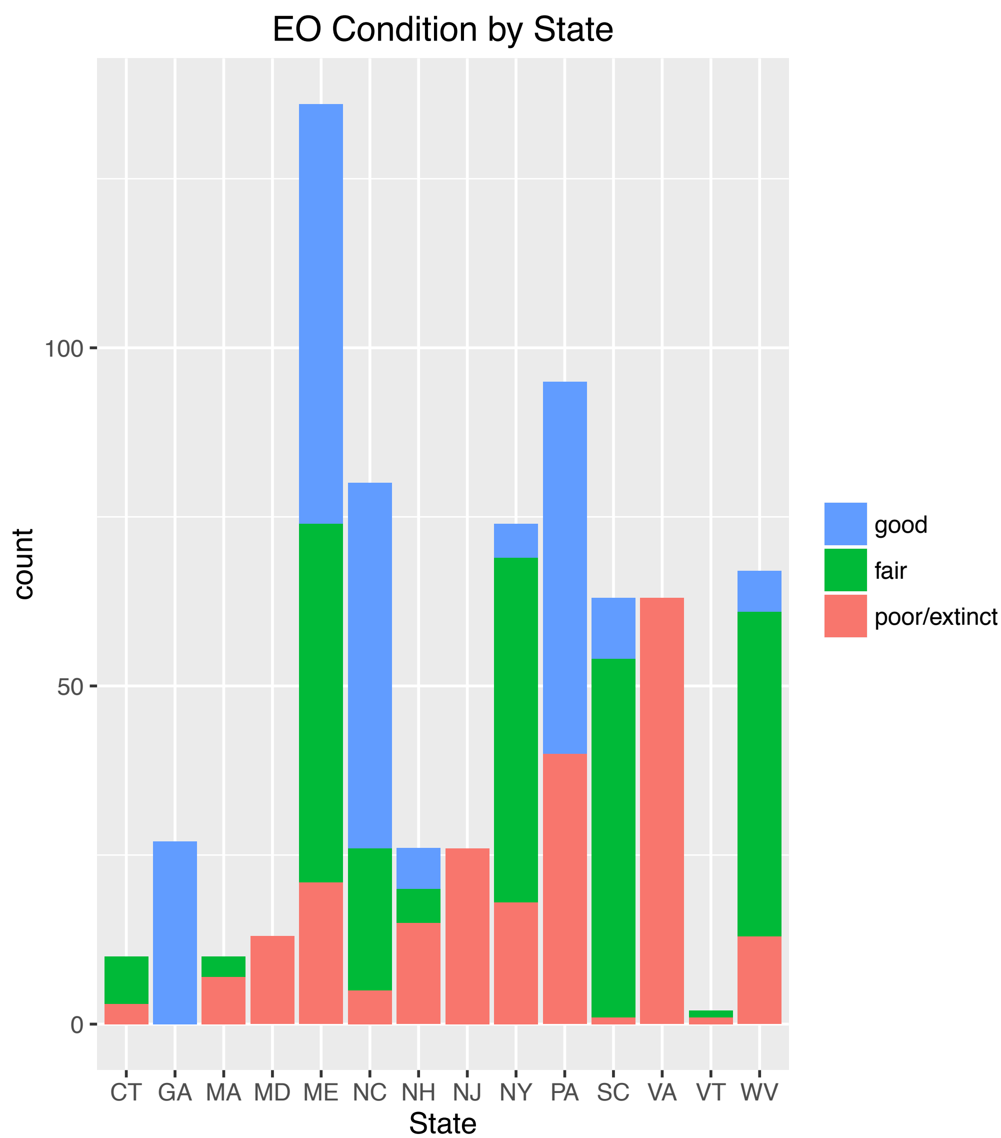


Figure 20. Per-state EO contributions to the stream-level modeling effort, including count and rank information.

Predictor Variables – Climate and land cover dominated our models as important variables. Changes in climate over the last century exhibit strong and varied spatial trends (Figures 21-23). For example, precipitation (Figure 21) tends to increase in the northern part of the study area, decrease in the Smoky Mountains, and remain fairly stable in non-coastal southern areas. Maximum temperature (Tmax) (Figure 22) has increased as much as 0.3 ^{0}C/decade in the Northeast, with the exception of Maine, which showed a weaker increase in Tmax compared to other states. As with precipitation, the southern states have been more resistant to changes in Tmax. Of the three climate trends we computed, we observed the greatest changes in minimum temperature (Tmin) (Figure 23). Maine, in particular, was affected by large changes in Tmin despite experiencing minimal changes to Tmax.

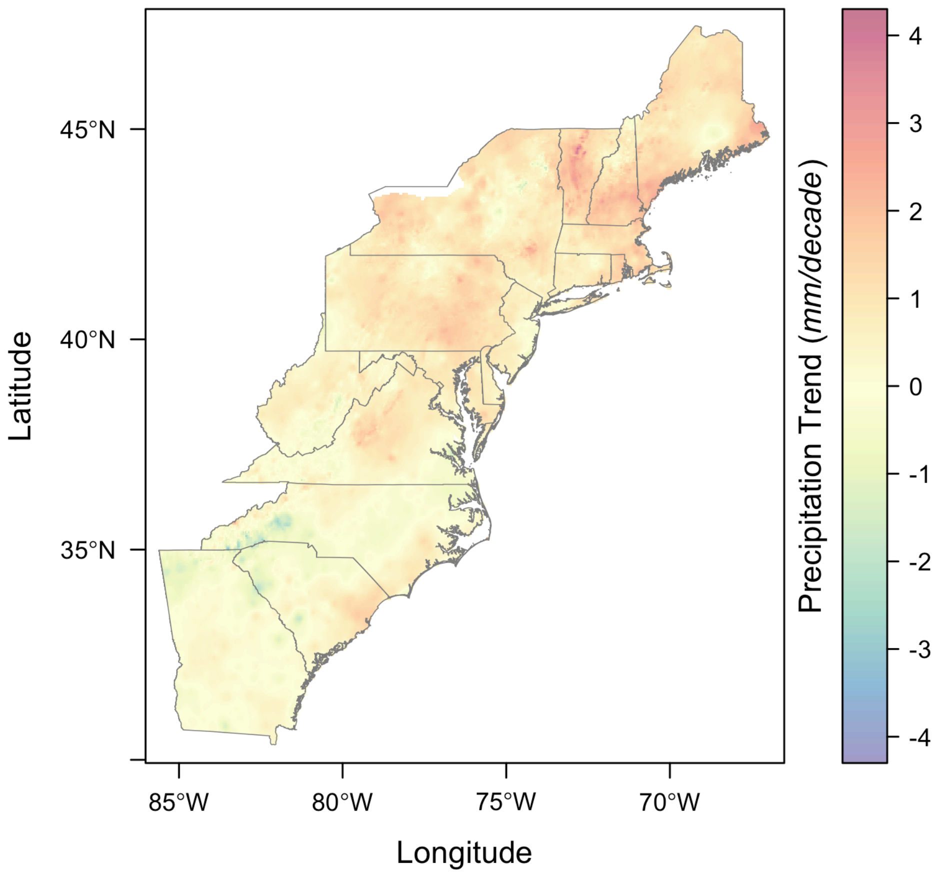


Figure 21. Decadal trends in precipitation between 1895-2012.

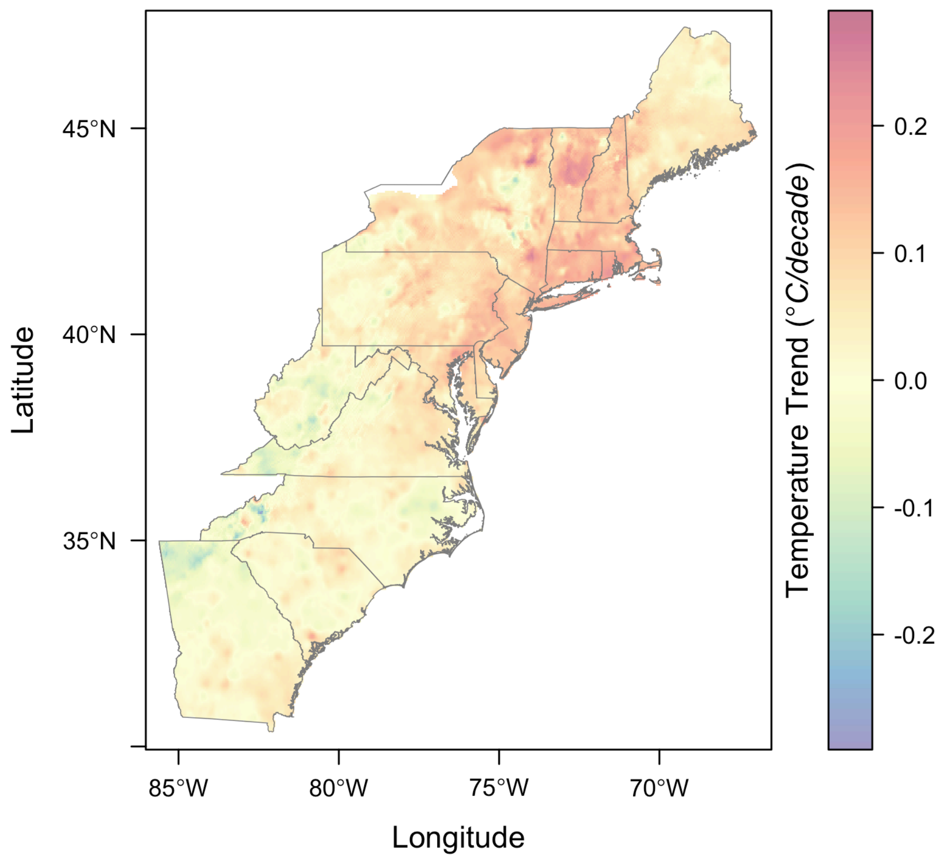


Figure 22. Decadal trends in maximum temperature between 1895-2012.

In some watersheds within the study area, land use also showed significant changes that could affect water quality and habitat suitability for *A. varicosa*. Figure 24 shows a binary change/no change classification over the entire study area between 2001 and 2011 based on the National Land Cover Database (Homer et al., 2015).

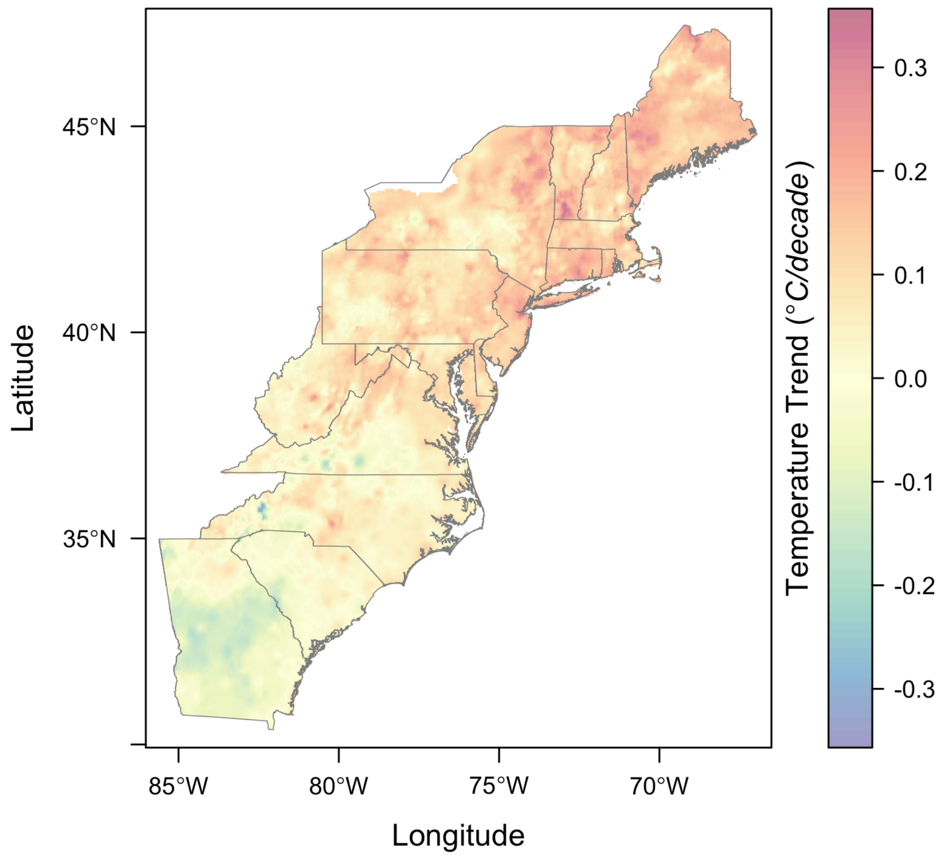


Figure 23. Decadal trends in minimum temperature between 1895-2012.

Once linked with the EO model training data, we were able analyze trends in predictor variables as they relate to *A. varicosa* condition. At the stream buffer scale (finest scale), stacked histograms of each predictor show the distribution of each model variable by condition (Figure 25). For example, within the distribution of forest cover (Figure 25, upper left, 1992 and upper right, 2011), higher forest cover is associated with better EO condition; conversely, there are more poor/extinct observations at lower levels of forest cover. For the climate trends variables, the greatest number of good and fair observations tends to occur where climate has been stable (near the middle of the histogram in Figure 25).

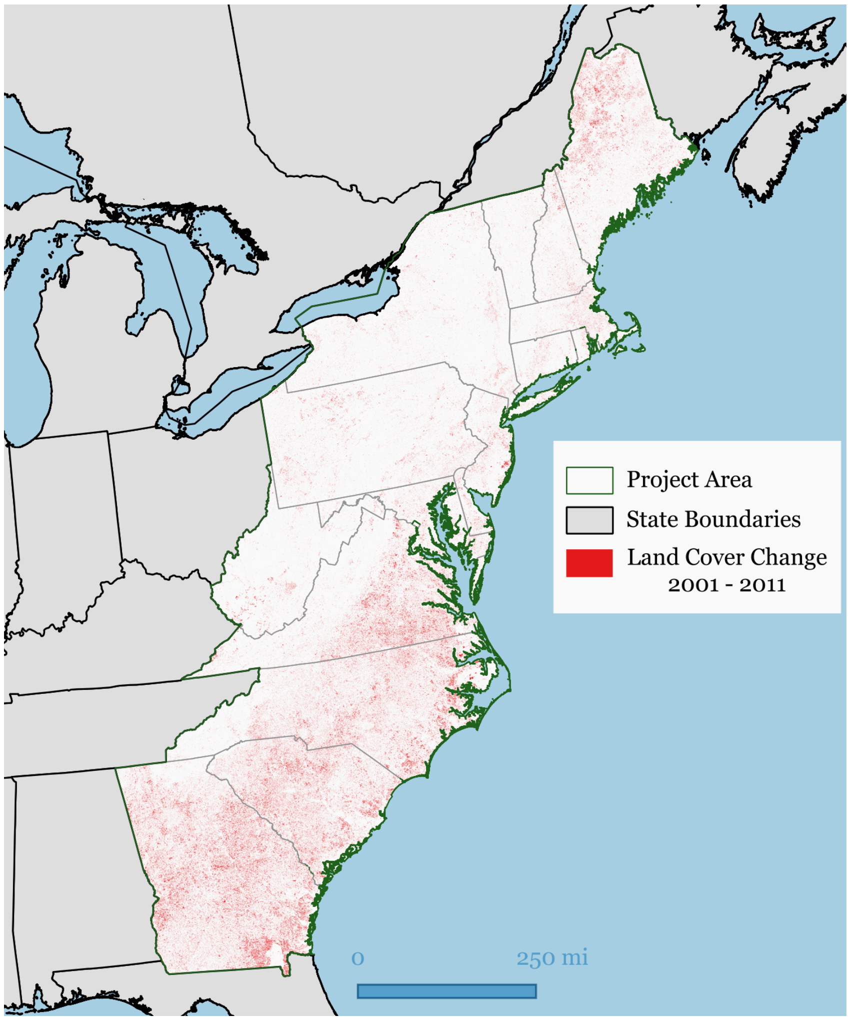


Figure 24. National Land Cover Database land cover change for the 2001-2011 time period. Red pixels indicate change, and white means no change.

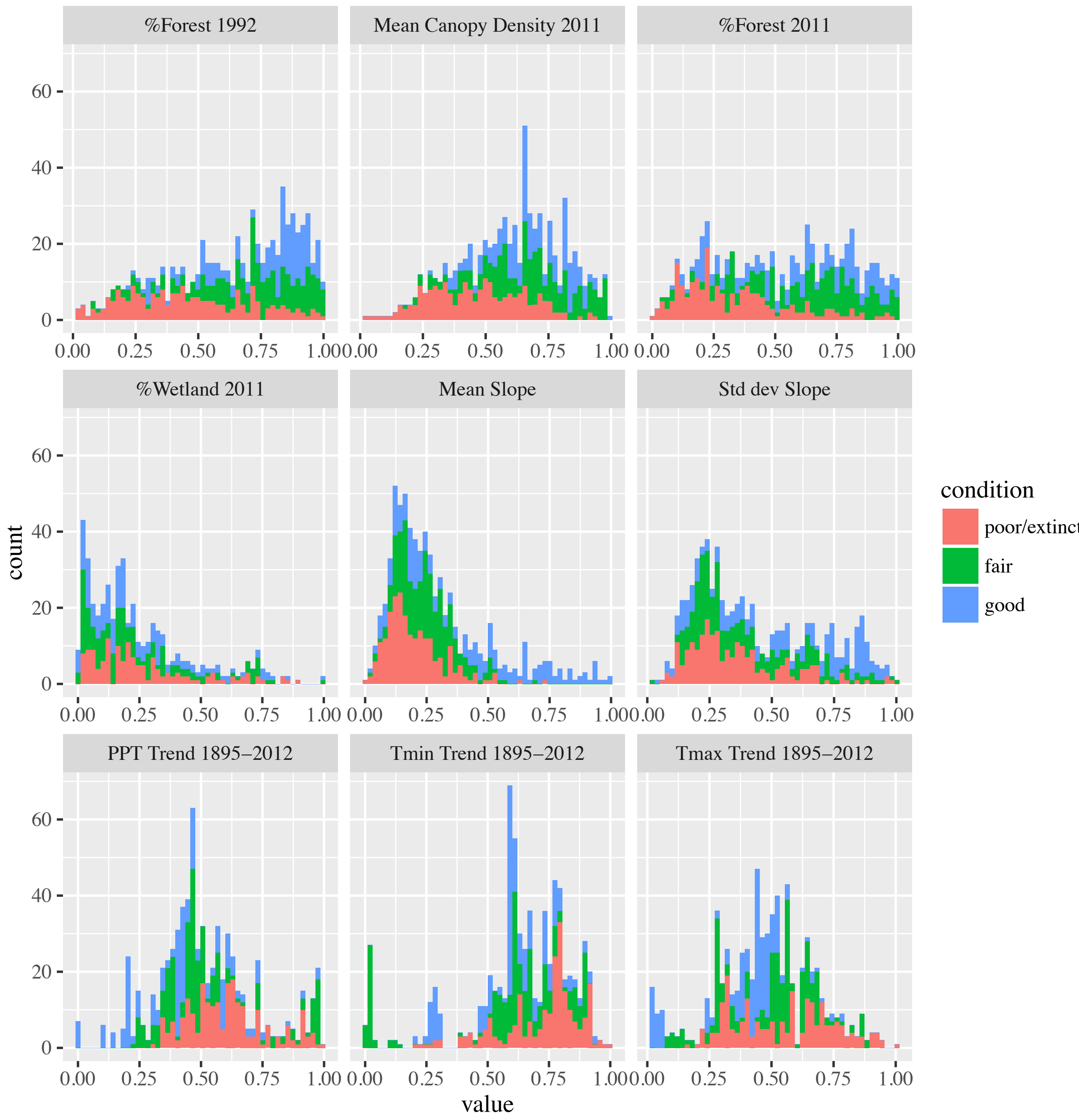


Figure 25. Histograms of each stream-level model predictor, stacked by EO condition. The x-axis has been normalized 0 - 1 for comparative purposes and for illustration only; the raw values were used for modeling. Due to the normalization, the climate variables tend toward stability near the middle of the histogram, with negative trends to the left, and positive trends to the right.

A. Watershed Model and Limitations – The randomForest watershed (HUC12) model had an R^2 of 0.86 (n=265). Individual class accuracies revealed very high classification accuracy for the predominant class (poor/extinct = 96%); however, the good and fair classes had 66% and 50% accuracy respectively. RandomForest is very sensitive to class imbalances, which likely accounted for these large differences in class accuracies.

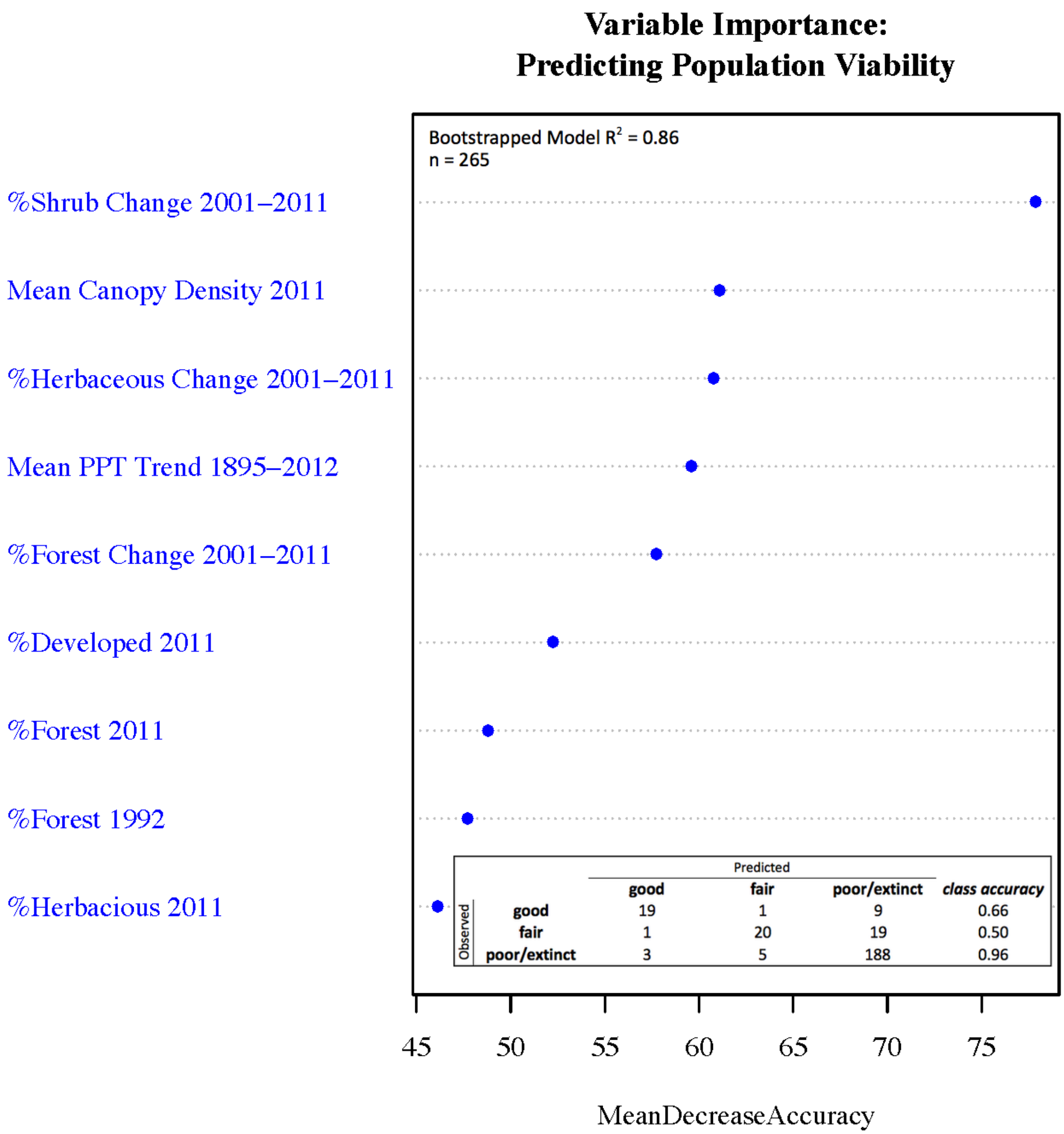


Figure 26. HUC12-scale randomForests model variable importance plot showing the average decrease in accuracy when each variable is removed. In this model, the change in shrub cover between 2001 and 2011 is the most important variable. The table provides per-class accuracy and shows where the model made errors (when the observed and predicted conditions differ).

Eight of the nine predictors in the model were land cover variables, indicating that at this scale, land cover is a very important correlate of EO condition (Figure 26). The Multi-dimensional Scaling (MDS) plot is a visual aid that shows how separable our classes are from one another (Figure 27). It illustrates a proximity measure calculated by randomForests to show how similar (or dissimilar) the training samples are. When class points are grouped into distinct clusters, the interpretation is that the model was better able to distinguish between classes than if the class points are spread throughout the plot. The computation is a Principle Components Analysis of the proximity table computed by randomForests that reduces the dimensionality down to 2-D and identifies any clear clusters in the data. Figure 27 reflects the challenges we had in separating the "fair" and “good” classes in the watershed model, revealing a high level of dispersion and confusion between classes.

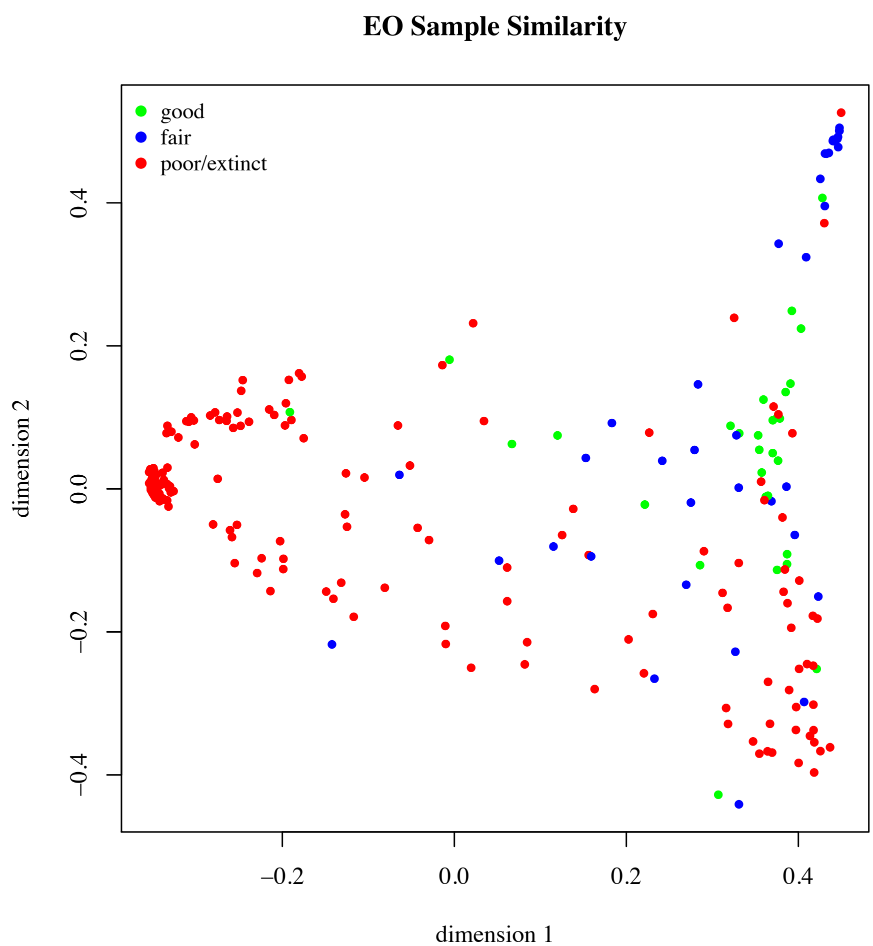


Figure 27. HUC12-scale randomForests model MDS plot shows relative separability between classes in the model.

Despite the challenges in achieving stable and consistent accuracy across classes with this model, the overall R^2 was high enough that we decided to predict each HUC12 watershed within the study area and produce a map (Figure 28). Given the apparent inter-class confusion, we do not have as much confidence in this map (or model) as compared to the stream-level map. It should therefore be used with caution for decision-making. Due to insufficient number of samples and confusion between training classes, this model may be improved with an even more simplified 2-class structure of "viable" and "nonviable," which may increase separability and reduce model confusion.

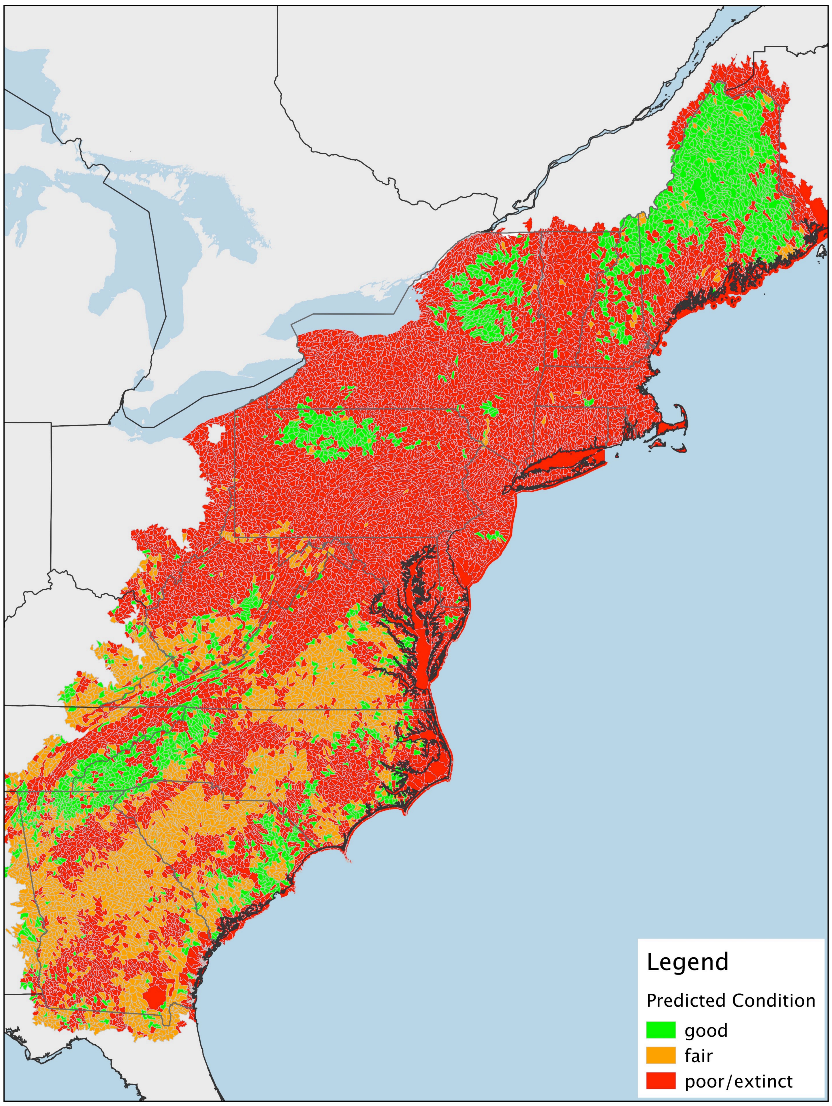
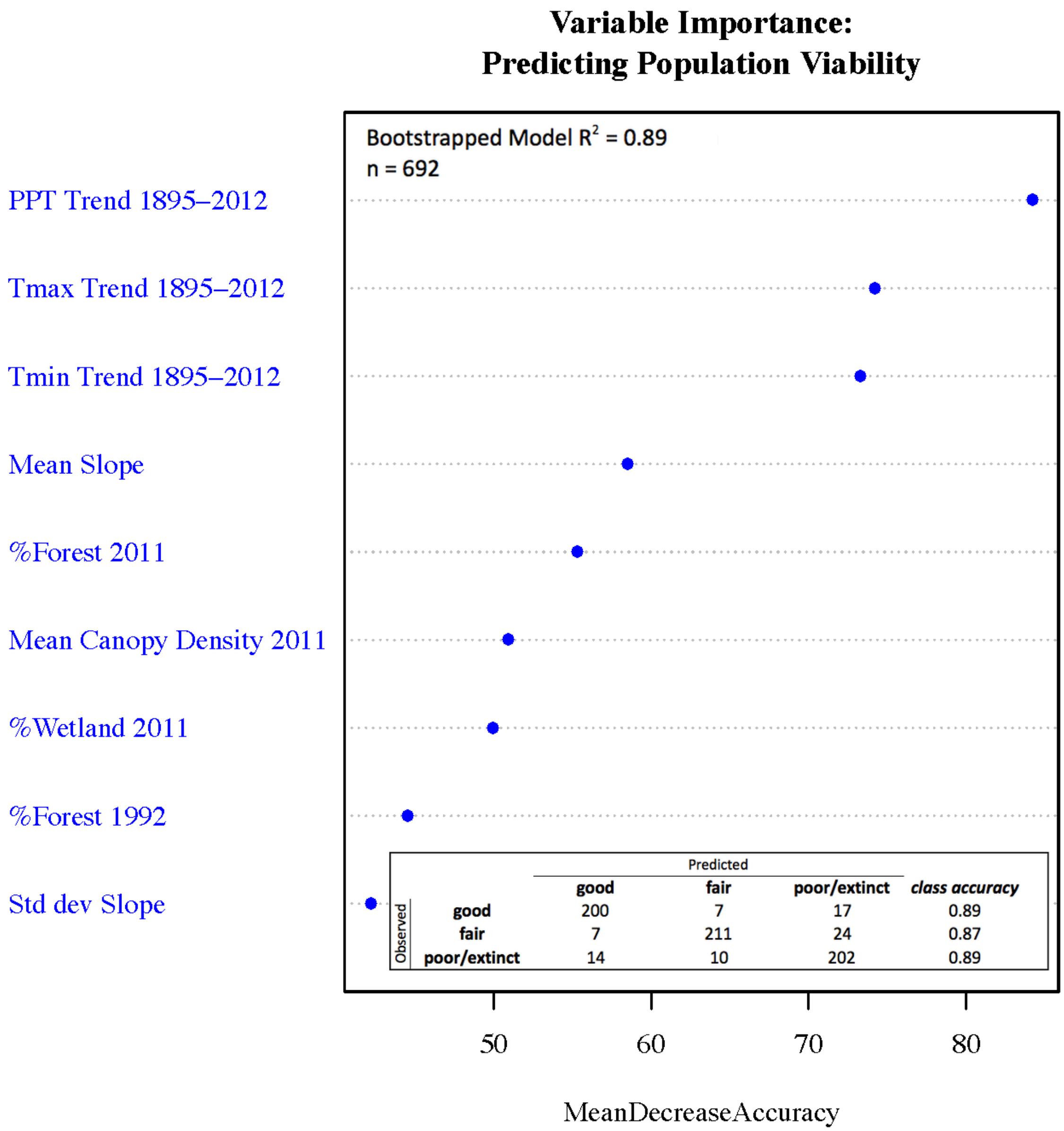


Figure 28. HUC12 model prediction. Each watershed in the study area was assigned a predicted condition based on the randomForests model. Note that the map covers area outside the Atlantic slope.

B. Stream Model, Prediction, and Limitations – The randomForest stream-level model had an R^2 of 0.89 (n=692), and class accuracies were much more balanced than the watershed model: 89%, 87%, and 89% for the good, fair, and poor/extinct classes, respectively. The most important variables in this model were a mix of climate, topography, and land cover layers (Figure 29). Worth noting is the dominance of climate variables in this model; climate trends represent the top three most important variables at the stream level. The MDS plot for the stream model shows much stronger sample clustering than the watershed model, which indicates a higher level of separability in this model (Figure 30). For these reasons, we have more confidence in the results of this model than in the watershed model results.



Observed	good	fair	poor/extinct	class accuracy
good	200	7	17	0.89
fair	7	211	24	0.87
poor/extinct	14	10	202	0.89

Figure 29. Stream-scale randomForests model variable importance plot showing the average decrease in accuracy when each variable is removed. In this model, the decadal precipitation trend is the most important variable. The table provides per-class accuracy and shows where the model made errors (when the observed and predicted conditions differ).

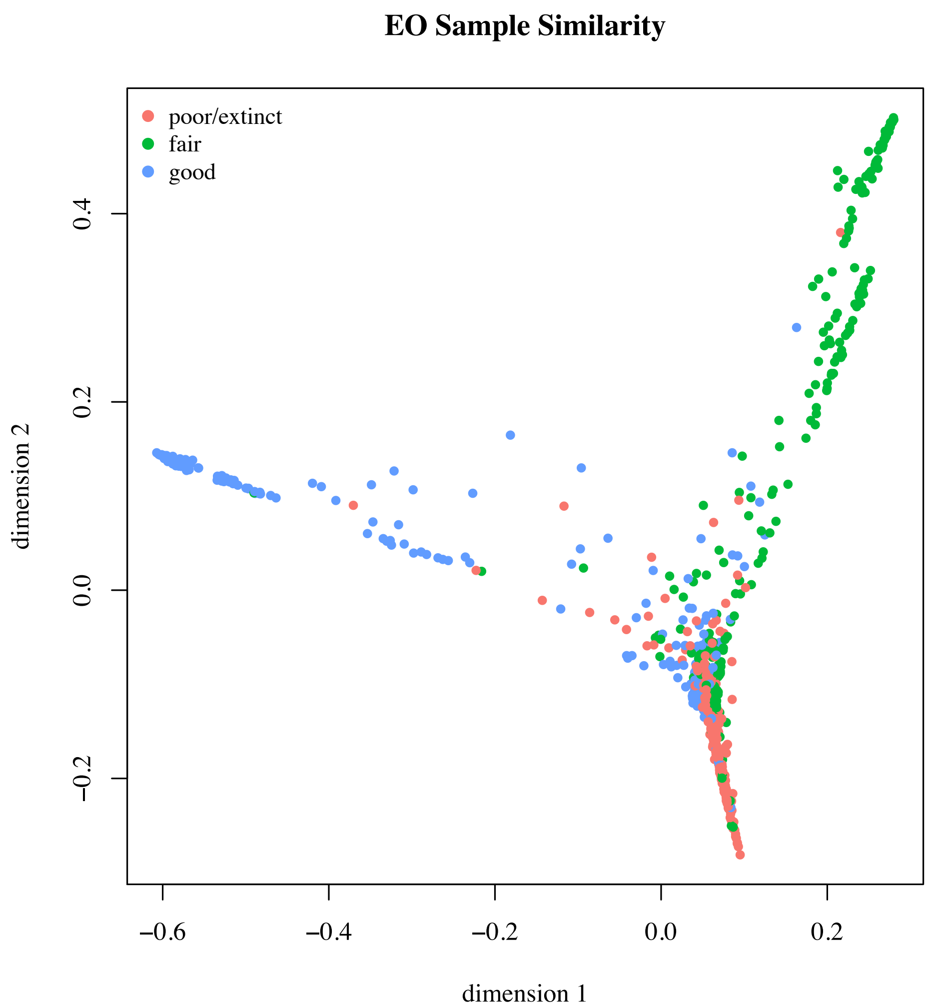


Figure 30. Stream-scale randomForests model MDS plot shows relative separability between classes in the model.

We predicted stream condition for each of the 3,257,359 stream segments in the NHD dataset within the study area. Streams were predicted as good, fair, or poor/extinct, and we present them as a single, project-wide map (Figure 31; Table 4), as well as by state due to the detail in each one (see Appendix 1 Figures 32–46). When comparing the HUC12-scale map (Figure 28) and the stream-scale map (Figure 31), we observe similar overall trends, which is encouraging given some of the challenges we had with the HUC12 model. The finer-scale map is more nuanced, as we would expect. The HUC12 map is necessarily more general due to the averaging of predictor data within entire watersheds, rather than the immediate area surrounding each EO. We have greatest confidence in areas where both maps predict the same condition.

	Good	Fair	Poor/Extinct	Total
Number of streams	479,844	641,829	2,135,713	3,257,386
Percent of total	14.7%	19.7%	65.6%	100.00%

Table 4. Stream-level model prediction summary.

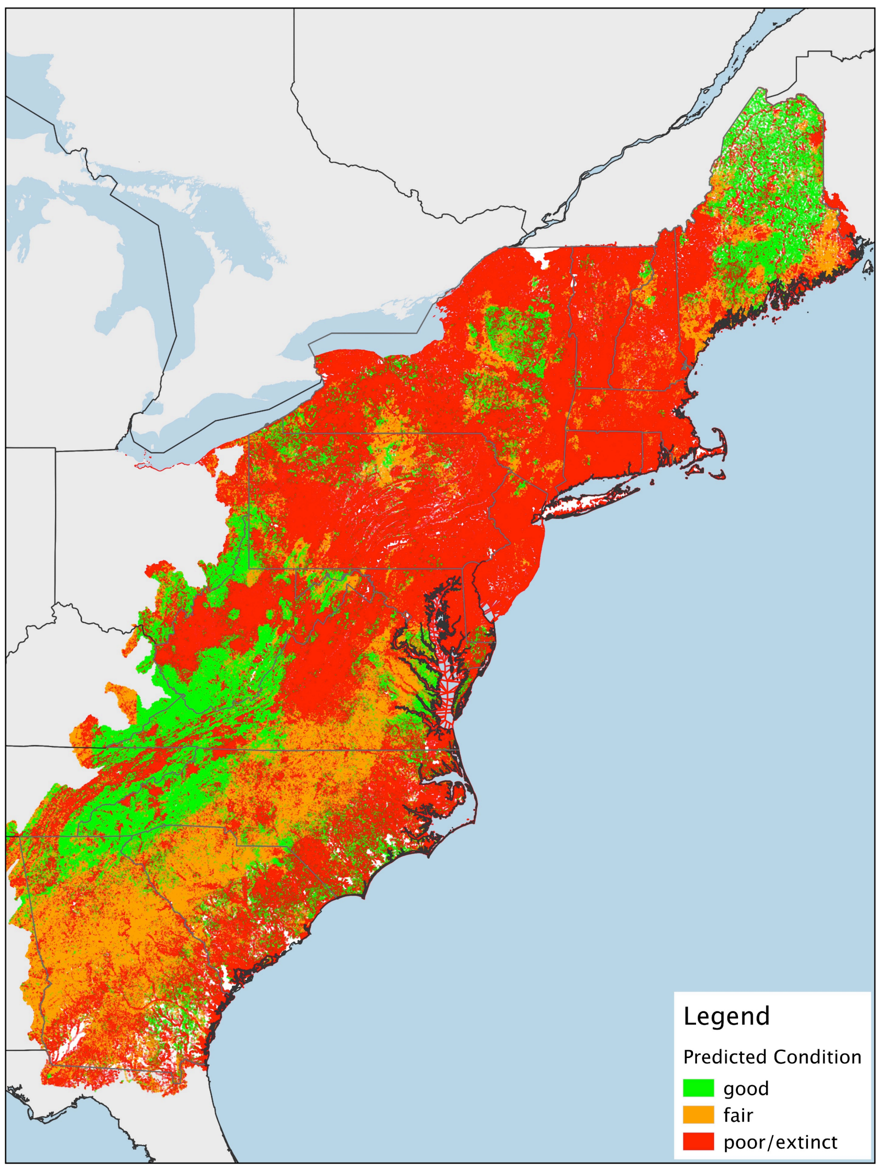


Figure 31. Stream-scale randomForest model prediction map for the project area. Note that the map covers area outside the Atlantic slope.

The prediction reflects environmental conditions that are associated with EO rank, but it has limitations that must be considered when using the model for decision-making. First, it is a model - a generalization of reality based on trends in the training data and predictor variables. As such, the model is not perfect and should be used as one of many tools for identifying potentially suitable conditions for *A. varicosa*; it does not predict *A. varicosa* presence or absence, but rather, characteristics that are associated with population condition based only on limited model inputs. The inputs are a set of available GIS layers that are uniform across the entire study area and behave as surrogates for important predictors of condition, including land cover, climate, and topography. These inputs vary in scale, specificity, and importance, and they are not all-inclusive. Examples of important and potentially constraining explanatory variables that were not available to our modeling effort include water quality, sediment type, invasive species, barriers to aquatic life passage, and predictors related to host fish presence and health, among others.

The predictions are based solely on the statistical relationships between EO condition and physical characteristics at specific locations; predicted *A. varicosa* condition is a result of matching physical characteristics at known locations with those in other locations throughout the study area. The models may be updated on a state-by-state basis (if there are enough training samples) with additional or improved state-level input data. Model prediction results should be reviewed and verified by state biologists. Biologist input is crucial in interpreting and using the models, as the models are based only on a limited set of physical characteristics, and expert review can help us identify areas where the prediction needs refinement or editing.

Other limitations include heterogeneity in the EO data itself, which were used to train the models. These data were collected and ranked by numerous people in each of the 15 states in the study area, necessarily resulting in inconsistencies. The data were delivered to us in multiple formats (points, lines, polygons) and with various attributes, and standardizing them necessarily introduced error. The EO data were also temporally diverse, with records collected as early as the 1800s and as recent as 2016. Matching those data with relevant GIS data from a single time period is challenging and likely a significant source of error. Despite these limitations and challenges we were able to achieve very accurate models of condition across the study area.

Model Conclusions

Our climate models are in general agreement with reports by Horton et al. (2014) for the Northeast and Carter at al. (2014) for the Southeast (Figures 21-23). Our models show that between 1895-2012, decadal trends in precipitation increased in the Northeast, as did trends in maximum temperature (0.3 ^{0}C/decade) and minimum temperature. Although Maine showed weak trends in maximum temperature it was affected by an especially strong increasing trend in minimum temperature. Horton et al. (2014) reported a 70% increase rainfall during extreme precipitation events between 1958-2012 as well as expected higher summer temperatures and droughts in the Northeast. The Southeast was more resistant to changes in both precipitation and temperature trends from 1895-2012; however, Carter et al. (2014) note that temperatures have increased by nearly 1.1 ^{0}C since 1970, heavy rainfall events and droughts have increased and extreme heat events will become more frequent, more intense and last longer. In our models minimum temperature showed the greatest increase of all three climate trends.

We used the National Land Cover Database to show changes in land cover between 2001-2011 (Figure 24). Changes in land cover can reflect timber harvest such as northern Maine as well as increased residential, agricultural, commercial and urban development. Increased human population growth – seen especially in the Southeast – is a strong driver of land cover change. For example rapid population growth accompanied by a rapid economic expansion in South Carolina has led to one of the fastest rates of land conversion to urban from rural in the nation (SC Wildlife Action Plan 2014, p. 1-1). We expect that these changes will adversely affect stream habitat and water quality and threaten *A. varicosa* populations.

Climate and land cover were important model variables at both the: 1) HUC12 watershed scale and 2) stream scale, encompassing a 100 m wide buffer of each stream that extends 1 km upstream from each EO.

HUC 12 Watershed Scale Predictors – Land cover was the predominant predictor of EO condition at the HUC 12 level. Eight of nine predictors at the watershed level were land use variables; the ninth was mean precipitation trend, 1895-2012 (Figure 26). Although the overall model accuracy was high with an R^2 of 0.86 (96% accuracy for poor/extinct EO classes), the principle components analysis of the proximity table computed by randomForests showed a high level of dispersion and confusion between “good” and “fair” EO classes, which showed accuracies of 66% and 50% respectively (Figure 27).

Stream Level Predictors – Overall accuracy at this scale was high with an R^2 of 0.89, and balanced with accuracies of 89%, 87%, and 89% for the good, fair, and poor/extinct classes, respectively (Figures 29 and 30). Forest cover was an important predictor of EO condition at the stream level (Figure 25). We found that better EO condition was strongly associated with higher mean canopy density and higher percent forest cover, whereas poor/extinct EO condition was correlated with lower canopy density and lower percent forest cover. We consider replacement of riparian forests with residential, agricultural, commercial and urban development as a major range-wide threat to *A. varicosa* populations (see section 3). Climate also was an important predictor at the stream level. Although most of the good and fair EO classes were associated with stable climate variables (precipitation trend, maximum temperature trend and minimum temperature trend) there was considerable overlap with poor/extinct EO classes. However, a cluster of poor/extinct EO classes was associated with higher minimum temperatures. Most EO classes showed a negative association with both mean slope and standard deviation of slope indicating a preference for lower gradient streams, however a large number in the good EO class were associated with higher slopes (that is, higher gradient streams). These streams would likely hold courser sediments and have greater interstitial water flow (Strayer 2008, p. 153). We also expect that landscapes with higher slopes may be less developable and therefore retain more forest cover than in landscapes with lower slopes. All EO classes showed a negative association with percent wetland.

Habitat Suitability Analysis – Using randomForests we assigned a predicted stream condition for each of the 3,257,359 stream segments in the NHD dataset within the study area. The model predicted only 14.7% of all stream segments in the study area to have conditions necessary to support "good" populations of *A. varicosa* (Table 4). Conversely, well over 2.1 million stream segments (> 65%) were classified as "poor."

We then mapped potential suitable conditions throughout the range at both HUC 12 watershed scale and the stream scale (Figures 28 and 31). Maps at both levels are similar in depicting areas of potential EO condition although the stream level map is more defined. For example, the predicted good condition on maps correspond to known good EO classes in the Penobscot River basin, Maine, the West Branch of the Susquehanna, Pennsylvania, the upper Potomac River basin, West Virginia, the upper Catawba River basin, North Carolina and the Chattooga River, South Carolina/Georgia (see section 2). The maps may be useful in depicting areas that *A. varicosa* is least likely to persist. However, these maps have limitations and should be used cautiously by resource managers (see model results section). Further, both the regional maps and individual state maps (see Appendix) show predicted stream condition for the entire 15 states including areas outside the Atlantic slope range of *A. varicosa*.

Part 5. Brook Floater Population Threats Survey

Summary

Our survey included seventy questions that rank threats to *A. varicosa* populations including spatial extent, severity, immediacy, certainty and reversibility of threats. There were 32 respondents. Altered hydrology due to dams, habitat fragmentation due to dams or other inhospitable impacts, loss of forested riparian buffers and agricultural runoff of nutrients or toxins all had high mean scores for spatial extent and severity of threat. The retention of riparian forests and reforestation of riparian buffers can ameliorate the impact of runoff from agriculture, residential development and impervious surfaces (see section 2). The threat from urbanization and development also had a high mean score for severity; threats of increased flood events and residual sediment contamination scored slightly lower. Most threats had high scores for immediacy but low scores for certainty. The highest mean scores for certainty were for threats of altered hydrology due to dams, habitat fragmentation due to dams or other inhospitable impacts, loss of forested riparian buffers, agricultural runoff of nutrients or toxins, urbanization and development and bridge and road construction. The highest four mean scores for reversibility (between reversibility difficult and irreversible) were: increased flood events, drought-induced mortality, residual sediment contamination and impacts from invasive animals. We consider the impact of invasive animals as a potential future threat to *A. varicosa* populations.

We also queried biologists for what they consider the most important research/management priorities aimed at preventing the decline of *A. varicosa* populations. The reintroduction/population augmentation and quantitative surveys and long-term monitoring of *A. varicosa* populations were considered the most important management priorities for the species; better enforcement of water quality regulations and measures to enhance riparian buffers were also deemed as management priorities. Reintroduction and population augmentation are important conservation tools; however, finding streams that have improved enough to warrant *A. varicosa* reintroduction may be challenging (see section 2). Additionally, our model predicts only 14.7 % of all stream segments in the study area to have conditions necessary to support "good" populations of *A. varicosa.* Nevertheless, reintroduction or augmentation may be the only means available to offset the disappearance of many *A. varicosa* populations.

Respondents judged demographic studies, ecological studies of habitat, studies to explain spatial patterns of populations as well as biology/life history studies as important in shaping strategies for conserving *A. varicosa* populations. Although range wide genetic research was deemed less important, we strongly suggest: (1) the use of eDNA to detect *A. varicosa* in streams where densities may be low and (2) the development of a genetic study – perhaps using mitochondrial cytochrome oxidase c – to assess range wide divergence of *A. varicosa.*

This research can help identify management units that may be present or perhaps reveal cryptic species.

Methods

Using Qualtrics survey software we developed and distributed survey questions to 48 state and federal and academic mussel experts from Maine to Georgia. Several state biologists did not receive the survey due to software filters and several more could not answer the survey questions because they had not worked with *A. varicosa.* This left a total of 32 respondents. We used threat characteristics and categories developed by Crisfield, E. and the Northeast Fish and Wildlife Diversity Technical Committee (2013, p. 30). Our survey included seventy questions that rank threats to *A. varicosa* populations including spatial extent, severity, immediacy, certainty and reversibility of threats. We also asked the name and location of streams where water quality and habitat have improved enough to consider reintroduction or augmentation of *A. varicosa* populations; the name and location of conservation priority sites due to healthy *A. varicosa* populations; and the name and location of conservation priority sites due to immediate threat to *A. varicosa* populations (we integrated the results of these three survey questions into the state summaries in section 2). Additionally, we queried biologists for what they consider the most important research/management priorities aimed at preventing the decline of *A. varicosa* populations.

We used the following summary graphs to show threat rankings and research/management priorities. The detailed survey results are found in Appendix 2.

Results

Spatial extent of threat – The percent habitat/population negatively affected by the threat was scored as 1 = localized (<10%); 2 = dispersed/patch (10-50%); 3 = pervasive (>50%). The loss of riparian forests received the highest scores for spatial extent followed by habitat fragmentation, agricultural runoff of nutrients and toxins, altered hydrology due to dams and urbanization and development (Figure 1).

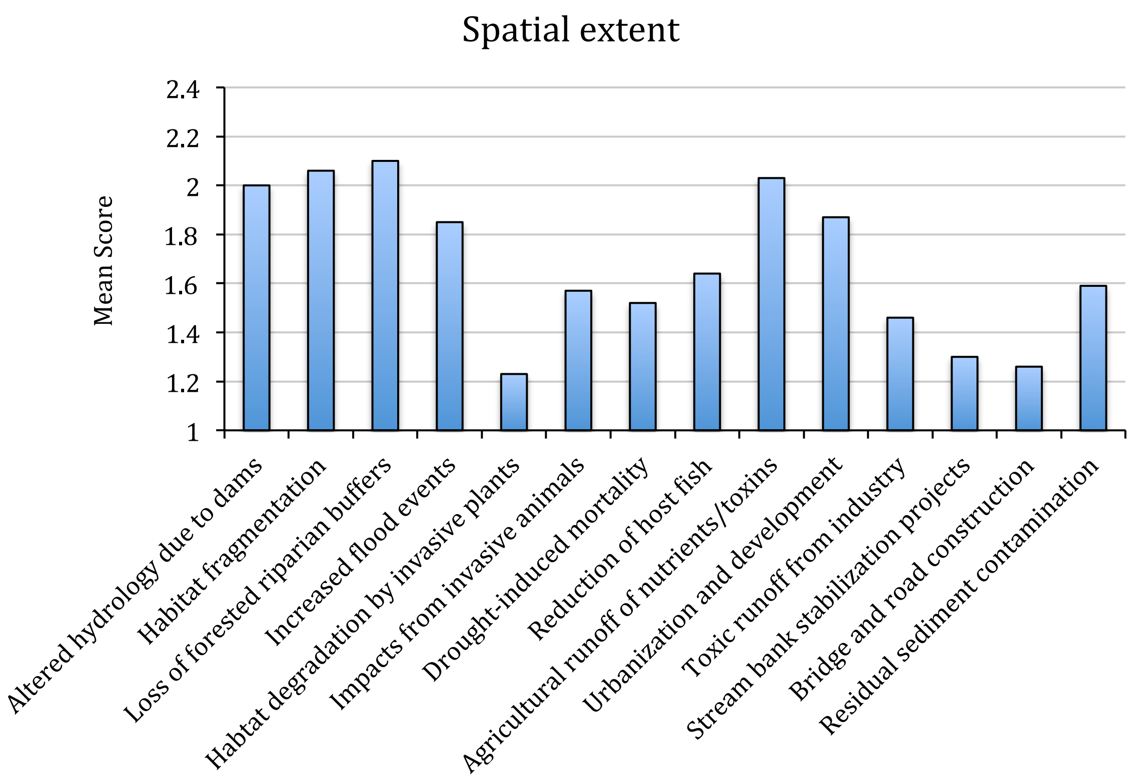


Figure 1. Mean scores for spatial extent of threat (% of the habitat/population negatively affected by the threat). Scores: 1 = localized (<10%); 2 = dispersed/patch (10-50%); 3 = pervasive (>50%).

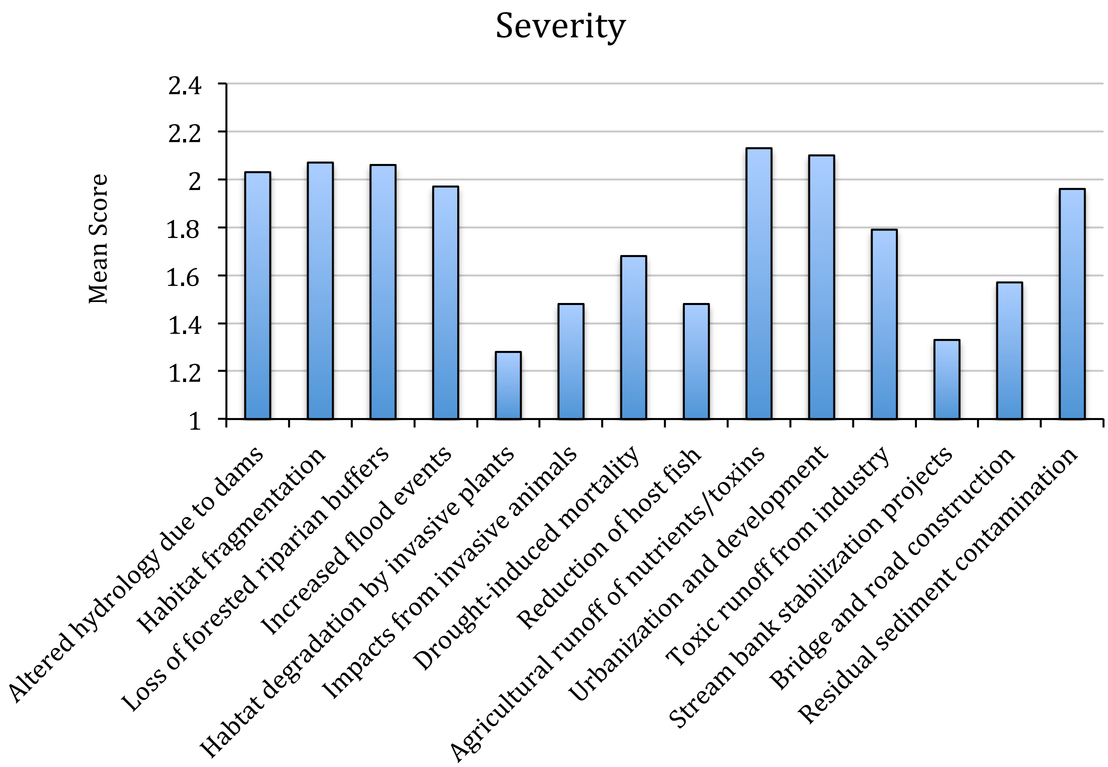


Figure 2. Mean scores for severity of threat (intensity of threat impacting exposed target under spatial extent). Scores: 1 = slight/minor; 2 = moderate/substantial; 3 = severe.

Severity of threat – Scores for intensity of threat impacting exposed target under spatial extent were: 1 = slight/minor; 2 = moderate/substantial; 3 = severe. Agricultural runoff of nutrients and toxins received the highest score for severity of threat and was followed closely by urbanization and development, habitat fragmentation, loss of riparian buffers, flood events and residual sediment contamination (Figure 2).

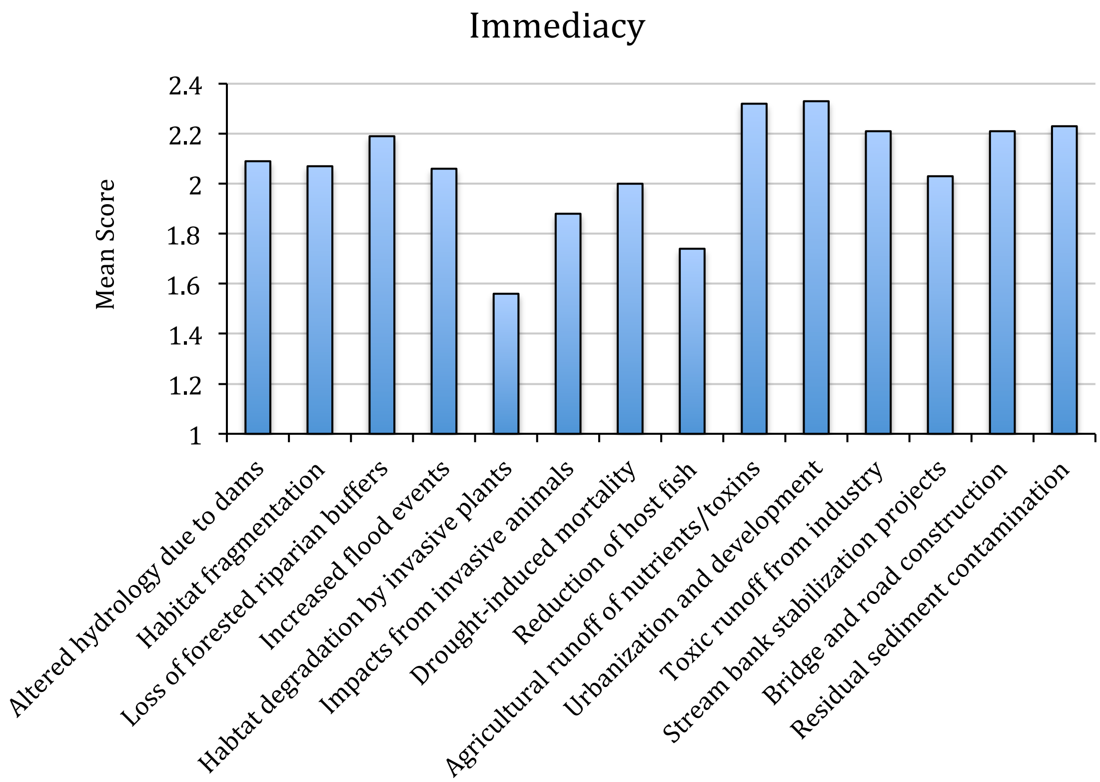


Figure 3. Mean scores for immediacy of threat time scale over which the impacts will be observable were 1 = long-term (10-100 years); 2 = near-term (1-10 years); 3 = immediate.

Immediacy of threat – Scores for the time scale over which the impacts will be observable were: 1 = long-term (10-100 years); 2 = near-term (1-10 years); 3 = immediate. Agricultural runoff of nutrients and toxins as well as urbanization and development received the highest scores for immediacy, however most threats received high scores for this category (Figure 3).

Certainty of threat – Scores for the amount of information/understanding of threat and response were: 1 = low; 2 = moderate; 3 = high. Although loss of riparian forests, agricultural runoff of nutrients and toxins as well as urbanization and development received the highest scores for certainty of threat, all categories scored between low and moderate (Figure 4).

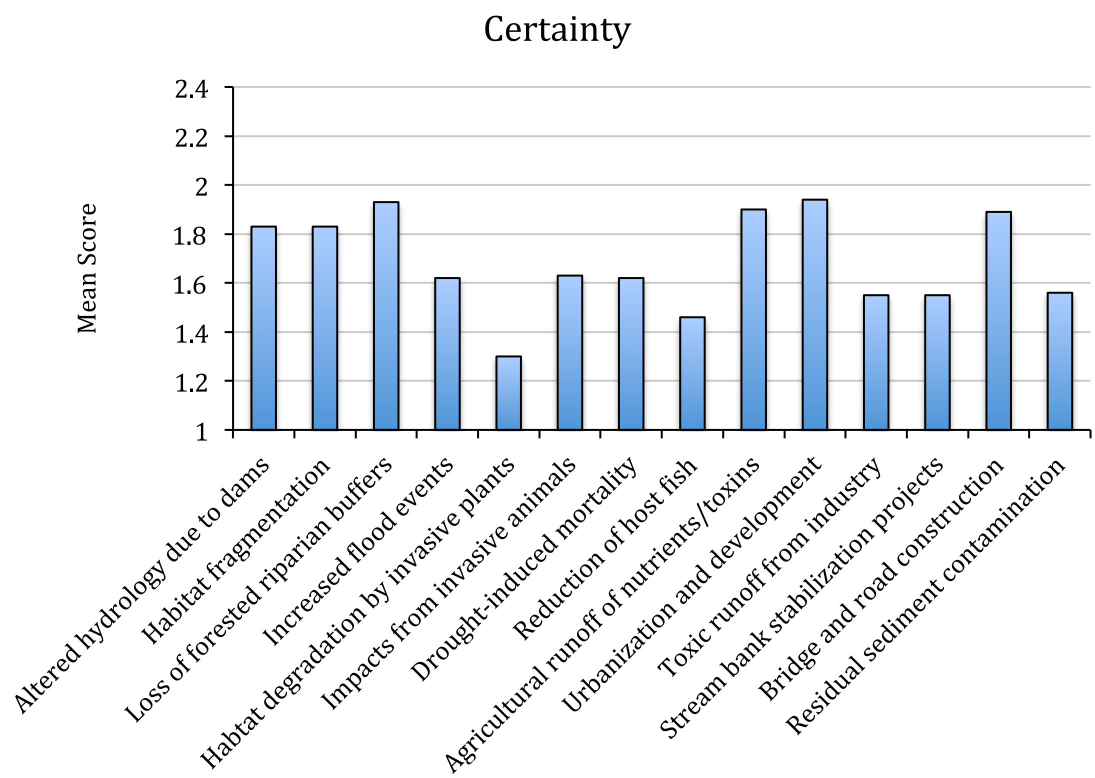


Figure 4. Mean scores for certainty of threat (amount of information/understanding of threat and response). Scores: 1 = low; 2 = moderate; 3 = high.

Reversibility of threat – Scores for the likelihood of reversing the impact within 10 years were: 1 = reversible; 2 = reversibility difficult; 3 = irreversible. Impacts from invasive animals, increased flood events, drought-induced mortality and residual sediment contamination all received high scores – meaning these categories were the most difficult to reverse. Bridge and road construction, stream bank stabilization projects, agricultural runoff of nutrients and toxins and loss of forested riparian forests scored as the most reversible categories (Figure 5).

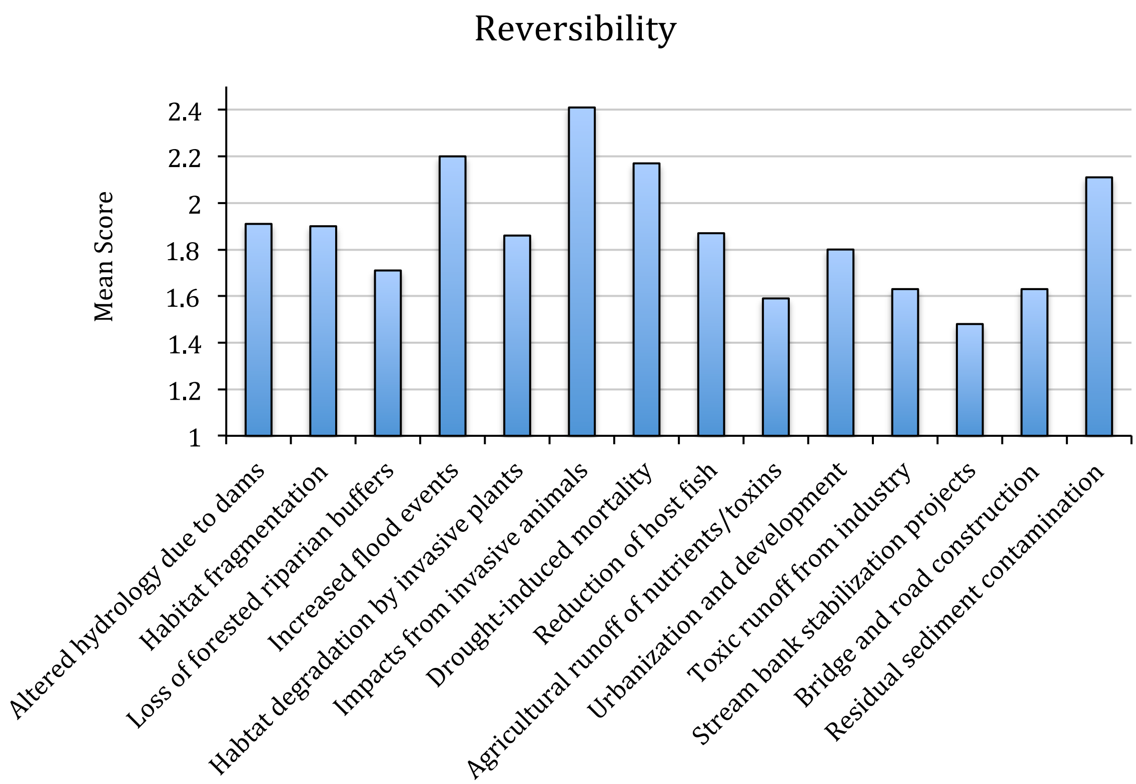


Figure 5. Mean scores for reversibility of threats (the likelihood of reversing the impact within 10 years). Scores: 1 = reversible; 2 = reversibility difficult; 3 = irreversible.

Research and Management Priorities – Respondents were asked to rate 12 research/management priorities (Table 1). Fifty percent or more of our respondents chose reintroduction or population augmentation, quantitative surveys and long-term monitoring, demographic studies, ecological studies of habitat requirements, better enforcement of water quality regulations, ecotoxicological studies and measures that enhance riparian buffers as priority research/management priorities. Over 38% of respondents chose studies to explain spatial patterns (patchiness) of populations and biology/life history studies as priority research/management priorities. Twenty-nine percent and 19% of respondents chose range wide genetics and additional water quality monitoring as research/management priorities, respectively (Table 1). Respondents added the following as research/management priorities: (1) seek protection of *A. varicosa* under the federal Endangered Species Act, (2) investigate possible hybridization between *A. varicosa* and *A. marginata*, (3) increase the percent forest at the watershed level, (4) protection of watersheds where *A. varicosa* occurs, (5) improved regulation of dam operation.

#	Answer	Bar	Response	%
1	Biology and life history studies		12	38.71%
2	Demographic studies (e.g. size, survivorship, sex ratio, age, recruitment)		19	61.29%
3	Quantitative surveys and long-term monitoring		20	64.52%
4	Ecotoxicological studies (the effects of chemical stressors on survivorship and recruitment)		16	51.61%
5	Ecological studies of habitat requirements		18	58.06%
6	Range wide genetic studies		9	29.03%
7	Reintroduction or population augmentation		21	67.74%
8	Strengthening of water quality regulations		14	45.16%
9	Better enforcement of water quality regulations		17	54.84%
10	Studies to explain spatial patterns (patchiness) of populations		14	45.16%
11	Additional water quality monitoring		6	19.35%
12	Measures that enhance riparian buffers		16	51.61%
13	Other		10	32.26%
	Total		192	100.00 %

Table 1. Respondent choices of research and management priorities aimed at protection of *A. varicosa*.

Conclusions

Respondents scored the loss of riparian forests, habitat fragmentation, agricultural runoff of nutrients and toxins, urbanization and development as both the most spatially extensive threats and the most severe threats. We believe that the retention of riparian forests will reduce impacts from agricultural runoff urbanization and development (see sections 2 and 3). Flood events and residual sediment contamination also received high scores for severity of threat. Whereas most categories received high scores for immediacy of threat, habitat degradation by invasive plants, impacts from invasive animals and reduction of host fish scored low for immediacy, severity and spatial extent of threat. Although we consider the impact of invasive animals as a potential future threat to *A. varicosa* populations, once established invasive animals are extremely difficult or impossible to eliminate. Respondents scored the impact of invasive animals as the most irreversible threat. Respondents also scored flood events and droughts – projected to increase in the future – as among the most irreversible impacts.

Respondents considered the reintroduction/population augmentation and quantitative surveys and long-term monitoring of *A. varicosa* populations as the most important management priorities for the species; better enforcement of water quality regulations and measures to enhance riparian buffers were also deemed as management priorities (Table 1). Using genetic guidelines (Jones et al. 2006, p. 529; Hoftyzer 2008, p. 1225) captive propagation, reintroduction and population augmentation have become important conservation tools in maintaining or rescuing mussel populations (see Haag 2012, pp. 406-418). However, substantial improvements in water quality, habitat, numbers of host fish, adequate food resources and other factors may be needed for these measures to be successful. For example, in the Czech Republic, despite a 25-year effort to enhance non-recruiting aged populations of the pearl mussel *Margaritifera margaritifera* by releasing fish bearing millions of glochidia and the release of 53,000 three to five year old juveniles, no natural reproduction has taken place (Simon et al. 2015, p. 18). The apparent cause was poor habitat quality. Finding streams that have improved enough to warrant *A. varicosa* reintroduction may be challenging (see section 2). Additionally, our models predict that only 14.7% of the over three million stream segments in the study area (all 15 states but including areas outside the Atlantic slope region) have conditions necessary to support "good" populations of *A. varicosa*. Nevertheless, reintroduction or augmentation may be the only means available to offset the disappearance of many *A. varicosa* populations.

Demographic studies, ecological studies of habitat, studies to explain spatial patterns of populations as well as biology/life history studies were all judged as important in shaping strategies for conserving *A. varicosa* populations. Only 29% of respondents chose range wide genetic studies as a research priority. However, we believe that based on the distribution range of *A. varicosa,* there may be several management units present or perhaps several cryptic species. We strongly suggest the development of a genetic study – perhaps using mitochondrial cytochrome oxidase c – to assess range wide divergence of *A. varicosa*.

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Appendix I. Stream-scale randomForest model prediction maps for the project area. Note that some maps cover areas outside the Atlantic slope.

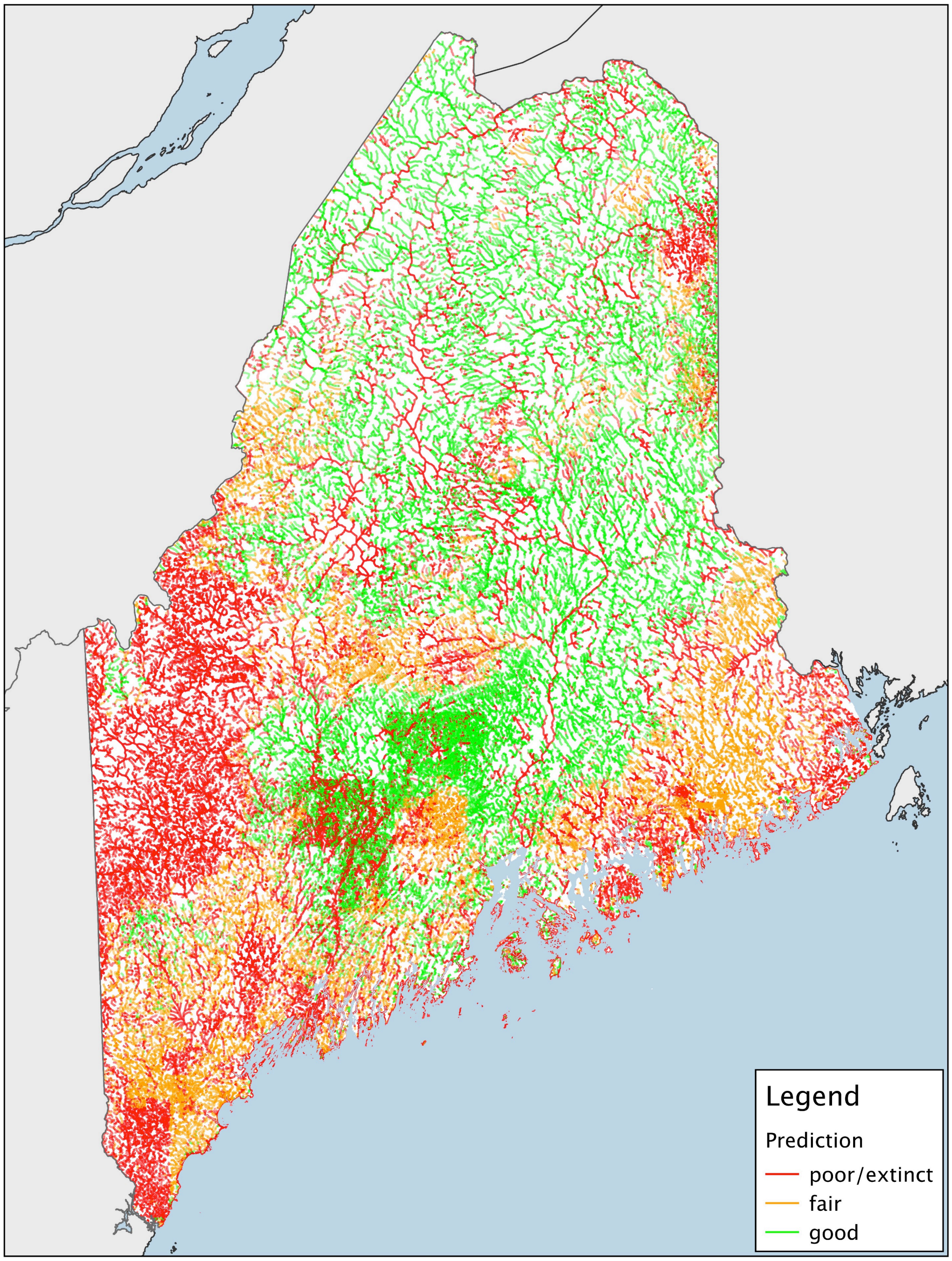

Figure 1. Stream-scale randomForest model prediction map for Maine. Note that the map covers area outside the Atlantic slope.

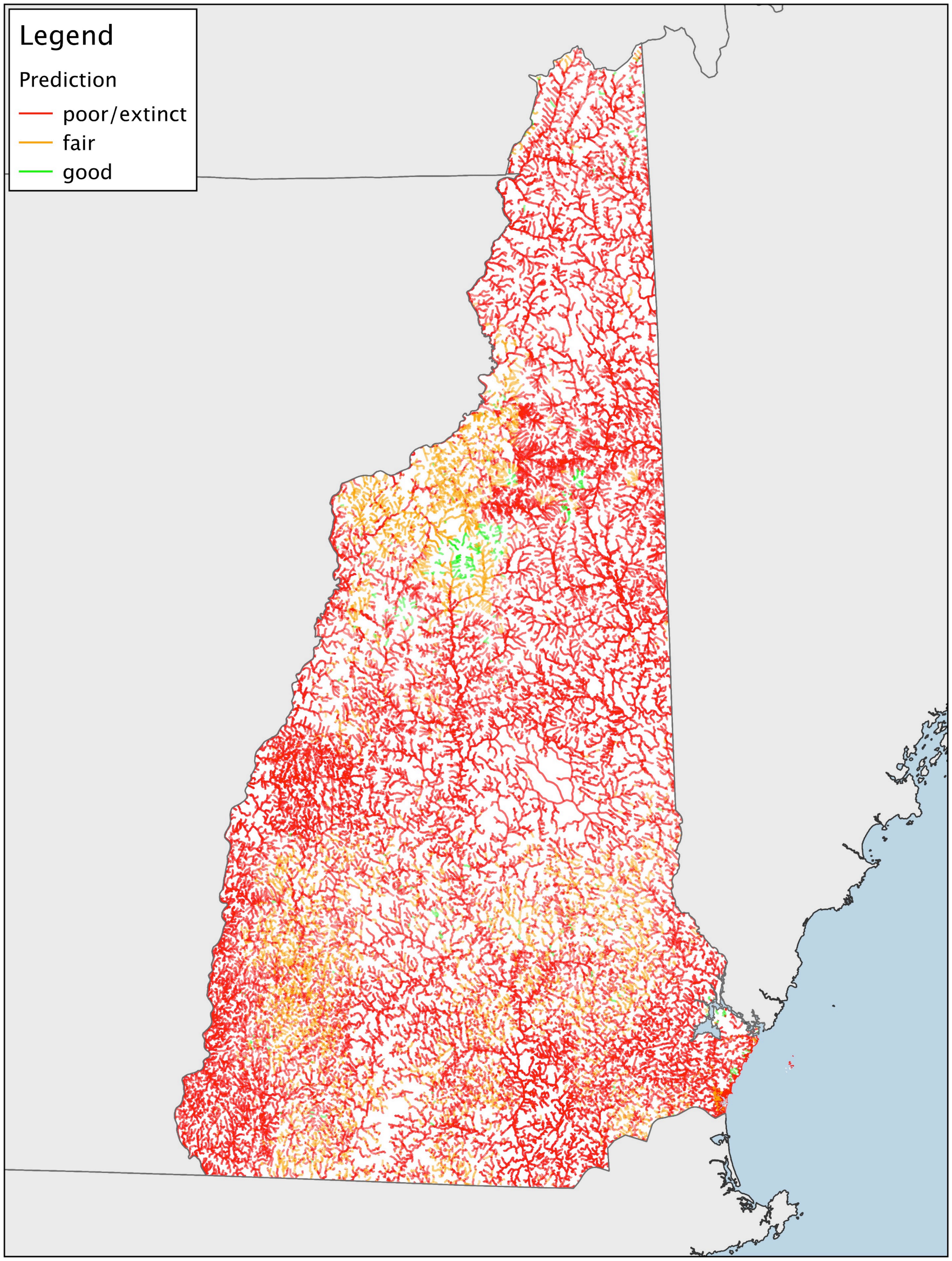

Figure 2. Stream-scale randomForest model prediction map for New Hampshire. Note that the map covers area outside the Atlantic slope.

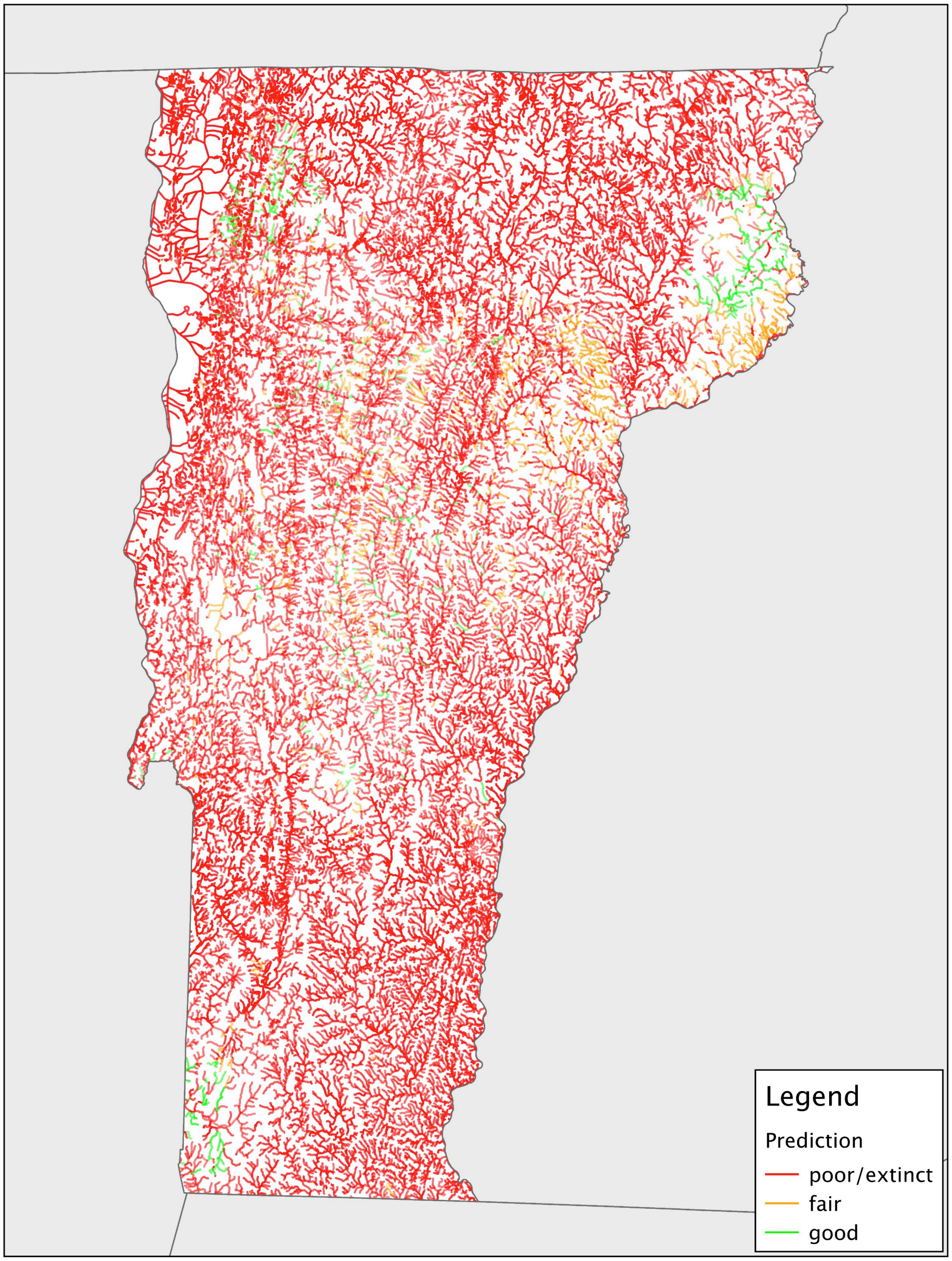

Figure 3. Stream-scale randomForest model prediction map for Vermont. Note that the map covers area outside the Atlantic slope.

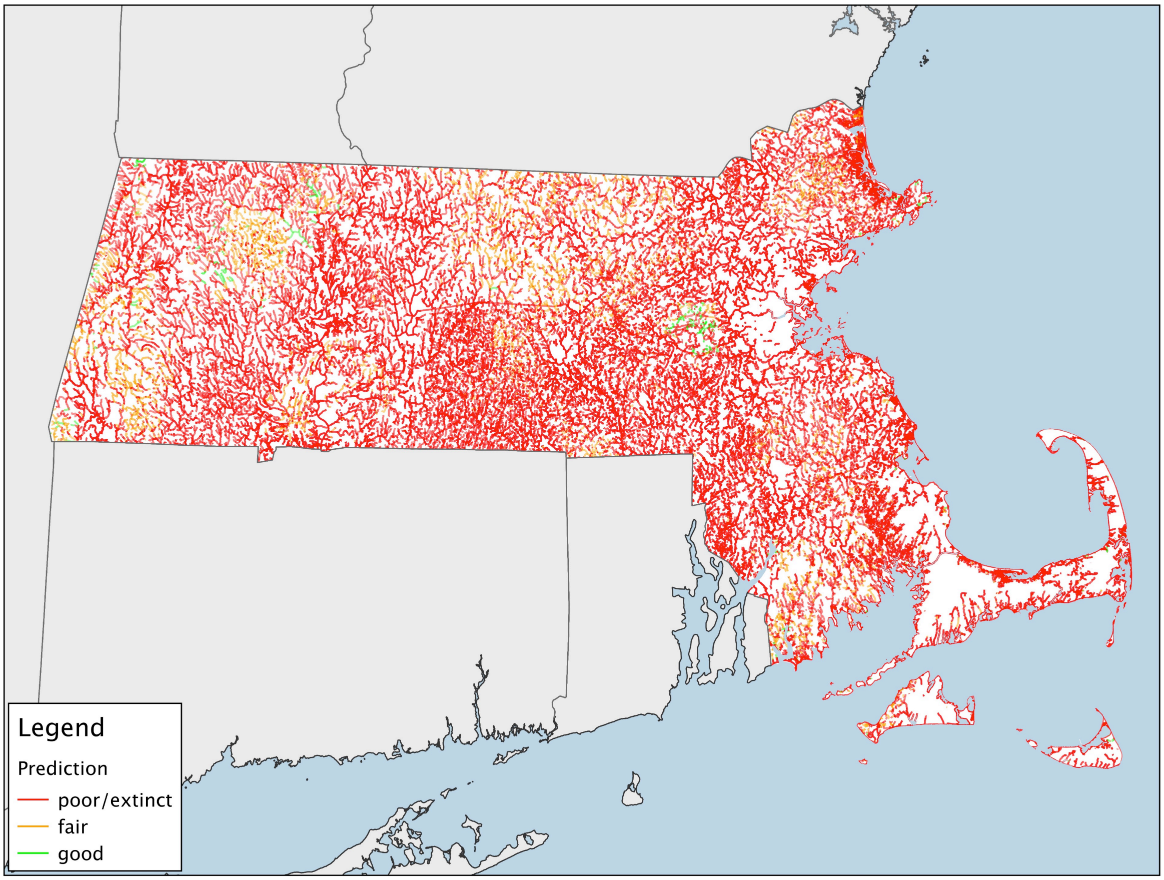

Figure 4. Stream-scale randomForest model prediction map for Massachusetts.

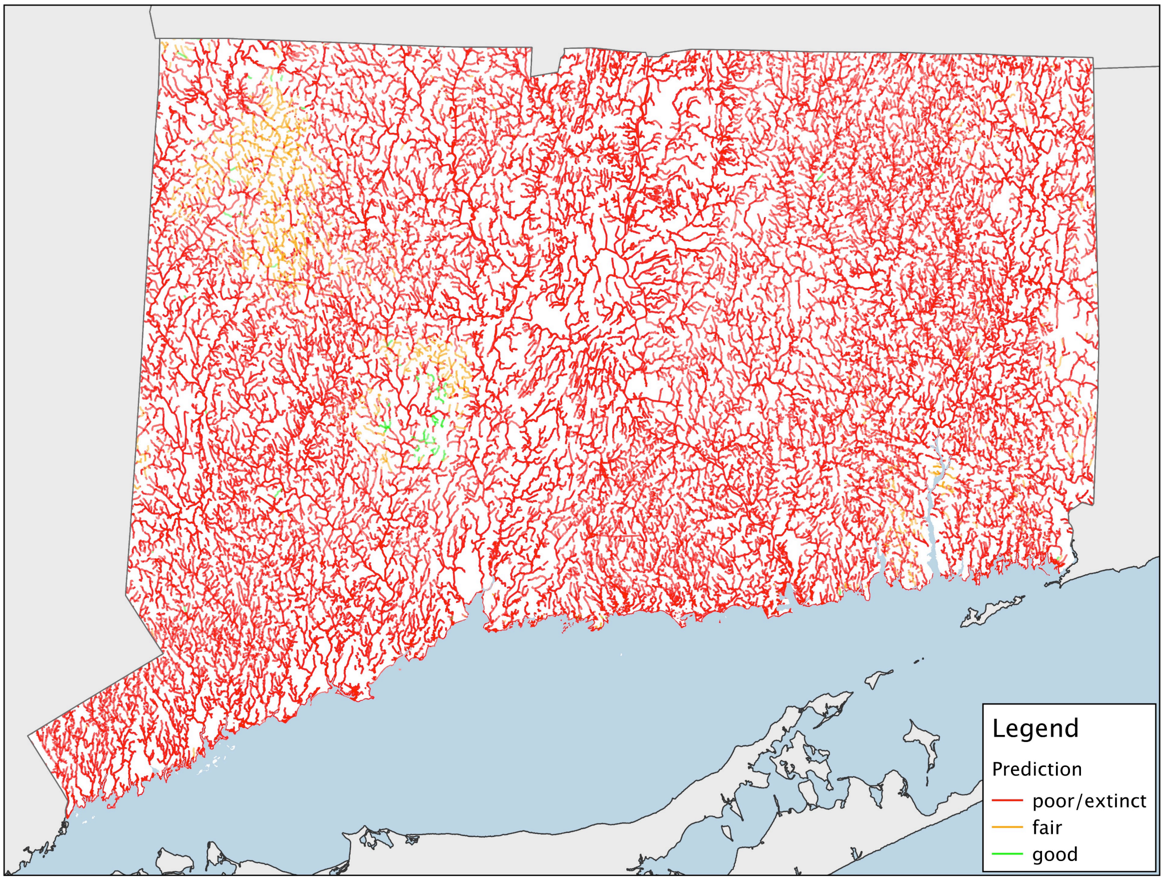

Figure 5. Stream-scale randomForest model prediction map for Connecticut.

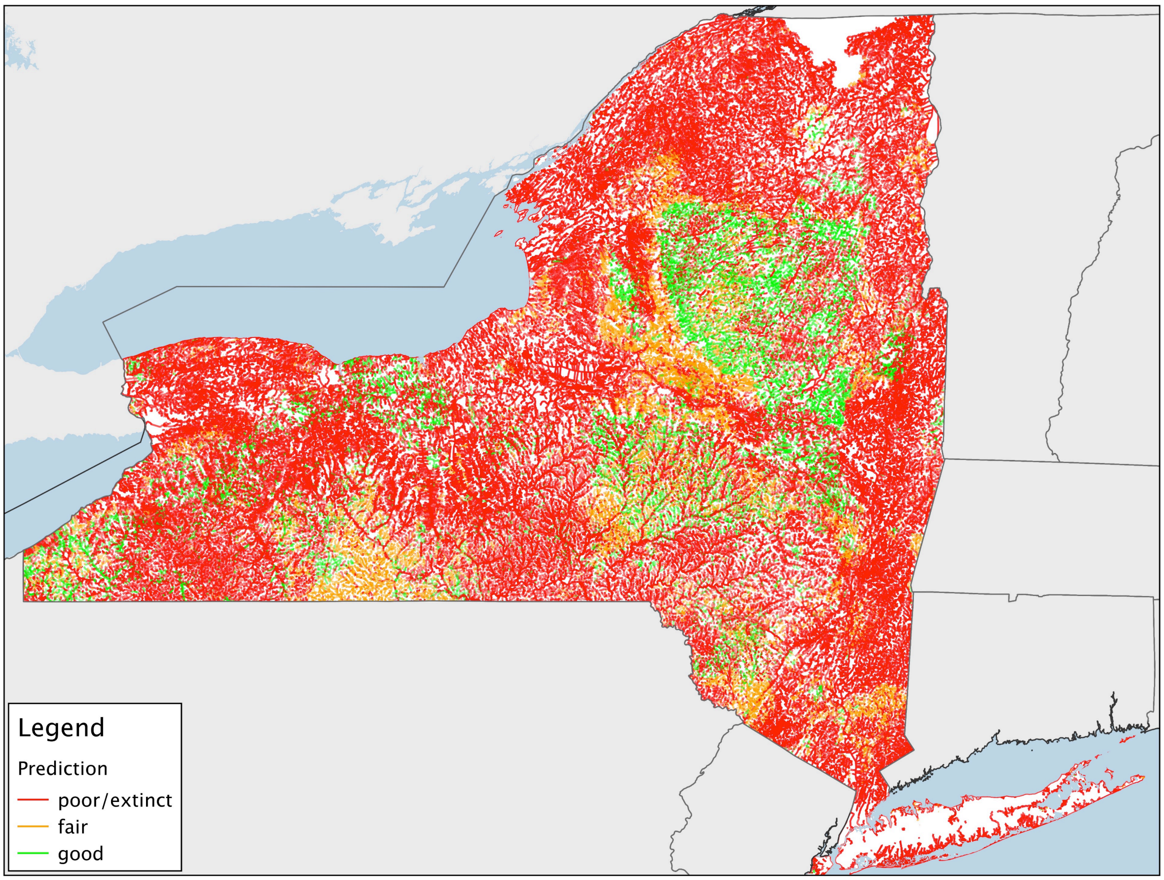

Figure 6. Stream-scale randomForest model prediction map for New York. Note that the map covers area outside the Atlantic slope.

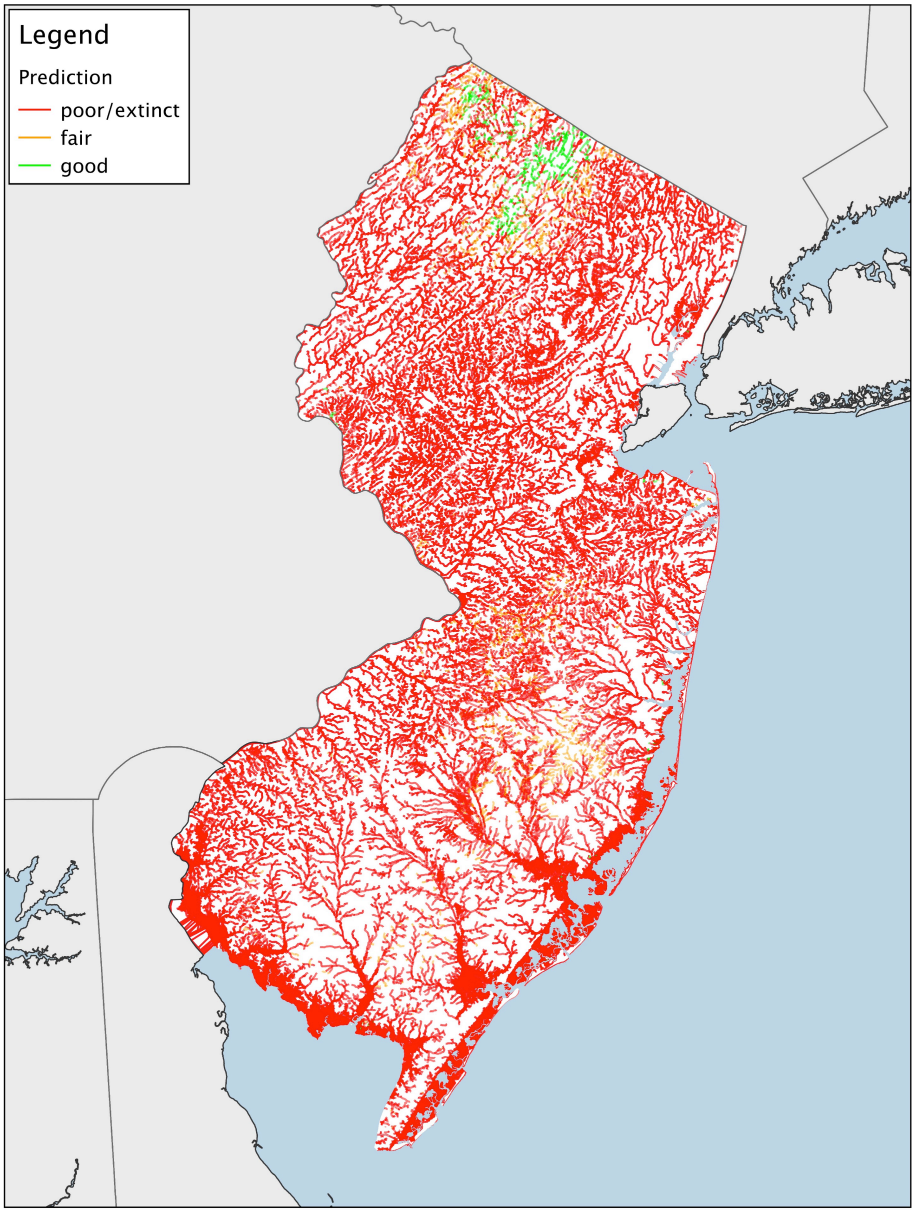

Figure 7. Stream-scale randomForest model prediction map for New Jersey.

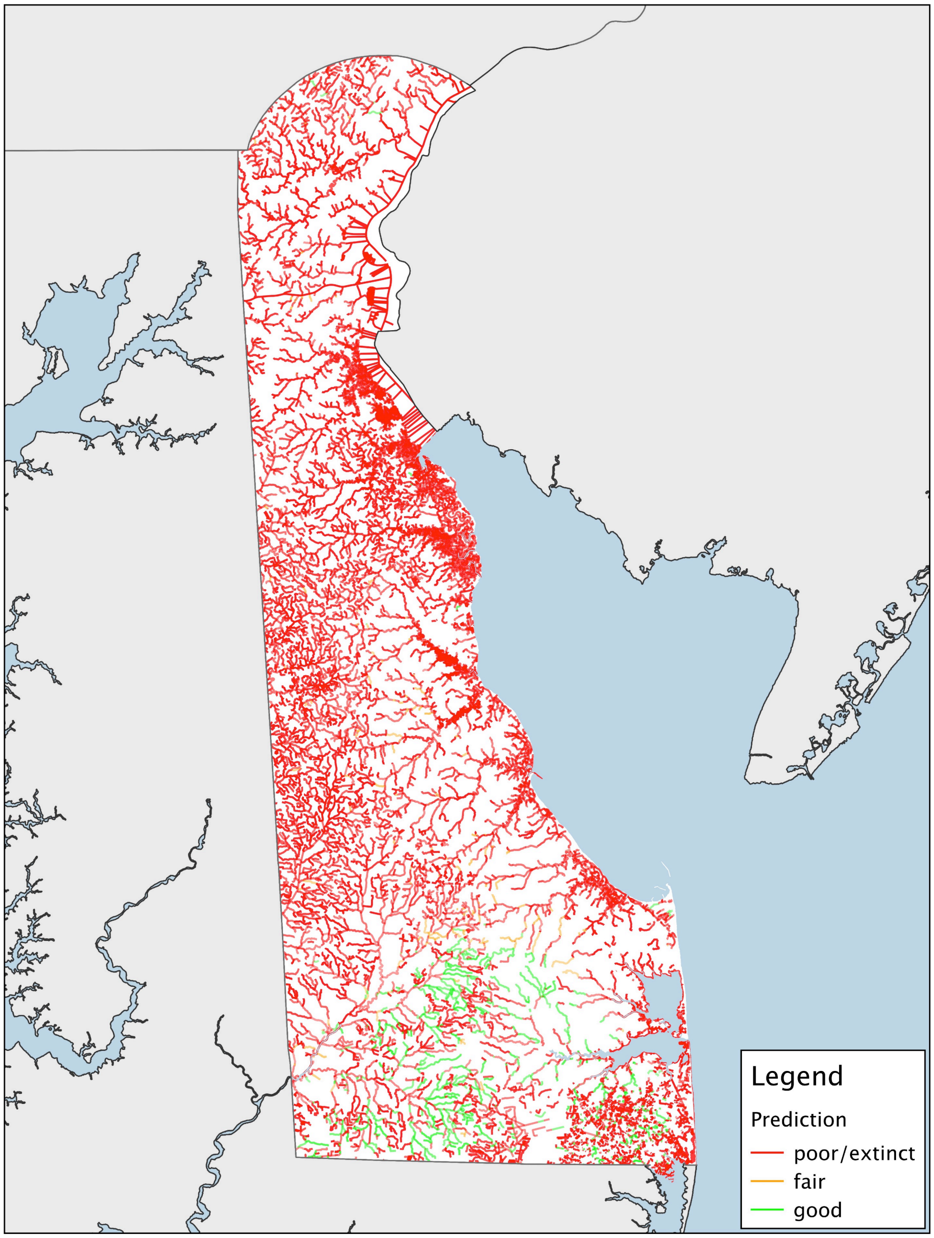

Figure 8. Stream-scale randomForest model prediction map for Delaware.

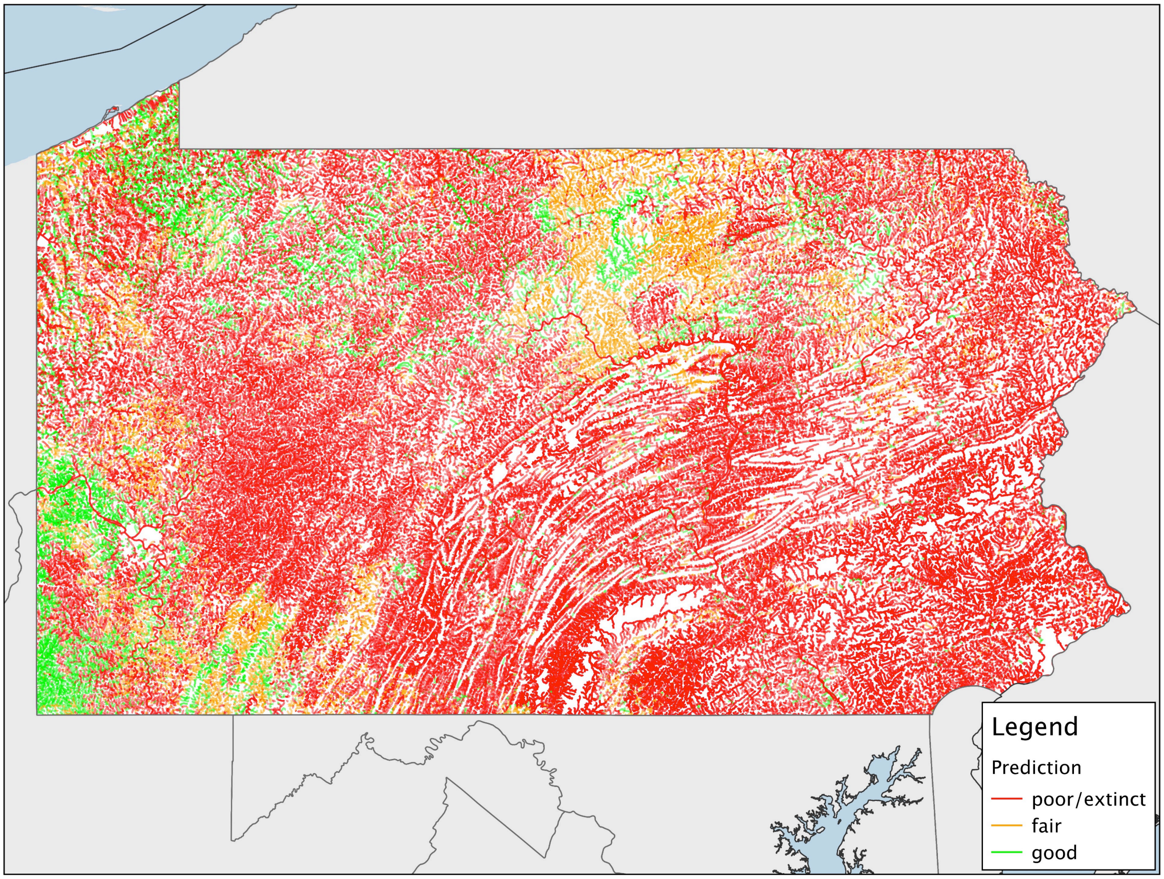

Figure 9. Stream-scale randomForest model prediction map for Pennsylvania. Note that the map covers area outside the Atlantic slope.

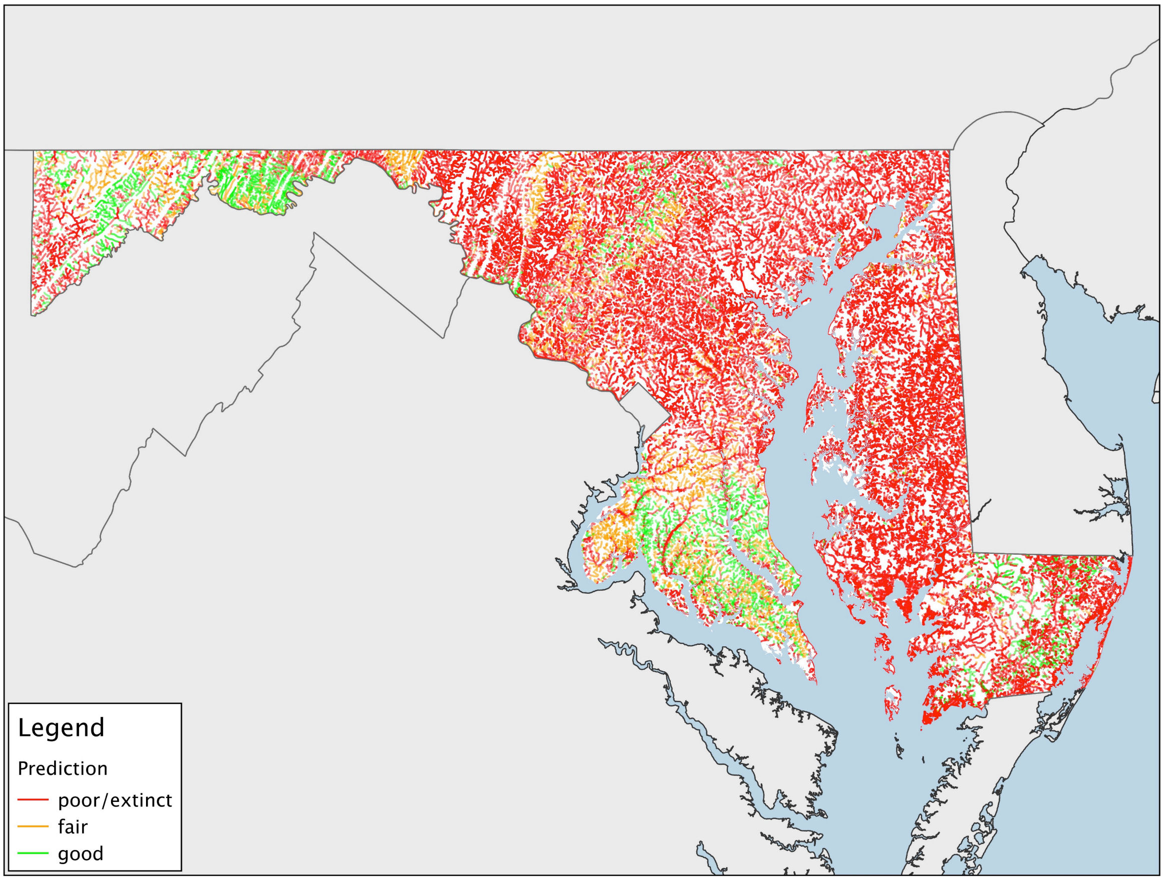

Figure 10. Stream-scale randomForest model prediction map for Maryland.

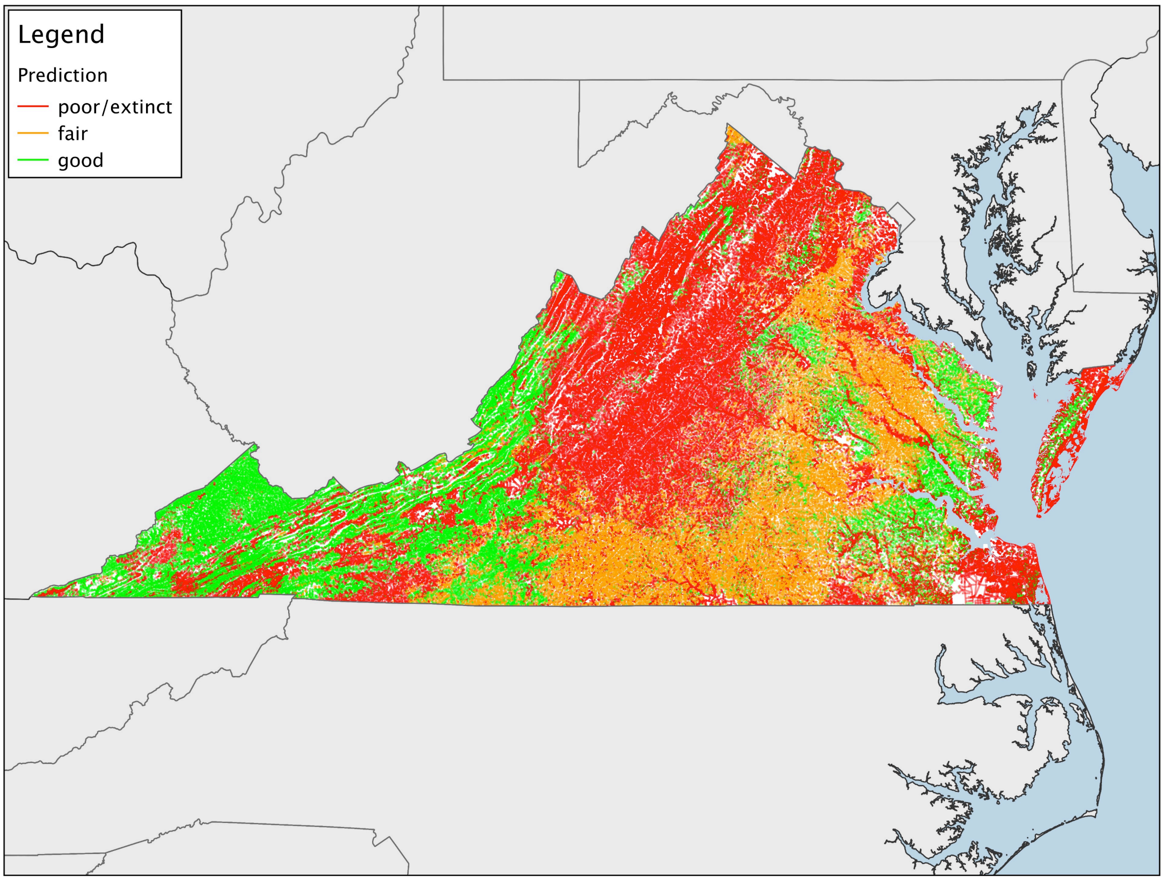

Figure 11. Stream-scale randomForest model prediction map for Virginia. Note that the map covers area outside the Atlantic slope.

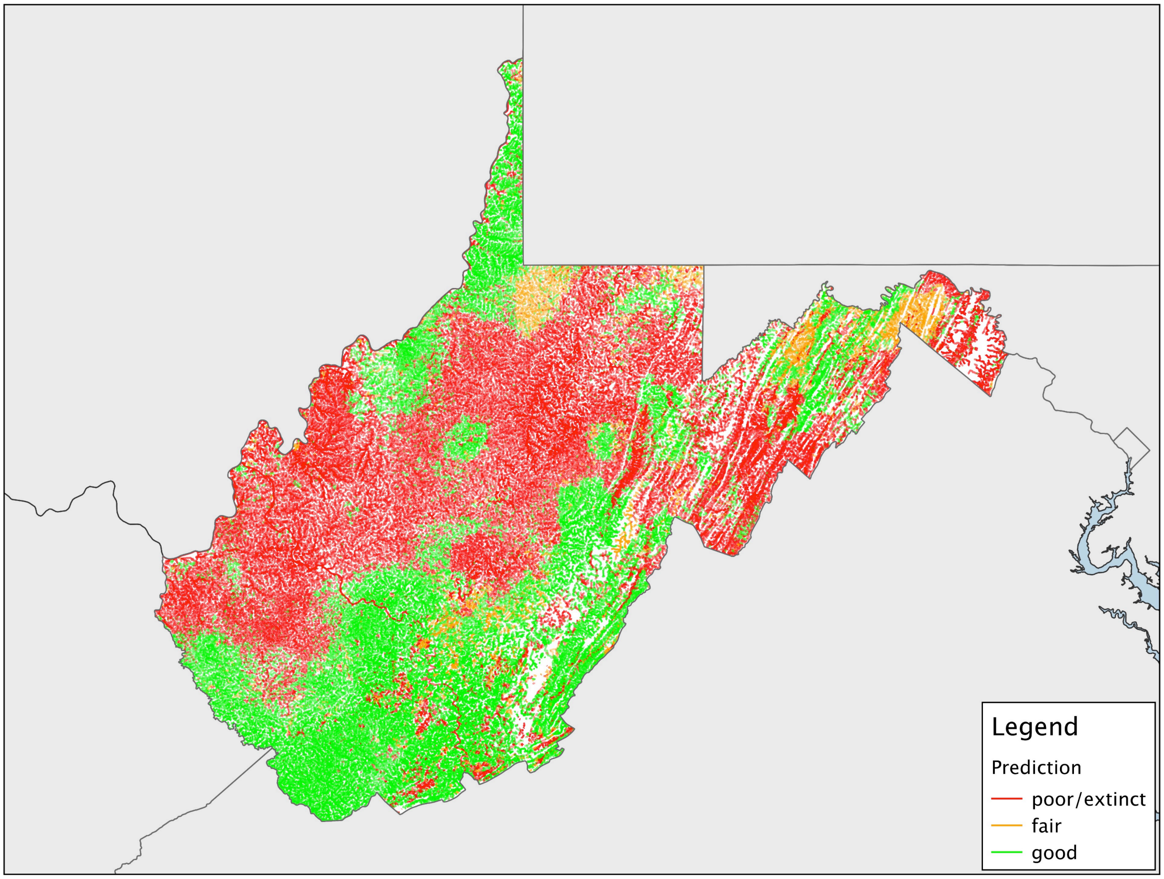

Figure 12. Stream-scale randomForest model prediction map for West Virginia. Note that the map covers area outside the Atlantic slope.

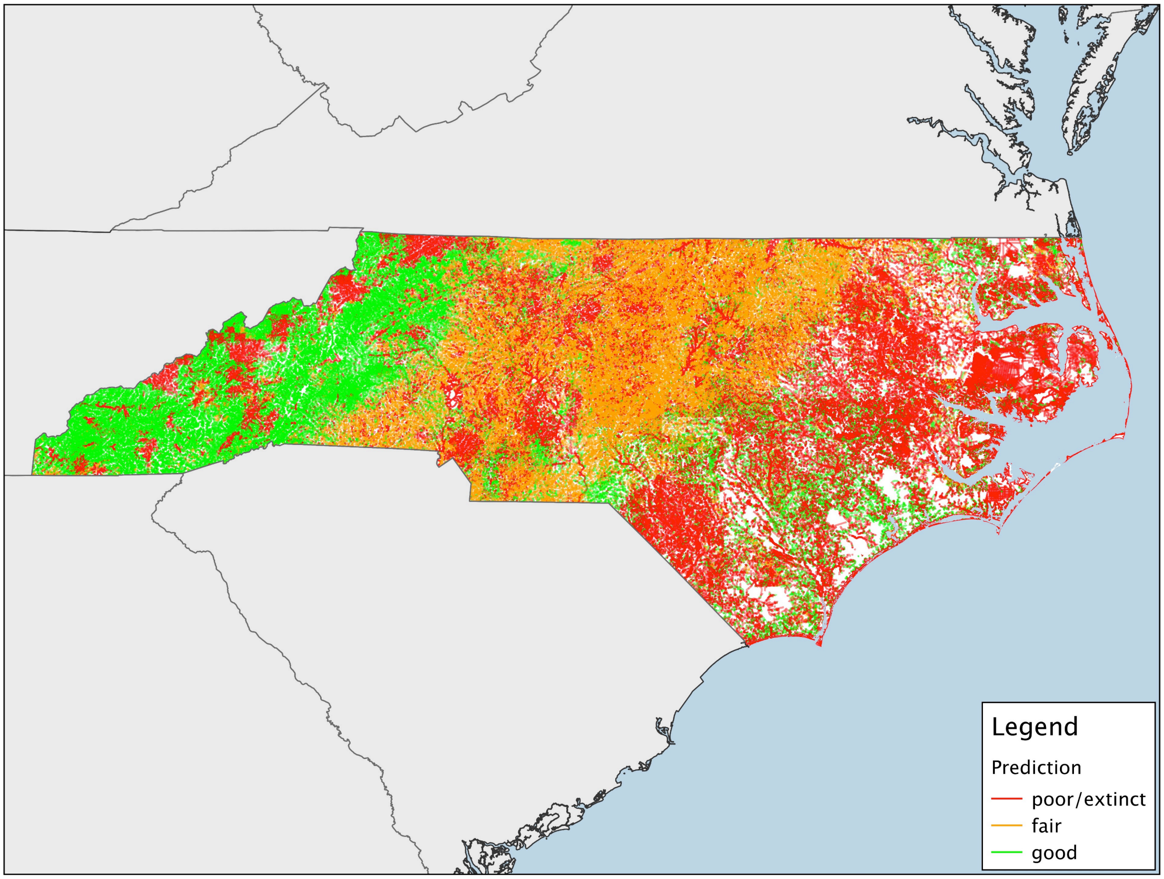

Figure 13. Stream-scale randomForest model prediction map for North Carolina. Note that the map covers area outside the Atlantic slope.

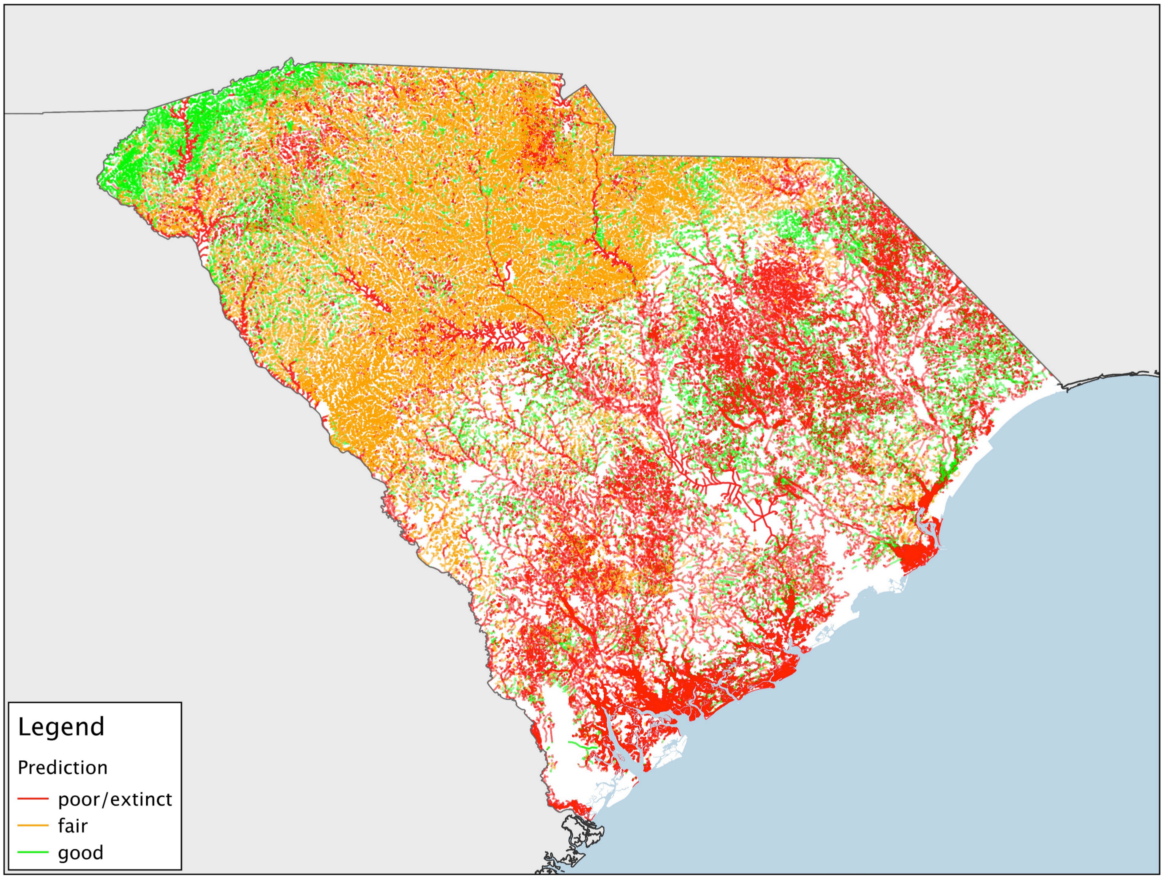

Figure 14. Stream-scale randomForest model prediction map for South Carolina.

Appendix II. Brook Floater Biologist Survey Report.

1. What is your affiliation (check all that apply)?

#	Answer	Bar	Response	%
1	Educator		3	9.09%
2	Researcher		5	15.15%
3	State biologist		13	39.39%
4	Federal biologist		10	30.30%
5	Environment or natural resource consultant		5	15.15%
6	Conservation organization		1	3.03%
7	Other		4	12.12%
	Total		41	100.00%

Other
bookseller
state biologist in NC from 1992-2002, consultant since that time
Retired
Interstate biologist

2. In what state(s) are you most familiar with brook floater populations (check all that apply)?

#	Answer	Bar	Response	%
1	Connecticut		2	6.06%
2	Delaware		0	0.00%
3	Georgia		2	6.06%
4	Massachusetts		4	12.12%
5	Maryland		4	12.12%
6	Maine		2	6.06%
7	New Hampshire		4	12.12%
8	New Jersey		1	3.03%
9	New York		2	6.06%
10	North Carolina		5	15.15%
11	Pennsylvania		6	18.18%
12	South Carolina		3	9.09%
13	Vermont		3	9.09%
14	Virginia		5	15.15%
15	West Virginia		4	12.12%
	Total		47	100.00%

3. Please rank the threat of altered hydrology from dams both upstream (impoundments) and downstream (scouring or temperature change).

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	7	15	7	29	2.00

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under spatial extent)	8	12	9	29	2.03

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100 years)	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	12	8	15	35	2.09

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	8	18	3	29	1.83

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	5	25	2	32	1.91

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	3	3
Mean	2	2.03	2.09	1.83	1.91
Variance	0.5	0.61	0.79	0.36	0.22
Standard Deviation	0.71	0.78	0.89	0.6	0.47
Total Responses	29	29	35	29	32
Total Respondents	29	28	26	29	29

4. Please rank the threat of fragmentation of habitat from dams or other inhospitable impacts.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	7	15	9	31	2.06

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under spatial extent)	6	15	8	29	2.07

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100 years)	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	10	10	16	36	2.17

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	11	13	6	30	1.83

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	4	26	1	31	1.90

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	3	3
Mean	2.06	2.07	2.17	1.83	1.9
Variance	0.53	0.5	0.71	0.56	0.16
Standard Deviation	0.73	0.7	0.85	0.75	0.4
Total Responses	31	29	36	30	31
Total Respondents	29	28	28	29	28

5. Please rank the threat from loss of forested riparian buffers

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	5	17	8	30	2.10

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under spatial extent)	5	20	7	32	2.06

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	7	12	13	32	2.19

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	6	20	4	30	1.93

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Rversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	12	16	3	31	1.71

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	3	3
Mean	2.1	2.06	2.19	1.93	1.71
Variance	0.44	0.38	0.61	0.34	0.41
Standard Deviation	0.66	0.62	0.78	0.58	0.64
Total Responses	30	32	32	30	31
Total Respondents	29	29	29	29	29

6. Please rank the threat of increased flooding events that degrade habitats or destroy mussel beds.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	11	9	7	27	1.85

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under spatial extent)	9	13	8	30	1.97

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	9	11	11	31	2.06

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	12	16	1	29	1.62

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	2	20	8	30	2.20

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	3	3
Mean	1.85	1.97	2.06	1.62	2.2
Variance	0.67	0.59	0.66	0.32	0.3
Standard Deviation	0.82	0.76	0.81	0.56	0.55
Total Responses	27	30	31	29	30
Total Respondents	27	28	28	29	28

7. Please rank the threat of habitat degradation from invasive plants.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	21	4	1	26	1.23

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under spatial extent)	22	6	1	29	1.28

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	17	5	5	27	1.56

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	19	8	-	27	1.30

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	8	16	4	28	1.86

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	2	3
Mean	1.23	1.28	1.56	1.3	1.86
Variance	0.26	0.28	0.64	0.22	0.42
Standard Deviation	0.51	0.53	0.8	0.47	0.65
Total Responses	26	29	27	27	28
Total Respondents	26	27	26	27	26

8. Please rank the threat of habitat degradation, competition or predation from zebra mussels, Asian clams or other invasive animals.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	17	6	5	28	1.57

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under	18	8	3	29	1.48

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	15	8	11	34	1.88

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	13	15	2	30	1.63

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	2	13	14	29	2.41

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	3	3
Mean	1.57	1.48	1.88	1.63	2.41
Variance	0.62	0.47	0.77	0.38	0.39
Standard Deviation	0.79	0.69	0.88	0.61	0.63
Total Responses	28	29	34	30	29
Total Respondents	28	28	28	29	28

9. Please rank the threat of mussel mortality from drought-induced desiccation or exposure to opportunistic predators.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	16	11	2	29	1.52

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under	13	11	4	28	1.68

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	9	12	9	30	2.00

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	11	18	-	29	1.62

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	3	18	8	29	2.17

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	2	3
Mean	1.52	1.68	2	1.62	2.17
Variance	0.4	0.52	0.62	0.24	0.36
Standard Deviation	0.63	0.72	0.79	0.49	0.6
Total Responses	29	28	30	29	29
Total Respondents	27	27	26	28	27

10. Please rank the threat of reduction of host fish from degraded habitat or species composition changes.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	12	10	3	25	1.64

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under	14	10	1	25	1.48

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	13	8	6	27	1.74

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	15	10	1	26	1.46

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	5	16	2	23	1.87

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	3	3
Mean	1.64	1.48	1.74	1.46	1.87
Variance	0.49	0.34	0.66	0.34	0.3
Standard Deviation	0.7	0.59	0.81	0.58	0.55
Total Responses	25	25	27	26	23
Total Respondents	24	24	22	25	22

11. Please rank the threat of runoff containing excess nutrients or toxins from agricultural activities.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	7	14	8	29	2.03

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Pervasive 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under spatial extent)	4	18	8	30	2.13

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	8	9	20	37	2.32

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	8	16	5	29	1.90

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 5	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	12	17	-	29	1.59

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	3	2
Mean	2.03	2.13	2.32	1.9	1.59
Variance	0.53	0.4	0.67	0.45	0.25
Standard Deviation	0.73	0.63	0.82	0.67	0.5
Total Responses	29	30	37	29	29
Total Respondents	28	28	28	29	28

12. Please rank the threat of runoff containing excess nutrients or toxins from residential development and urbanization.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	10	14	6	30	1.87

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under spatial extent)	5	16	8	29	2.10

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100 years)	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	7	10	19	36	2.33

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	9	15	7	31	1.94

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	7	22	1	30	1.80

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial extent)	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	3	3
Mean	1.87	2.1	2.33	1.94	1.8
Variance	0.53	0.45	0.63	0.53	0.23
Standard Deviation	0.73	0.67	0.79	0.73	0.48
Total Responses	30	29	36	31	30
Total Respondents	28	28	28	29	28

13. Please rank the threat of runoff containing pollutants from industrial activities.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	17	9	2	28	1.46

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under spatial extent)	11	12	5	28	1.79

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100 years)	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	8	6	14	28	2.21

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	13	16	-	29	1.55

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	10	17	-	27	1.63

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial extent)	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	2	2
Mean	1.46	1.79	2.21	1.55	1.63
Variance	0.41	0.54	0.77	0.26	0.24
Standard Deviation	0.64	0.74	0.88	0.51	0.49
Total Responses	28	28	28	29	27
Total Respondents	28	28	25	29	27

14. Please rank the threat of stream bank stabilization projects on reducing habitat quality.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	20	6	1	27	1.30

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under spatial extent)	18	9	-	27	1.33

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	8	12	9	29	2.03

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	13	16	-	29	1.55

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	16	12	1	29	1.48

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	2	3	2	3
Mean	1.3	1.33	2.03	1.55	1.48
Variance	0.29	0.23	0.61	0.26	0.33
Standard Deviation	0.54	0.48	0.78	0.51	0.57
Total Responses	27	27	29	29	29
Total Respondents	27	26	24	28	27

15. Please rank the threat of bridge and road construction on reducing habitat quality.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	21	5	1	27	1.26

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under	12	16	-	28	1.57

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	8	10	15	33	2.21

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	Hight 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	6	19	3	28	1.89

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	11	15	1	27	1.63

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	2	3	3	3
Mean	1.26	1.57	2.21	1.89	1.63
Variance	0.28	0.25	0.67	0.32	0.32
Standard Deviation	0.53	0.5	0.82	0.57	0.56
Total Responses	27	28	33	28	27
Total Respondents	27	27	27	27	27

16. Please rank the threat of residual sediment contamination from past pollution episodes.

Spatial extent (% of habitat/population negatively affected by threat)						
#	Question	Localized 1 (<10%)	Dispersed/Patch 2 (10-50%)	Pervasive 3 (>50%)	Response	Average Value
1	Spatial extent (% of habitat/population negatively affected by threat)	15	8	4	27	1.59

Severity (intensity of threat impacting exposed target under spatial extent)						
#	Question	Slight/Minor 1	Moderate/Substantial 2	Severe 3	Response	Average Value
2	Severity (intensity of threat impacting exposed target under spatial extent)	8	11	7	26	1.96

Immediacy (the time scale over which the impacts will be observable)						
#	Question	Long-term 1 (10-100	Near-term 2 (1-10 years)	Immediate 3	Response	Average Value
3	Immediacy (the time scale over which the impacts will be observable)	8	7	15	30	2.23

Certainty (amount of information/understanding of threat and response)						
#	Question	Low 1	Moderate 2	High 3	Response	Average Value
4	Certainty (amount of information/understanding of threat and response)	16	7	4	27	1.56

Reversibility (likelihood of reversing the impact within 10 years)						
#	Question	Reversible 1	Reversibility Difficult 2	Irreversible 3	Response	Average Value
5	Reversibility (likelihood of reversing the impact within 10 years)	3	19	6	28	2.11

Statistic	Spatial extent (% of habitat/population negatively affected by threat)	Severity (intensity of threat impacting exposed target under spatial	Immediacy (the time scale over which the impacts will be observable)	Certainty (amount of information/understanding of threat and response)	Reversibility (likelihood of reversing the impact within 10 years)
Min Value	1	1	1	1	1
Max Value	3	3	3	3	3
Mean	1.59	1.96	2.23	1.56	2.11
Variance	0.56	0.6	0.74	0.56	0.32
Standard Deviation	0.75	0.77	0.86	0.75	0.57
Total Responses	27	26	30	27	28
Total Respondents	26	25	25	27	26

17. What do you consider are the most important research/management priorities aimed at preventing the decline of brook floater populations (check all that apply)?

#	Answer	Bar	Response	%
1	Biology and life history studies		12	38.71%
2	Demographic studies (e.g. size, survivorship, sex ratio, age, recruitment)		19	61.29%
3	Quantitative surveys and long-term monitoring		20	64.52%
4	Ecotoxicological studies (the effects of chemical stressors on survivorship and recruitment)		16	51.61%
5	Ecological studies of habitat requirements		18	58.06%
6	Range wide genetic studies		9	29.03%
7	Reintroduction or population augmentation		21	67.74%
8	Strengthening of water quality regulations		14	45.16%
9	Better enforcement of water quality regulations		17	54.84%
10	Studies to explain spatial patterns (patchiness) of populations		14	45.16%
11	Additional water quality monitoring		6	19.35%
12	Measures that enhance riparian buffers		16	51.61%
13	Other		10	32.26%
	Total		192	100.00%

Other
Removal of the threats to the species is the most priority to prevent the decline of the brook floater. Secondarily, if warranted, applying species protection via state and federal endangered species regulations (and by association, critical habitat designations) allow specific protection from threats. If you don't protect the species using the existing tools (again, if warranted) you risk literally studying the species to death.
investigate limiting factors, including both micro and landscape level
hybridization with A. marginata should be studied
protect existing viable population habitat
large gaps in survey data in Savannah, Saluda and Cape Fear River basins, thus there is the potential for "new" populations to be present
Protection of exisiting watershed where the species occurs
In addition to providing appropriate riparian forest buffers, maintaining and restoring sufficient watershed forest cover (vs. impervious surface, ag lands, etc.) should be a very high management priority; riparian buffers alone will not provide sufficient habitat protection. As with riparian buffers, this will require a combination of better water quality regs, enforcement, local/county/state land use planning, and public land mgt, along with continuation/improvement of landowner incentive programs and I&E. Question(s) regarding the lack of sufficient watershed forest cover as a threat should've been included in this survey. Federal listing should be given serious consideration.
increase in % forest cover in each watershed, decrease in % impervious surfaces
Improved regulation of dam operations, including federal