Species Status Assessment (SSA) Report

for the

Candy Darter

(*Etheostoma osburni*)

Version 1.5

Candy darter (Photo credit: Dr. Stuart Welsh, U.S. Geological Survey)

March 2018
U.S. Fish and Wildlife Service
Northeast Region
Hadley, MA

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Changes From The Previous Species Status Assessment (SSA) Report

We have updated the SSA Report to rectify an inaccuracy in how we had applied the criterion used to assess the risk of hybridization (see Chapter 3), which was one of the eight criteria used to evaluate the species' condition (see Appendix B), in version 1.4 of the SSA Report (Service 2017). This discrepancy affected only the modelled condition of the Upper Gauley metapopulation. We have resolved the discrepancy in this version of the SSA Report (version 1.5; Service 2018) by updating the hybridization criterion, re-running the model, and updating the results section, as appropriate.

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EXECUTIVE SUMMARY

This report summarizes the results of a species status assessment (SSA) conducted for the candy darter (*Etheostoma osburni*), which includes relevant information about the species' life history characteristics and how those characteristics are effected by stressors and conservation measures to address those stressors. This report is intended to provide the biological support for the decision on whether or not to propose to list the species as threatened or endangered under the Endangered Species Act of 1973, as amended (Act). The process and this SSA report do not represent a decision by the U.S. Fish and Wildlife Service (Service) whether or not to list a species under the Act. Instead, this SSA report provides a review of the best available information strictly related to the biological status of the candy darter.

Background

The candy darter is a small, freshwater fish endemic to 2nd order and larger streams and rivers within portions of the upper Kanawha River basin, which is synonymous with the Gauley and greater New River watersheds in Virginia and West Virginia. The species is considered by both Virginia and West Virginia in their State Wildlife Action Plans to be a species of greatest conservation need.

The species is described as a habitat specialist, being most often associated with faster flowing stream segments with coarse bottom substrate (*e.g.*, gravel, cobble, rocks, and boulders), that provides shelter for individual darters. Candy darters are intolerant of excessive sedimentation and stream bottom embeddedness (the degree to which gravel, cobble, rocks, and boulders are surrounded by, or covered with, fine sediment particles). The available candy darter occurrence data, all of which were collected after the aquatic habitat in the region was degraded by widespread forest clearing in the late 1800s, indicate the species prefers cool or cold water temperatures, but that warm water conditions may also be tolerated. The fish are opportunistic feeders, eating mostly benthic macroinvertebrates such as mayflies and caddisflies. In streams maintaining favorable habitat conditions, through natural or managed condition, candy darters can be abundant throughout the stream continuum. Examples of managed stream conditions include the State of West Virginia's "stream liming" projects that add calcium carbonate sand or gravel to streams to neutralize acidic water conditions in the Upper Gauley watershed (see Chapter 3—Water Chemistry), and the U.S. Forest Service's implementation of a variety of stream restoration projects in the Monongahela National Forest specifically to reduce sedimentation in the Greenbrier watershed (see Chapter 3—Sedimentation).

Candy darters are sexually mature at 2 years of age and live to a maximum age of 3 years. They are classified as brood-hiding, benthic spawners. In this reproductive strategy, the female deposits her eggs in the pebble and gravel substrate between larger cobbles and boulders and an attendant male simultaneously fertilizes the eggs as they are released. During spawning, males become aggressively territorial and in all observed instances of spawning aggression, the larger male prevailed and fertilized the female's eggs. Female candy darters are reported to produce a relatively low number of eggs (average 170 per individual) as compared to other fish, with no significant deviation from 1:1 sex ratios. We are uncertain whether individual candy darters complete their lifecycle within single riffles or riffle complexes spanning just a few hundred

meters or are capable of longer, seasonally mediated movements within suitable habitat. While data are sparse regarding the minimum habitat size and degree of genetic connectivity required for candy darter population viability, the historical distribution of the species and the fundamentals of conservation biology suggest these factors are important to the species.

An emergent threat to the candy darter is hybridization with the closely related variegate darter (*E. variatum*). The variegate darter is native to the upper and middle Ohio River drainage including the lower Kanawha River basin below Kanawha Falls in Fayette County, West Virginia. Kanawha Falls serves as a natural barrier to fish dispersal from the lower Kanawha River basin (and greater Ohio River basin) upstream into the range of the candy darter in the upper Kanawha River basin. However, in the late 20th century, the variegate darter was introduced into the upper Kanawha basin, likely by "bait bucket transfer." Since their introduction, variegate darters have expanded within the range of the candy darter and genetic studies have demonstrated that where variegate and candy darter ranges now overlap, the two species will hybridize, quickly resulting in "genetic swamping" (the homogenization or replacement of native genotypes) of the endemic candy darter population and eventually its complete replacement by variegate darters or hybrids.

Methodology

To assess the biological status of the candy darter across its range, we used the best available information, including peer reviewed scientific literature and academic reports, and survey data provided by State and Federal agencies in Virginia and West Virginia. Additionally, we consulted with several candy darter experts who provided important information and comments on candy darter life history, genetics, and habitat. Fundamental to our analysis of the candy darter was the determination of scientifically sound analytical units at a scale useful for assessing the species. In this report, we defined candy darter analytical units based primarily on known occurrence locations and stream connectivity, identifying a total of 35 individual candy darter populations within 7 metapopulations. We acknowledge that specific candy darter demographic and genetic data are sparse with which to support this construct; however the species experts generally agreed that this was a valid approach for assessing the species' condition in this SSA report.

After consulting with the species experts, we identified the factors (i.e., stressors and ongoing conservation measures to address stressors) most likely affecting the candy darter. These stressors include hybridization with the introduced variegate darter, excessive sedimentation, warming water temperatures, habitat fragmentation, changes in water quality and flow, catastrophic events, and competition or predation associated with other introduced species. Conservation actions to address stressors include survey efforts, genetic research, hybridization monitoring, public land management plans (i.e., National Park Service and National Forests), stream liming, best management practices, riparian restoration, and angler guidelines. Because no consistent, rangewide assessment of the candy darter is available, we developed a semiquantitative model to estimate the condition of each candy darter population. The model relies on three categories of interrelated metrics, including habitat parameters (i.e., water quality and forest cover), non-native species parameters (i.e., the presence of brown trout and/or variegate darters), and estimates of the candy darter's demographic status within each unit.

Information about applicable stressors and any existing conservation measures to address those stressors were included in our analysis (see Chapter 3—Current Conditions, Chapter 4—Species Viability, and Appendix B—Model). These metrics were selected because the supporting data were consistent across the range of the species and at a resolution suitable for assessing the species at the population level. The model output was a condition score for each candy darter population that was then used to assess the candy darter's current condition across its range under the "3Rs," described below:

> **Resiliency** means having sufficiently large populations for the species to withstand stochastic events (arising from random factors). We can measure resiliency based on metrics of population health; for example, birth versus death rates and population size, if that information exists. Resilient populations are better able to withstand disturbances such as random fluctuations in birth rates (demographic stochasticity), variations in rainfall (environmental stochasticity), or the effects of human activities.
>
> **Redundancy** means having a sufficient number of populations for the species to withstand catastrophic events (such as a rare destructive natural event or episode involving many populations). Redundancy is about spreading the risk and can be measured through the duplication and distribution of populations across the range of the species. Generally, the greater the number of populations a species has distributed over a larger landscape, the better it can withstand catastrophic events.
>
> **Representation** means having the breadth of genetic makeup of the species to adapt to changing environmental conditions. Representation can be measured through the genetic diversity within and among populations and the ecological diversity (also called environmental variation or diversity) of populations across the species' range. The more representation, or diversity, a species has, the more it is capable of adapting to changes (natural or human caused) in its environment.

The same methodology was used to assess the species' condition and potential viability under five future scenarios. We chose to model these scenarios out to 25 years because we have data to reasonably predict potential habitat changes and hybridization rates and their effects on the candy darter within this timeframe. Scenarios 1 and 2 modeled the potential effects of significant positive and negative habitat changes on the species. Because these scenarios assume the degree of variegate darter hybridization does not change from the current condition, which is not probable, we do not consider these scenarios to be feasible on their own. We do however consider them useful for exploring the relative magnitude of habitat changes to the species in light of continued variegate darter expansion, which is modeled under Scenarios 3, 4, and 5.

Under Scenarios 3, 4, and 5, we relied on the best available data to estimate the rate of variegate darter expansion and hybridization within the three extant candy darter metapopulations currently affected by that species, and to estimate when variegate darters would be introduced into two extant candy darter metapopulations that are currently unaffected. Scenario 3, which we conclude is the most likely scenario, modeled the effects of variegate darter expansion in isolation (i.e., no concurrent changes to the habitat metrics), while Scenarios 4 and 5 combined

the variegate darter expansion model (Scenario 3) with the positive habitat change model (Scenario 1) and negative habitat change model (Scenario 2), respectively.

We made our draft report available to the species experts, State and Federal partners, and four independent peer reviewers with expertise in fisheries biology; fisheries, population, and landscape ecology; speciation as it relates to conservation biodiversity; and genetics. These reviewers generally found that the SSA report provided an accurate and adequate review and analysis of potential stressors or threats to the candy darter; that our analyses was logical and supported by the evidence we provide; and that we cited all necessary and pertinent literature to support our scientific analyses. We incorporated editorial and specific suggestions for additional clarification or analysis as available and appropriate.

Conclusions

Current Condition

Historically, the candy darter occurred in 35 populations distributed across 7 metapopulations located in the Bluestone, Lower New River, Upper Gauley, Lower Gauley, and Middle New watersheds in the Appalachian Plateaus physiographic province and the Upper New River and Greenbrier watersheds in the Valley and Ridge physiographic province.

Within these two physiographic provinces, the candy darter has been extirpated from almost half of its historical range; 17 (49 percent) of 35 known populations (and 2 (29 percent) of 7 known metapopulations), with the extirpations representing a complete loss of resiliency in those populations. We qualitatively assessed the 18 remaining (extant) populations, placing them in "low," "moderate," or "high" categories that represent the populations' potential to bounce back after stochastic events. These categories were based on a combination of physical habitat metrics, non-native competition metrics, and candy darter demographic metrics. Of the 18 extant populations, 5 (28 percent) have a current score of high or moderate to high resiliency, 9 (50 percent) have moderate resiliency, and 4 (22 percent) have low or moderate to low resiliency. The five populations with higher resiliency occur in three metapopulations (the Upper Gauley in the Appalachian Plateaus physiographic province and the Greenbrier and Middle New in the Valley and Ridge physiographic province); the remaining two extant metapopulations (the Lower Gauley in the Appalachian Plateaus physiographic province and the Upper New River in the Valley and Ridge physiographic province) maintain populations with moderate and low resiliency. Therefore, we conclude the candy darter currently maintains moderate <u>resiliency</u>.

This loss of candy darter populations and the areas they represented within the species' historical range, as well as the fragmentation of extant populations, has compromised the species' ability to repatriate those areas or avoid species level effects from a catastrophic event. Based on the species' current distribution (5 (71 percent) of 7 known metapopulations and 18 (51 percent) of 35 known populations) across its historical range, and the species' distribution and condition within each of the 7 metapopulations (1 with moderate to high internal redundancy, 1 with moderate internal redundancy, 1 with low internal redundancy, and 2 with no internal redundancy), we conclude that the candy darter's current <u>redundancy</u> is moderate to low.

The best available data for the candy darter indicate that there is a high level of genetic differentiation between the Greenbrier River and Upper and Lower Gauley River metapopulations. These metapopulations currently have moderate resiliency, however the loss of either would represent a substantial reduction in the species' genetic representation. While the candy darter currently maintains representation in both the Appalachian Plateaus and Valley and Ridge physiographic provinces, only a single metapopulation in the Appalachian Plateaus province has a moderate to high resiliency score. As related to the species' diversity of environmental settings, candy darters have lost representation from lower mainstem rivers and tributaries. Although the candy darter retains representation in both of the Appalachian Plateaus and Valley and Ridge physiographic provinces, the species has a different distribution than it had historically, and likely a different ability to respond to stochastic and catastrophic events, thereby putting the species at increased risk of extinction from any such events. Therefore, we conclude that the species' representation is currently moderate to low.

Future Condition

Under Scenario 1 (positive habitat changes only), the condition of 12 candy darter populations is predicted to remain relatively unchanged from the current condition, and 6 are predicted to have improved. Under Scenario 1, the species' redundancy and representation remained unchanged (e.g., all current metapopulations remained extant), and the resiliency of the six improved populations is predicted to increase. However, we do not consider this a feasible prediction in light of ongoing variegate darter hybridization.

Under Scenario 2 (negative habitat changes only), 10 populations are predicted to remain relatively unchanged, and eight are predicted to decrease in condition. Five of the populations are predicted to fall into the low condition category and three are predicted to remain in the low category. It should be noted that two of these populations are isolated and at an increased risk of extirpation due to environmental or demographic stochasticity. The loss of either of these populations would also represent the loss of their respective metapopulation, thereby reducing the redundancy and representation of the species and increasing the candy darter's risk of extinction. Therefore, under Scenario 2, while the candy darter would maintain extant populations over the next 25 years, the species would lose resiliency in three (60 percent) of the five current metapopulations, with two of these being at a high risk of extirpation. We do not consider this a feasible prediction in light of ongoing variegate darter hybridization.

Under Scenario 3 (variegate darter expansion), within the next 15 years, ongoing hybridization and genetic swamping is expected to cause the decline and loss of pure candy darters from three metapopulations. Within this timeframe, we expect that additional bait bucket transfer(s) will result in variegate darters spreading within the Upper Gauley metapopulation, and expect the pattern of candy darter hybridization and loss previously observed in other metapopulations to be repeated there. We also expect that in 20 years, variegate darters will be introduced into an additional unaffected candy darter metapopulation, where we anticipate the pattern of hybridization with candy darters will continue. Therefore, based on the modeled assumptions for variegate darter expansion, the redundancy of the candy darter will decline from five to two metapopulations within the next 25 years. Notably, two of the expected to be extirpated metapopulations currently include perhaps the best connected and abundant candy darter

populations known. The loss of these metapopulations eliminates the species' representation in the Appalachian Plateaus physiographic province. Under this scenario, four isolated candy darter populations are predicted to remain extant. However, these populations are predicted to have low resiliency and be at an increased risk of extirpation from catastrophic or stochastic events. Therefore variegate darter expansion and hybridization, which we conclude is the most likely future scenario, significantly increases the candy darter's risk of extinction over the next 25 years.

Under Scenario 4 (variegate darter expansion with positive habitat change), the pattern of candy darter metapopulation extirpations is predicted to remain the same as described under Scenario 3. This is because both the candy and variegate darters share general habitat requirements. Therefore, improvements in habitat quality that benefit the candy darter will also likely benefit the variegate darter. Significant positive habitat changes may increase the resiliency of two of the four candy darter populations that are expected to remain extant following 25 years of variegate darter expansion and hybridization. But because of its expected exposure to variegate darters by this time, one candy darter population is expected to decrease in condition score even with positive habitat changes. Therefore, habitat improvements alone do not significantly reduce the risk of extinction resulting from variegate darter expansion.

Under Scenario 5 (variegate darter expansion with negative habitat change), the pattern of candy darter metapopulation extirpations again remain the same as described under Scenario 3. The negative habitat changes are expected to reduce the resiliency of all four candy darter populations that are expected to remain extant following 25 years of variegate darter expansion and hybridization, increasing their risk of extirpation from stochastic events. Furthermore, it is possible that variegate darters are tolerant of a wider range of habitat conditions, as is suggested by that species' wide range in much of the greater Ohio River basin. Therefore degraded habitat conditions might selectively favor the variegate darter over the candy darter, placing the candy darter at additional risk of extinction.

Summary

We considered what the candy darter needs to maintain viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation. For the purpose of this assessment, we generally define viability as the ability of the species to sustain populations in natural stream ecosystems within a biologically meaningful timeframe: in this case, 25 years. Based on the candy darter's life history and habitat needs, and in consultation with the species' experts, we identified the potential stressors (negative influences), the contributing sources of those stressors, and conservation measures to address those stressors that are likely to affect the species' current condition and viability. We evaluated how these stressors may be currently affecting the species and whether, and to what extent, they would affect the species in the future. Water temperature, excessive sedimentation, habitat fragmentation, water chemistry, water flow, and nonnative competition likely influenced the species in the past and contributed to its current condition, and may continue to affect some individual populations in the future. Hybridization with the closely related variegate darter appears to be having, and will continue to have, the greatest influence on candy darter populations and its overall viability within the next 25 years. While we acknowledge there is uncertainty regarding some of the scientific data and

assumptions used to assess the biological condition of the candy darter, the species' experts generally agreed with the overall methodology and confirmed that the results were reflective of their observations of the candy darter and its habitat.

CHAPTER 1 – INTRODUCTION

Background

This report summarizes the results of a species status assessment (SSA) conducted for the candy darter (*Etheostoma osburni*). We, the U.S. Fish and Wildlife Service (Service), were petitioned to list 404 aquatic, riparian, and wetland species, including the candy darter, as endangered or threatened under the Endangered Species Act of 1973, as amended (Act) on April 20, 2010, by the Center for Biological Diversity, Alabama River Alliance, Clinch Coalition, Dogwood Alliance, Gulf Restoration Network, Tennessee Forests Council, West Virginia Highlands Conservancy, Tierra Curry, and Noah Greenwald. In September of 2011, the Service found that the petition presented substantial scientific or commercial information indicating that the listing of 374 species, including the candy darter, may be warranted. A subsequent complaint for not meeting the statutory petition finding deadlines was filed on April 15, 2015. Per a court approved settlement agreement, we agreed to send a 12-month petition finding for the candy darter to the Federal Register by September 30, 2017. Thus, we conducted a SSA to compile the best scientific and commercial data available regarding the species' biology and factors that influence the species' viability.

Analytical Framework

The SSA report, the product of conducting a SSA, is intended to be a concise review of the species' biology and factors influencing the species, an evaluation of its biological status, and an assessment of the resources and conditions needed to maintain long-term viability. The intent is for the SSA report to be easily updated as new information becomes available, and to support all functions of the Endangered Species Program. As such, the SSA report will be a living document upon which other documents, such as listing rules, recovery plans, and 5-year reviews, would be based if the species warrants listing under the Act.

This SSA report for the candy darter is intended to provide the biological support for the decision on whether or not to propose to list the species as threatened or endangered and if so, whether or not to propose designating critical habitat. The process and this SSA report do not represent a decision by the Service whether or not to list a species under the Act. Instead, this SSA report provides a review of the best available information strictly related to the biological status of the candy darter. The listing decision will be made by the Service after reviewing this document and all relevant laws, regulations, and policies, and a decision will be announced in the Federal Register.

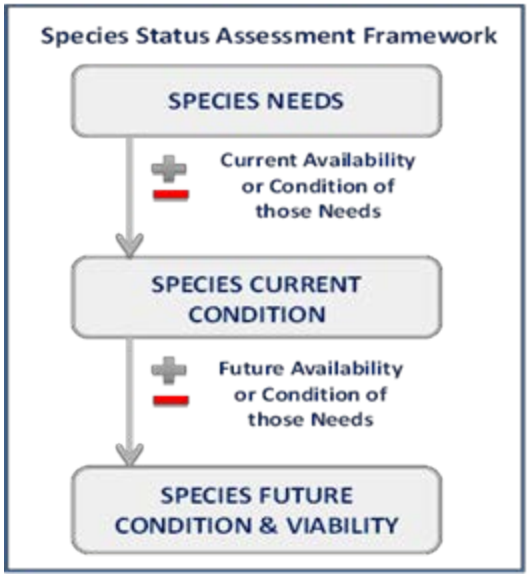

Figure 1. Species Status Assessment Framework

Using the SSA framework (figure 1), we consider what a species needs to maintain viability by characterizing the biological status of the species in terms of its resiliency, redundancy, and representation (Shaffer *et al*., 2002, pp. 139–140; Wolf *et al*. 2015, entire). For the purpose of this assessment, we generally define viability as the ability of the species to sustain populations in natural stream ecosystems within a biologically meaningful timeframe: in this case, 25 years. We chose 25 years because the available data allow us to reasonably predict the potential significant effects of stressors within the range of the candy darter within this timeframe. Resiliency, redundancy, and representation are defined as follows:

- **Resiliency** means having sufficiently large populations for the species to withstand stochastic events (arising from random factors). We can measure resiliency based on metrics of population health; for example, birth versus death rates and population size, if that information exists. Resilient populations are better able to withstand disturbances such as random fluctuations in birth rates (demographic stochasticity), variations in rainfall (environmental stochasticity), or the effects of human activities.

- **Redundancy** means having a sufficient number of populations for the species to withstand catastrophic events (such as a rare destructive natural event or episode involving many populations). Redundancy is about spreading the risk and can be measured through the duplication and distribution of populations across the range of the species. Generally, the greater the number of populations a species has distributed over a larger landscape, the better it can withstand catastrophic events.

- **Representation** means having the breadth of genetic makeup of the species to adapt to changing environmental conditions. Representation can be measured through the genetic diversity within and among populations and the ecological diversity (also called environmental variation or diversity) of populations across the species' range. The more representation, or diversity, a species has, the more it is capable of adapting to changes (natural or human caused) in its environment. In the absence of species-specific genetic

and ecological diversity information, we evaluate representation based on the extent and variability of habitat characteristics within the geographical range.

The decision whether to list a species is based *not* on a prediction of the most likely future for the species, but rather on an assessment of the species' risk of extinction. Therefore, to inform this assessment of extinction risk, we describe the species' current biological status and assess how this status may change in the future under a range of scenarios to account for the uncertainty of the species' future. We evaluate the current biological status of the candy darter by assessing the primary factors negatively and positively affecting the species to describe its current condition in terms of resiliency, redundancy, and representation (together, the 3Rs). We then evaluate the future biological status of the candy darter by describing a range of plausible future scenarios representing a range of conditions for the primary factors affecting the species and forecasting the most likely future condition for each scenario in terms of the 3Rs. As a matter of practicality, the full range of potential future scenarios and the range of potential future conditions for each potential scenario are too large to individually describe and analyze. These scenarios do not include all possible futures, but rather include specific plausible scenarios that represent examples from the continuous spectrum of possible futures. Consequently, the results of this SSA do not describe the overall risk to the species. Recognizing these limitations, the results of this SSA nevertheless provide a framework for considering the overall risk to the species in listing decisions.

CHAPTER 2 – SPECIES INFORMATION

Taxonomy and Genetics

The candy darter (previously the "finescale saddled darter") (*Etheostoma osburni*) belongs to the Percidae (true perches) family of fishes and was first described by Hubbs and Trautman (1932, entire) from a specimen collected in 1931 from Stony Creek, a tributary of the Greenbrier River in Pocahontas County, West Virginia. Other specimens used to describe the species were collected in 1885 and 1931 from Reed Creek, a tributary of the New River in Wythe County, Virginia (Hubbs and Trautman 1932, pp. 34–35). The candy darter is recognized by the American Fisheries Society (Page *et al*. 2013, p. 139) as a valid taxon and is listed as such in the Integrated Taxonomic Information System (ITIS) database (*http://www.itis.gov*) (accessed October 13, 2016). We have no information to suggest there is scientific disagreement about the candy darter's taxonomy.

An analysis of candy darter genetics across its range has not been conducted; however, analyses of specimens from the Greenbrier and Gauley River watersheds (figure 2) show that these two populations are significantly differentiated, indicating "long-standing" isolation from each other (Switzer *et al*. 2007, pp. 23–24; Gibson 2017, pp. 28–34). These genetic data also indicate that the demographic histories of the populations differ, with the Gauley River population showing evidence of undergoing a past genetic bottleneck and subsequent population expansion, and the Greenbrier population maintaining a larger, more stable population over time. The genetic differences noted between these two populations suggests they may be in the early stages of speciation, but the best available information indicates they are the same taxonomic entity (i.e., both the Gauley River and Greenbrier River populations are definitively candy darter (*Etheostoma osburni*)) (Switzer et al., 2007, pp. 23–24; Service 2016; Gibson 2017, p. 34).

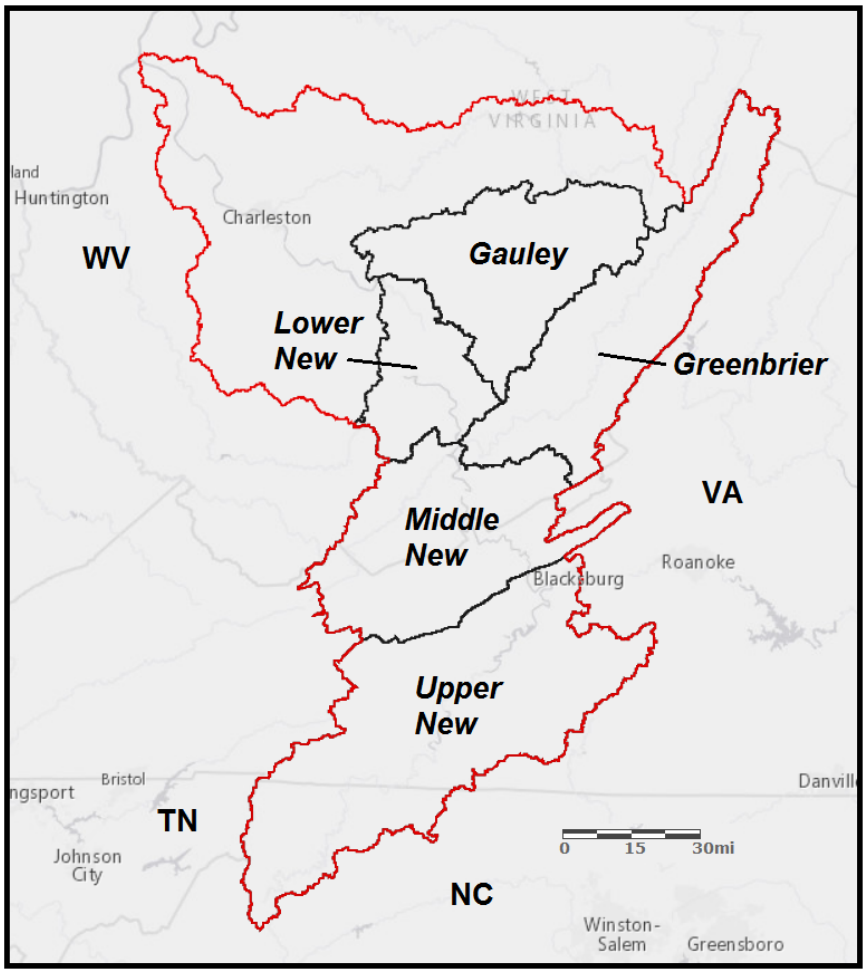

Figure 2. Kanawha River basin outlined in red with major watersheds of the upper Kanawha basin indicated.

The candy darter is considered a member of the *Etheostoma variatum* species group, which includes six closely related darter species: the candy darter (*E. osburni*), variegate darter (*E. variatum*), Kanawha darter (*E. kanawhae*), Arkansas saddled darter (*E. euzonum*), Missouri saddled darter (*E. tetrazonum*), and Meramec saddled darter (*E. erythrozonum*) (Hubbs and Black 1940, entire; Raney 1941, entire; McKeown *et al*. 1984, entire; Switzer 2004, entire; Switzer and Wood 2009, entire). The six species are historically allopatric (meaning they occupied geographically separate, non-overlapping areas), with the candy, variegate, and Kanawha darters being endemic to the Ohio River drainage and the Arkansas, Missouri, and Meramec saddled darters being endemic to the Missouri and White River drainages in Arkansas and Missouri (figure 3). Within the Ohio River group, the candy and Kanawha darters are known only from the upper Kanawha River basin in West Virginia and Virginia (candy darter) and Virginia and North Carolina (Kanawha darter). Within the upper Kanawha basin, the candy darter is endemic to the Gauley, Greenbrier, and New River watersheds (see figure 2) and the Kanawha darter is endemic to the uppermost portions of the Upper New River watershed. While their ranges are adjacent, the candy and Kanawha darters are not known to co-occur (Jenkins and Burkhead 1993, pp. 826–827).

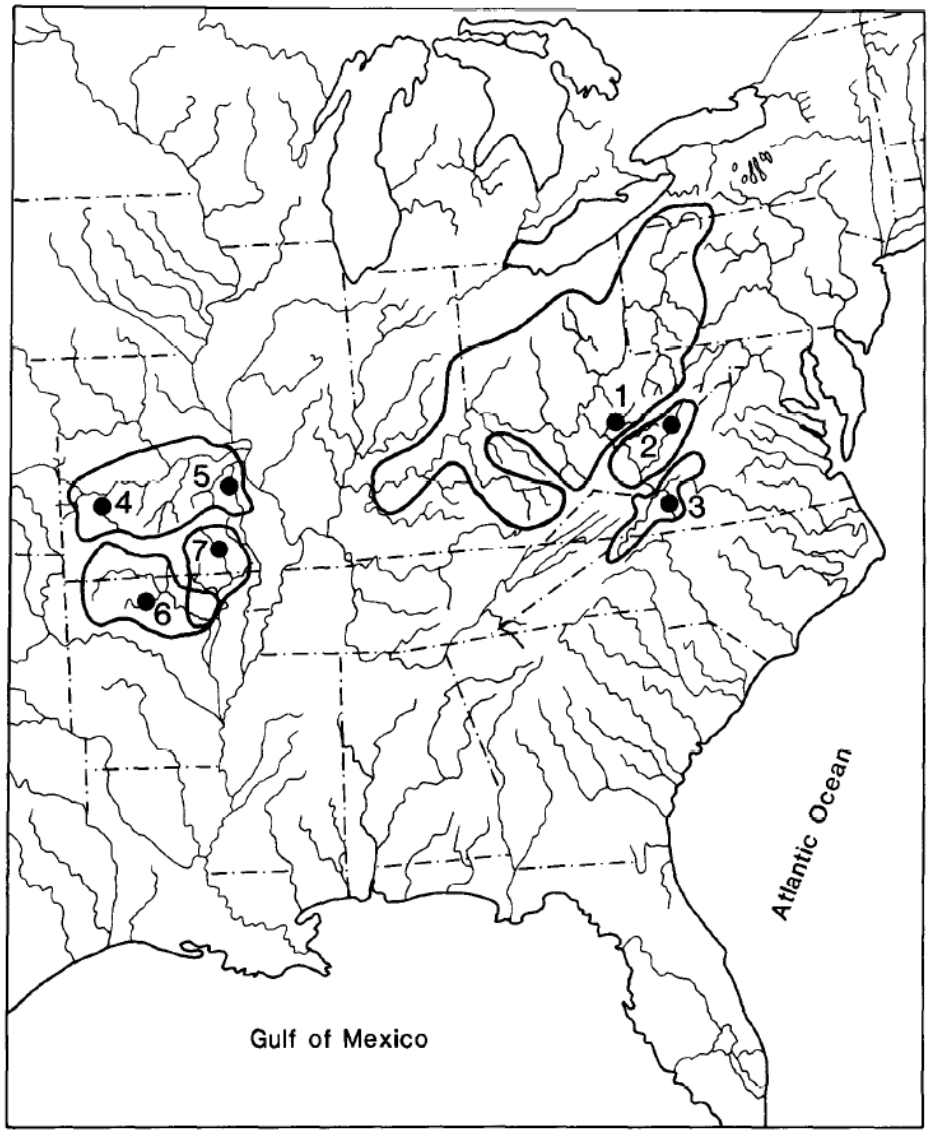

Figure 3. Historical distribution of (1) variegate darter, (2) candy darter, (3) Kanawha darter, (4) Missouri saddled darter, (5) Meramec saddled darter, and (6 and 7) Arkansas saddled darter (modified from McKeown *et al*. 1984).

The variegate darter is the most widely distributed of the group and naturally occurs throughout much of the upper and middle Ohio River drainage (see figure 3). Historically, the 6-meter (m) (20-foot (ft)) high Kanawha Falls in Fayette County, West Virginia served as a physical barrier and prevented the natural dispersal of the variegate darter into the waters of the upper Kanawha River basin (Hubbs and Trautman 1932, pp. 31–33; Raney 1941, pp. 7–8; Addair 1944, pp. 170–172, 174, 220; Jenkins and Burkhead 1993, pp. 823–830; Stauffer *et al*. 1995, p. 315). However, in the late 20th century variegate darters were introduced into the upper Kanawha basin, likely by "bait bucket transfer" (Ludwig and Leitch 1996, entire; Messinger and Chambers 2001, p. 6; Service 2016), and are now found within the range of the candy darter, specifically the Gauley, Greenbrier, and Lower New River watersheds of West Virginia. Genetic studies have demonstrated that where variegate and candy darter ranges now overlap the two species will hybridize, with the outcome being "genetic swamping" (the homogenization or replacement of native genotypes) of the endemic candy darter population and eventually its complete

replacement by variegate darters or hybrids (Switzer 2004, p. 111; Switzer *et al*. 2007, pp. 3–6, 22–25; Gibson 2017, pp. 13–19). These findings have important conservation implications for the candy darter and are discussed in **Chapter 3** (Current Condition) and **Chapter 4** (Species Viability).

Species Description

Candy darters are small (55–86 millimeters (mm) standard length (SL; the length measured from the tip of the snout to the last vertebra, which excludes the length of the caudal fin (tail)) (2.2–3.4 inches (in)), freshwater fish that tend to occupy the riffle (i.e., shallow areas of fast, turbulent flow) bottoms of fast moving, cool or cold water streams (Hubbs and Trautman 1932, p. 35; Jenkins and Burkhead 1993, p. 827). Males are very brightly colored, especially during the breeding season (figure 4). The species can be identified by 5 distinctive black saddles on its back and 9 to 11 vertical blue-green bars alternating with narrow bright red-orange bars along its sides. The females maintain a similar general pattern, but the colors are much more subdued, appearing a general olive hue overall (West Virginia Division of Natural Resources (WVDNR) undated factsheet).

Figure 4. Adult male (top) and female (bottom) candy darter (*Etheostoma osburni*) (photos courtesy of Dr. Stuart Welsh, USGS).

Life History

***Life cycle and Longevity*—**Adult candy darters breed in the spring, juvenile rearing takes place in the summer and fall, and young and adults overwinter together. Adults are sexually mature at 2 years of age and live to a maximum age of 3 years (Jenkins and Burkhead 1993, p. 828).

***Reproduction*—**Candy darters spawn in mid- to late spring, approximately late April through June (Kuehne and Barbour 2015, p. 80) and are classified as brood-hiding, benthic spawners (Jenkins and Kopia 1995, pp. 4–5). Female candy darters select patches of finer substrates (i.e., pebble and gravel) situated among larger cobble and boulder substrates in riffles and swift runs to deposit eggs (Jenkins and Kopia 1995, pp. 4–5), and males simultaneously deposit sperm to fertilize the eggs as they are deposited in the substrate (Jenkins and Kopia 1995, pp. 4–5). One species expert observed that female candy darters may lay multiple clutches of eggs (Dunn 2017, pers. comm.). During spawning, males become aggressively territorial, as evidenced by observations of torn fins and other signs of aggression following the breeding season (Jenkins and Burkhead 1993, p. 828). Helfrich *et al*. (1996, p. 4) observed spawning pairs interrupted by a third large male, after which the two males would chase and nip at each other until one was driven away; in all observed instances of spawning aggression, the larger male prevailed. Schoolcraft *et al*. (2002, p. 6) observed that females had a relatively low number of mature ova (average 170 per individual), and analysis showed no significant deviation from 1:1 sex ratios.

Time to hatching for fertilized eggs is not reliably described for candy darters, but for most *Etheostoma* species, incubation time lasts approximately 5 to 25 days, with variations based largely on water temperature (Hubbs *et al*. 1969, p. 184; Burr and Page 1979, p. 9). Newly hatched individuals are considered "young-of-year" until age 1, when total length (TL; the length measured from the tip of the snout to the end of the tail) is ~45 mm (1.8 in). They are then considered juveniles until females reach a TL greater than 60 mm (2.4 in) and males a TL greater than 65 mm (2.6 in), at which point they are considered adults.

***Movement/Dispersal*—**No information exists characterizing the movement patterns of candy darters among suitable habitat patches (Dunn and Angermeier 2016, p. 1278). The scientific literature suggests that many other small-bodied, riffle-dwelling fish species complete their lifecycle within single riffles or riffle complexes spanning just a few hundred meters (Hill and Grossman 1987, pp. 377–378; Roberts and Angermeier 2007, p. 422); however, some darter species have been documented moving upstream and downstream between riffles and between riffles and pools, with within-year movements generally ranging from 36 to 420 meters (m) (118 to 1,378 feet (ft)), but with some movements of up to 4.8 km (3.0 mi) (May 1969, pp. 86–87, 91; Freeman 1995, p. 363; Roberts and Angermeier 2007, pp. 422, 424–427). Longer migratory movements are suggested for other darter species. The bluebreast darter (*Etheostoma camurum*), a species that inhabits moderate- to large-sized streams and is typically found in riffles, similar to the candy darter, were found to be well-distributed throughout a 51-km (32-mi) reach of river during the breeding season (Trautman 1981, pp. 673–675). However, from September to early spring, the bluebreast darter's numbers appeared to shift from the upper half of the reach to the lower half of the reach (Trautman 1981, pp. 673–675). Individual bluebreast darters captured in the spring were documented to have moved 152 m (500 ft) in a single day. The author

concluded that bluebreast and other darter species migrated upstream in spring and downstream in the fall (Trautman 1981, pp. 673–675). We are uncertain if similar long distance, seasonally mediated movements are significant to the candy darter.

***Feeding*—**Candy darters are opportunistic "invertivores." Schoolcraft *et al*. (2007, entire) studied the food habits of candy darters in the Cherry River (Upper Gauley watershed) and found that adult candy darters fed almost exclusively on benthic macroinvertebrates (small, bottom-dwelling animals lacking a backbone), primarily mayflies and caddisflies, with some variation in food selection and feeding rates across seasons.

***Sheltering*—**Adult candy darters are often observed near rock cover (figure 5) (Chipps *et al.* 1994, p. 131; Jenkins and Kopia 1995, pp. 5–6) and may overwinter under the cover of rocks or woody debris in deeper water habitats (Leftwich *et al*. 1996, p. 6). After their first year, juveniles are presumed to use similar shelter as adults. Young-of-the-year and newly hatched individuals seek cover in benthic substrates in slower, shallower stream habitats than those used by adults (Dunn and Angermeier 2016, pp. 1271–1276). Table 1 provides a summary of the candy darter's life history needs based on the best available information.

Figure 5. Two views of adult male candy darters sheltering among rocky bottom substrate (photos courtesy of Corey Dunn, University of Missouri).

Table 1. Summary of candy darter life history information by life stage.

Life Stage	Resource and/or circumstance needs and related information
Eggs	• Spawning occurs from late April to mid-June, depending on location. • Eggs are buried by females in patches of fine substrates. • Mean substrate size for egg deposition sites was between 3 and 50 mm. • Spawning sites characterized by small gravel deposited behind large cobble and boulders where velocity was adequate to prevent siltation.
Young of Year	• Classified by size, approximately 35–40 mm, depending on sex and location. • Select slower, more marginal habitats with higher fine sediment concentration than older individuals. • Select slower water velocities (0.0–0.80 m/s).
Juveniles	• Classified by size, approximately 35–58 mm, depending on sex and location. • Select habitats with velocities more similar to those of adults in fall compared to spring. • Selected small substrates and were less averse to fine sediments than adults. • Selected intermediate water velocities (0.40–1.20 m/s) in both fall and spring.
Adults	• Classified by size, approximately 51–92 mm, depending on sex and location. • Sexual maturity reached by age 2. • Oldest known specimen was age 3. • Found among rubble and boulder in runs and riffles at depths of 0.4–1.0 m. • Select larger substrates than younger life stages, and avoid areas with fine sediments. • Select habitats with the swiftest water velocities available (>1.20 m/s in spring, >0.60 m/s in fall). • Males display antagonistic/aggressive behavior towards one another during spawning periods. • Individual pairs spawned one to four times before separating. • At times the spawning act would be interrupted by a large male, which was accompanied by aggression, chasing, and nipping until one male was driven away. • In all observed cases of male-male aggression, the larger male prevailed.
All	• Habitat specialists. • Most life stages select microhabitats with moderate flow (>0.19 m/s), shallow depth (<0.5 m), coarse substrates (>sand), and non-embedded and non-silted substrates (<26%). • Distribution among habitats: 29% riffle, 40% run, 30% glides, 4% pool. • Embeddedness consistently lower (<6%) in streams with robust. populations than in streams with localized or extirpated populations (6-25%). • Individuals tend to segregate among habitats based on life stage rather than behavior mode (i.e., activity type). • The strongest and most consistent habitat relationships across life stages were negative relationships with embeddedness. • Densities reported of 0 to 30 individuals per 100 m^2.

Physical Setting

The candy darter is endemic to portions of the upper Kanawha River basin, which is synonymous with the Gauley and greater New River watersheds (figure 6). The New River begins in the highlands of western North Carolina and flows generally northward through southwest Virginia and into southwestern West Virginia. Major tributaries of the New River include the Greenbrier River, which flows in a southwesterly direction and joins the New River at Hinton, West Virginia, and the Gauley River, which flows in a westerly direction and joins the New River at Gauley Bridge, West Virginia. The confluence of the New and Gauley Rivers forms the Kanawha River (just above Kanawha Falls) which then flows to the northwest through southern West Virginia before joining the Ohio River at Point Pleasant, West Virginia.

The gradient of the rivers and streams in the basin generally varies from moderate to high. For example, the average gradient of the New River over its entire course is about 2.5 meters per kilometer (m/km) (13 feet per mile (ft/mi)), and that of the Gauley River is about 4.9 m/km (26 ft/mi). However, some localized segments are noted for their high-gradient rapids and cascades, such as those in the Gauley River National Recreation Area, where the average gradient is 16.7 m/km (88 ft/mi) (Purvis 2002, p. 34). Floods are a naturally recurring phenomenon in the basin and can range from relatively minor high water events brought on by seasonal thunderstorms or frontal passages to major flooding resulting from tropical storms or hurricanes (Messinger and Hughes 2000, p. 39). Four flood control or hydroelectric dams were built in the upper Kanawha basin between 1932 and 1966. The Hawks Nest Dam (completed in 1932), Bluestone Dam (1949), and Claytor Dam (1939) are on the mainstem of the New River, and the Summersville Dam (1966) is on the Gauley River.

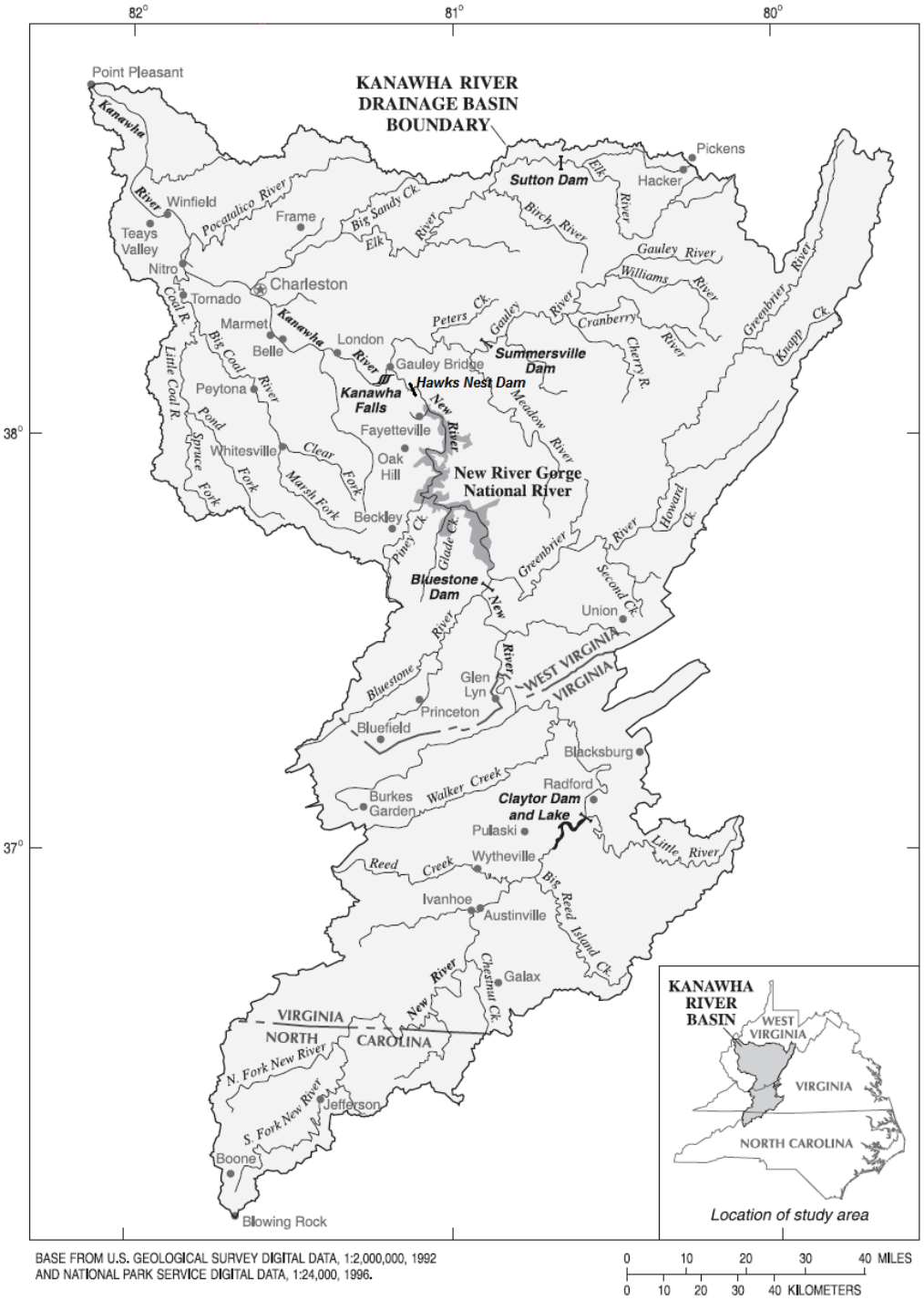

Figure 6. The Kanawha River basin (upper and lower) (modified from Messinger and Hughes 2000).

The range of the candy darter is located within portions of the Valley and Ridge and Appalachian Plateaus physiographic provinces (figure 7). These provinces are generally described as mountainous, with the Valley and Ridge province (primarily the Middle and Upper New and Greenbrier River watersheds) being characterized by long, linear ridges separating elongated valleys.

Valley and Ridge Physiographic Province

The general stream drainage pattern in the Valley and Ridge province is “trellised” (the streams follow the parallel valleys with the smaller tributaries feeding in from the steep slopes of the adjacent mountain sides). Because the geology of the valley floors is typically shale and limestone, the streams in the Valley and Ridge province are naturally neutral to slightly alkaline (pH = 7.0 to 8.0) (Messinger and Hughes 2000, p. 4; Chambers and Messinger 2001, p. 2).

Appalachian Plateau Physiographic Province

The Appalachian Plateaus physiographic province (primarily the Gauley and Lower New River watersheds) is characterized by steep rugged mountainsides separating deep narrow, disconnected valleys. The stream drainage patterns are “dendritic,” analogous to the branching of a tree, with smaller streams flowing together to form larger tributary streams, which likewise join to form larger streams or rivers. Because of the insoluble sandstone and shale bedrock underlying much of the Appalachian Plateaus province, stream water is often poorly buffered and subject to low pH conditions as a result of acid precipitation (Chambers and Messinger 2001, pp. 2–3). Additionally, because of the underlying geology, there are fewer groundwater springs in the Appalachian Plateaus province than in the Valley and Ridge province. This leads to more variable seasonal stream flows in the former province, often including very low flows in late summer (Angermeier 2017, pers. comm.). Stream gradients (*i.e.*, steepness) throughout are moderate to high and tend to increase in the higher elevation areas (Messinger and Hughes 2000, p. 4). Portions of the Appalachian Plateaus province in the Gauley and New River watersheds are underlain by minable coal deposits (Messinger and Hughes 2000, pp. 19–20).

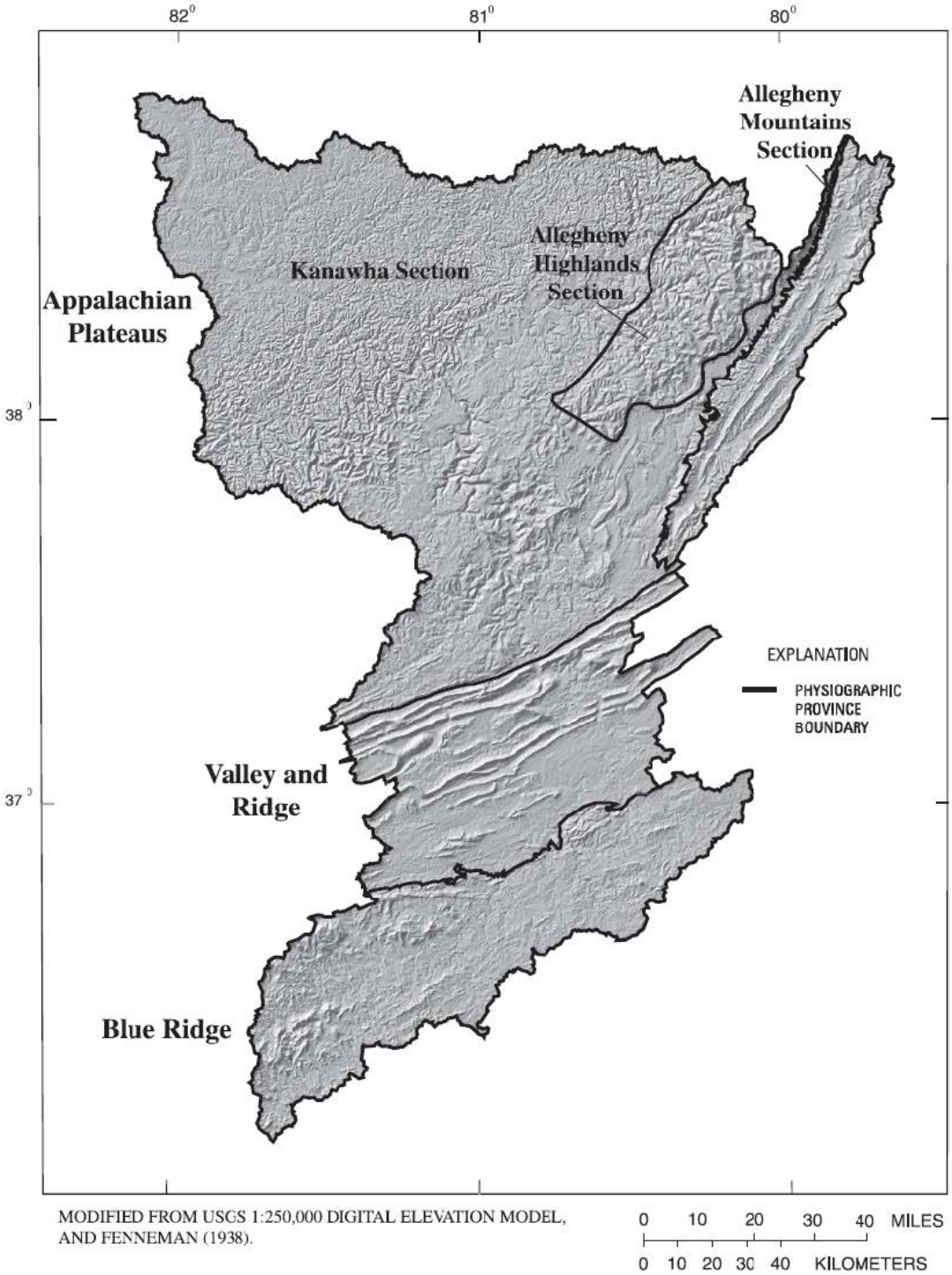

Figure 7. Physiography of the Kanawha River Basin (from Messinger and Hughes 2000).

The range of the candy darter falls within the Central Appalachian Broadleaf Forest, Coniferous Forest ecosystem province (Bailey 1980, pp. 19–20; McNab and Avers 1996, section M221; McNab *et. al*., 2007, p. 11). The climate is temperate with four distinct seasons, and the average annual precipitation varies from 890 mm (35 in) in the valleys to 2,040 mm (80 in) at some high elevation sites. Precipitation is fairly well distributed throughout the year, although dry periods

may occur during the late summer. The natural vegetation varies with topography and geological substrate, but is generally closed-canopy, mixed mesophytic (adapted to moderately moist conditions) forest. Typical forest compositions include oak-pine, oak-hickory-pine, Appalachian oak forest, northern hardwoods, and spruce-fir.

Landcover in the upper Kanawha River basin, which drains approximately 21,262 square kilometers (km^2) (8,209 square miles (mi^2)) is mostly forested, although substantial areas of some watersheds have been converted to agricultural use (table 2). Timber harvesting, which was historically ubiquitous in the region (Eller 1982, pp. 93–112), now generally occurs less intensively on a rotational basis throughout the basin (Piva and Cook 2011, entire). Because of the generally rugged terrain, most roads and railroads, residential, commercial, and industrial development, and agriculture, is concentrated in the valley bottoms, often directly adjacent to streams and rivers.

Table 2. Landcover within the watersheds of the upper Kanawha River basin (data from the National Land Cover Database 2011, see Homer *et al*. 2015).

	Total Area		Percent Landcover		
	km^2	mi^2	Forested*	Agricultural	Developed
Lower New	1,791	692	80%	8%	11%
Middle New**	4,231	1,634	76%	16%	7%
Upper New	7,300	2,819	61%	31%	8%
Gauley	3,680	1,421	90%	4%	5%
Greenbrier	4,260	1,645	83%	12%	5%

* Includes shrub/scrub and herbaceous vegetation

** Includes the Bluestone River watershed

Most land in the basin is privately owned, with some areas in Federal ownership. For example, substantive areas in the upper Gauley and Greenbrier watersheds are within the Monongahela National Forest, and the Gauley River passes through the Gauley River National Recreational Area. The New River dissects the Jefferson National Forest in Virginia, and most of the lower New River and its riparian area is part of the New River Gorge National River. The lower reach of the Bluestone River and its riparian area are within the Bluestone National Scenic River. Other publically managed lands within the basin include several state forests, wildlife management areas, and parks.

Habitat Needs

The candy darter is known from 2nd order and larger (generally wider than about 3.7 m (12 ft)) streams and rivers (including the mainstem of the New River) and is described as a habitat specialist, being most often associated with faster flowing stream segments with coarse bottom substrate (*e.g*., gravel, cobble, rocks, and boulders) and low levels of siltation (Addair 1944, p. 170; Jenkins and Burkhead 1993, pp. 828–829; Chipps *et al*. 1994, entire; Jenkins and Kopia 1995, pp. 5–6; Leftwich *et al.* 1996, pp. 6–12; Dunn 2013, pp. 16–17, 23–24; Dunn and Angermeier 2016, pp. 1267, 1272–1273).

In streams maintaining favorable habitat conditions, including abundant coarse bottom substrate, candy darters can occur throughout the stream continuum in relatively high numbers. For example, a survey of Stony Creek (a rocky, cool or cold water trout stream that flows into the

New River) (figure 8) found candy darters in 74 percent of the habitat units sampled (n = 942) throughout the 13.8-stream kilometer (skm) (8.6-stream mile (smi)) survey reach (Leftwich *et al*. 1996, pp. 7–12). Candy darters were observed in all stream habitat types (82 percent of riffles, 90 percent of runs, 79 percent of glides, and 41 percent of pools) with densities ranging from 0 to 30 candy darters per 100 square meters (CD/100 m^2) (0 to 2.79 CD/100 square feet (ft^2)). The highest densities were found in riffles, with an average of 10 CD/100 m^2 (0.93 CD/100 ft^2) (Leftwich *et al*. 1996, pp. 7–12). Studies have also noted that adult candy darters are often observed near rock cover (Chipps *et al.* 1994, p. 131; Jenkins and Kopia 1995, pp. 5–6) and may overwinter under the cover of rocks or woody debris in deeper water habitats (Leftwich *et al.* 1996, p. 6).

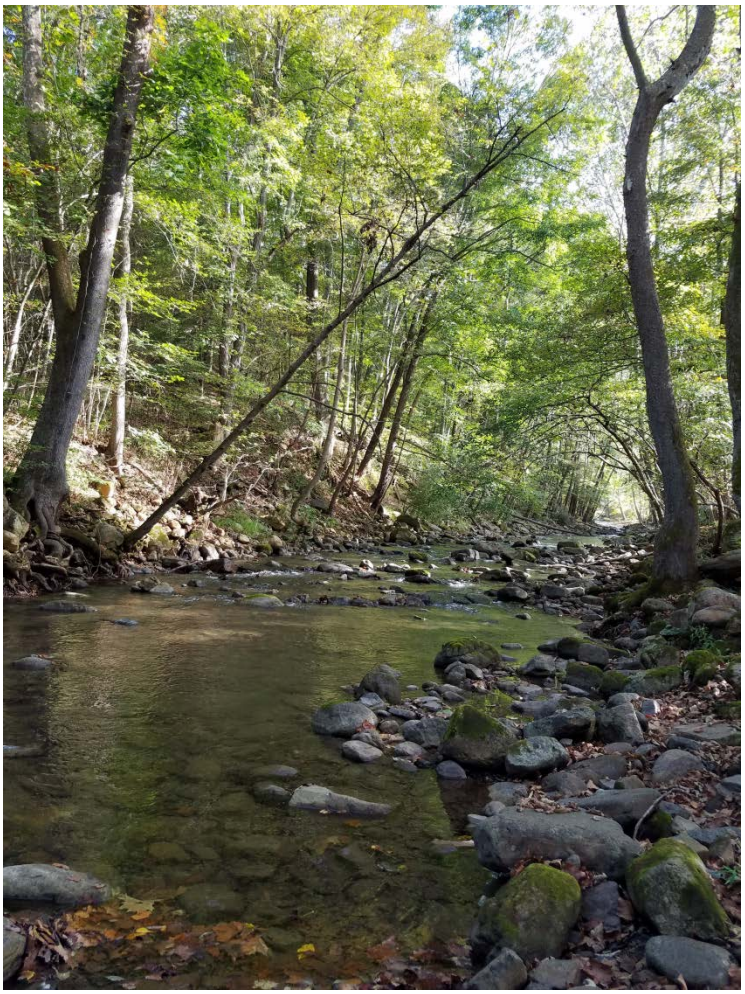

Figure 8. View of Stony Creek near Kimballton, Virginia (approximately 2 skm (1.2 smi) upstream of the New River confluence) (photo courtesy of Krishna Gifford, USFWS).

Candy darters appear to prefer shallower (depths less than 50 centimeters (cm) (19.7 in) and at least moderately flowing (velocities greater than19 centimeters/second (cm/s) (7.5 in/s) waters, with individual darters partitioning within stream microhabitat by age class (Chipps *et al.* 1994, entire; Helfrich *et al*. 1996, pp. 2–3; Dunn 2013, pp. 23–24; Dunn and Angermeier 2016, pp. 1272–1273). Observations of candy darter microhabitat use in the spring and fall of 2011 found that adults selected areas with faster flowing waters (greater than 120 cm/s (47.2 in/s) in the

spring; greater than 60 cm/s (23.6 in/s) in the fall), juveniles selected intermediate velocities (40 to 120 cm/s (15.7 to 47.2 in/s)), and first year fish selected slower flowing areas (0 to 80 cm/s (0 to 31.5 in/s)) (Dunn and Angermeier 2016, pp. 1272–1273). This pattern of habitat partitioning by life stage has been noted by other researchers (Jenkins and Kopia 1995, pp. 5–6).

Suitable candy darter habitat is also characterized by low levels of siltation and stream bottom embeddedness (the degree to which gravel, cobble, and boulders are surrounded by, or covered with, fine sediment particles) (Jenkins and Kopia 1995, pp. 5–6; Dunn and Angermeier 2016, entire). In 1991, researchers surveyed for the species at 22 locations in 10 streams in the upper Gauley and Greenbrier River watersheds and concluded that excessive siltation characterized areas where the species had declined or was absent (Chipps *et al*. 1993, p. 52). Dunn and Angermeier (2016, entire) observed candy darter microhabitat use in three occupied streams (one each in the Gauley, Greenbrier, and New River watersheds) and found that, in general, individuals selected sites with less than 26-percent silt cover and substrate embeddedness. The researchers also noted differences in microhabitat selection based on life stage. Adult candy darters almost completely avoided areas where silt cover and embeddedness were greater than 25 percent, while younger individuals were less averse to areas with fine sediments (Dunn and Angermeier 2016, p. 1273). Of the habitat variables examined in the study (depth, velocity, substrate, embeddedness, and silt cover), embeddedness was consistently the most important parameter determining individual candy darter microhabitat selection (regardless of life stage) and overall population robustness (Dunn and Angermeier 2016, p. 1275).

Based on candy darter occurrence records from cold, cool, and warm water streams, the species is probably best described as eurythermal (*i.e.*, able to tolerate a wide range of water temperatures). In 2012, Dunn (2013, pp.18–28) surveyed 43 sites within the historical range of the candy darter and determined that sites where candy darters were present had cooler daily temperatures in all seasons, as well as a greater range of annual and daily temperatures, than sites not harboring the species. In the summer, sites with candy darters had average maximum temperatures of 27.8 degrees Celsius (ºC) (82.0 degrees Fahrenheit (ºF)), with three candy darter sites having maximum summer temperatures over 30.0 ºC (86.0 ºF). In the winter, the candy darter sites had average minimum temperatures of 0.2 ºC (32.4 ºF). Between 1973 and 1974, Stauffer *et al.* (1976, pp. 8–9, 16) collected two candy darters from the New River near Glen Lyn, Virginia. One capture site was in an area where ambient water temperatures ranged from approximately 5 ºC to 25 ºC (41 ºF to 77 ºF), depending on the month. The other capture site was influenced by the heated discharge from an electrical power plant where monthly water temperatures were calculated to be higher, ranging from approximately 11 ºC to 32 ºC (52 ºF to 90 ºF), seasonally. Jenkins and Kopia (1995, pp. 5–6) commented on these records and reported the water temperatures where these specimens were captured to be 22.2 ºC to 27.8 ºC (72.0 ºF to 82.0 ºF). While the known distribution and abundance of the candy darter seems to indicate that cool or cold "trout" streams (*e.g.*, the headwaters of the Gauley and Greenbrier Rivers, Stony Creek) are preferred habitat, data suggest that if the habitat is otherwise favorable for the species (*e.g.*, abundant rocky, unembedded bottom substrate) warm water streams may also be suitable for the species (Dunn 2013, pp. 24–26).

Little is known regarding other water quality parameters tolerated or preferred by the candy darter; however, we have inferred suitable conditions for the species based on parameters

observed in high quality streams and rivers in the region, including those currently supporting candy darter populations. In general, the streams and rivers in the region are well oxygenated (greater than 8 milligrams per liter (mg/l) dissolved oxygen (DO)) and maintain circumneutral pH (pH 6.5 to 7.5) (pH is a measure of acidity or alkalinity). However, because of the underlying geology, some streams in the Gauley River watershed are somewhat naturally acidic (lower pH), and some in the Ridge and Valley watersheds (*e.g.*, Greenbrier and New River watersheds) are naturally slightly alkaline (higher pH) (Chambers and Messinger 2001, p. 2). Schoolcraft *et al*. (2002, p. 8) collected candy darters from Cherry River in the Upper Gauley watershed and reported DO concentrations of 8 to 9 mg/l and pH measurements of 7.0 to 8.0.

Little is known regarding the minimal habitat patch size or degree of habitat connectivity necessary to support persistent candy darter populations or subpopulations. However, it is generally understood in the field of conservation biology that larger and more-connected populations contribute to the long-term viability of a species and that smaller isolated populations are more at risk of decline or extirpation as a result of genetic drift, demographic or environmental stochasticity, and catastrophic events (Gilpin and Soulé 1986, pp. 32–34; Angermeier 1995, entire; Fagan 2002, p. 3248; Wiegand *et al*. 2005, entire; Letcher *et al*. 2007, 5–6; Peterson *et al*. 2014, pp. 564–565). Occurrence data dating back to 1885 show candy darters inhabited a variety of streams and rivers throughout their range. While there are natural areas of fast flowing cascades and rapids within the upper Kanawha River basin, they do not appear to pose a significant barrier to fish movement, as evidenced by the expansion of variegate darters in the Lower Gauley and Lower New River watersheds. Therefore it is reasonable to conclude that the candy darter evolved in a connected river system that allowed for potential population shifts (i.e., expansion and contraction of ranges, abandonment and recolonization of streams, longer seasonal movements, etc.) as environmental and/or demographic conditions changed. Under these conditions we expect the species maintained some level of genetic flow between the various populations, although genetic analyses of populations from the Gauley and Greenbrier watersheds indicate those two populations have been isolated from each other for an extended time period (Switzer *et al*. 2007, pp. 23–24; Gibson 2017, pp. 31–32). We have no information with which to characterize the degree of genetic flow within and among the other populations.

In summary, candy darters occur in 2nd order and larger streams and rivers with moderate to fast flowing water. The species appears to prefer relatively shallow stream reaches (e.g., riffles, runs, and glides) with rocky bottom substrates. Adult candy darters are intolerant of excessive bottom sedimentation. The available data indicate the species is tolerant of warm water conditions, but that cool or cold water temperatures are preferred. Based on the characteristics of high quality rivers and streams in the region, including those supporting abundant candy darter populations, we conclude candy darters likely need well oxygenated circumneutral pH waters. While data are sparse regarding the minimum patch size and degree of genetic connectivity required for candy darter population viability, historical occurrence data and the fundamentals of conservation biology suggest these factors are important to the species.

Historical Range and Distribution

The best available information supports that the candy darter is endemic to the upper Kanawha River basin. Records dating back to 1885 indicate that the historical range of the candy darter is limited to the Gauley and greater New River watersheds in West Virginia and Virginia (but not extending into the New River watershed in North Carolina) (see figures 2 and 6) (Hubbs and Trautman 1932, entire; Addair 1944, pp. 170–172; Burton and Odum 1945, pp. 191–192; Hocutt *et al*. 1978, pp. 61–64; Hocutt *et al*. 1979, 63–74; Chipps *et al*. 1993, pp. 52–54; Jenkins and Burkhead 1993, pp. 827–830; Stauffer *et al*. 1995, pp. 308–309, Helfrich *et al*. 1996, entire; Bye 1997, entire; Welsh *et al*. 2006, pp. 14–16, 22, 25). The first documented candy darter specimen was collected from South Fork Reed Creek in the Upper New River watershed of Virginia in 1885; however, the specimen was not identified until the species was formally described by Hubbs and Trautman in 1932 (p. 35). The holotype (the single specimen on which Hubbs and Trautman based the species' description) was collected from Stony Creek in the Greenbrier River watershed of West Virginia by Addair in 1931 (Hubbs and Trautman 1932, pp. 34–35). Subsequent fish surveys of the region documented candy darters in other upper Kanawha basin tributary watersheds, including the Gauley and Middle New River (which includes the Bluestone) watersheds (Addair 1944, pp. 170–172; Burton and Odum 1945, pp. 191–192; Hocutt *et al.* 1978, pp. 61–64; Hocutt *et al*. 1979, 63–74; Chipps *et al.* 52–54; 1993; Jenkins and Burkhead 1993, pp. 827–830; Jenkins and Kopia 1995, pp. 6–11; Stauffer *et al*. 1995, pp. 308–309, Helfrich *et al.* 1996, entire; Bye 1997, entire; Welsh *et al*. 2006, pp. 14–16, 22, 25). A single 1972 report of a candy darter in the Elk River below the Kanawha Falls was not confirmed and is considered dubious (Hocutt *et al.* 1979, p. 63). There are no other historical or recent reports of the species from below Kanawha Falls.

The candy darter is not confirmed to occur in the Lower New River watershed; however, the best available data suggest that the species did likely occupy this watershed. Jenkins and Kopia (1995, pp. 7–8) reported that Lower New River fish collections from 1964 and 1991 may have produced candy darters, but the specimens were never confirmed. Because the Lower New River watershed is geographically positioned between the Middle New and Greenbrier River watersheds (upstream) and the Gauley River watershed (downstream), each of which have confirmed candy darter populations and suitable habitat, it is reasonable to conclude that the species likely occurred in the Lower New River watershed.

Support that the Lower New River watershed was historically occupied by the candy darter is provided by the expansion of the variegate darter within this watershed and other connected candy darter habitats. The variegate darter is a closely related species with similar life history characteristics and habitat requirements as the candy darter (Jenkins and Burkhead 1993, pp. 824, 828–829; Stauffer *et al.* 1995, pp. 308–309, 315; Kuehne and Barbour 2015, pp. 66–67, 80–81, 86–87). Since its introduction above Kanawha Falls in the late 20th century, the variegate darter has colonized the Lower New River watershed, along with known candy darter streams in the lower Gauley and Greenbrier River watersheds (Wellman 2004, p. 10; WVDNR 2016, unpublished data; Switzer *et al.* 2007, entire; Gibson 2017, entire). Because the variegate darter and candy darter share general habitat requirements, this pattern of variegate darter expansion suggests that the Lower New River watershed likely maintains habitat conditions also suitable for the candy darter. Additionally, genetic analysis of phenotypical variegate darters collected

from three Lower New River tributaries (Glade Creek, Manns Creek, and Laurel Creek) between 2004 and 2006 confirmed the presence of candy darter alleles in these populations (Switzer 2004, pp. 93, 111; Switzer *et al*. 2007, pp. 28, 33). These candy darter genetic markers could have been introduced into the Lower New watershed via the movement of individual candy darters or hybrids from the Greenbrier watershed or they could be a remnant of resident New River candy darters that were subsequently extirpated by genetic swamping after variegate darters were introduced. This line of evidence (the report of candy darter specimens in 1964 and 1991, the historical connectedness of the Lower New River watershed to known candy darter populations, and the apparent availability of suitable habitat) leads us to conclude that the Lower New River watershed represents a historical candy darter metapopulation.

Although the available survey data appear to indicate the candy darter was always patchily distributed and perhaps rare within most of its historical range, it is important to consider that by the time of the earliest candy darter records (Addair 1944, p. 171), the species had likely already undergone a significant reduction in distribution and numbers (Jenkins and Kopia 1995, pp. 2, 11–12; Dunn 2013, p. 19; Dunn and Angermeier 2016, p. 1267). While early (pre-1900) survey data are sparse, fishery experts agree that by the late 1800s overall fish populations in the region had already undergone severe declines as a result of widespread aquatic habitat degradation (*e.g*., sedimentation, increased temperatures, chemical toxicity) caused by unregulated, industrial-scale logging, agriculture, coal mining, and sewage and chemical discharges (Ayres and Ashe 1905, pp. 17–23, 73–77; Goldsborough and Clark 1907, pp. 31–33; Addair 1944, pp. 7–9, 201–202, 205; Hocutt *et al*. 1978, p. 75; Eller 1982, pp. 93–112; Dolloff 1994, pp. 121–122; Messinger and Chambers 2001, p. 6). Several contemporaneous scientific accounts from within known candy darter areas describe habitat conditions and provide information on overall fish abundance during this period. In 1888, Reed Creek (from which the first known candy darter specimen had been collected 3 years previously) was described as a warm, muddy stream flowing through cultivated fields and pastures (Jordan 1889, p. 140). In 1900, researchers concluded that in West Virginia "the aquatic life in general, and fishes in particular, had been and are now in many streams being greatly injured and in others practically destroyed by the unwise and destructive operations of the lumberman and the miner" (Goldsborough and Clark 1907, p. 31). While Goldsborough and Clark (1907, pp. 31–32) reported that fish were still abundant in many of the less disturbed headwater streams of the Greenbrier River, they concluded that fish in the lower Greenbrier and New River tributaries had suffered severe declines. In the Bluestone River, Goldsborough and Clark (1907, pp. 31–32) noted that coal mining operations in the upper watershed had "greatly reduced" fish throughout nearly the entire river. Therefore, by the time researchers first began documenting the candy darter in the 1930s, the abundance and distribution of the species had likely been significantly reduced as a result of widespread habitat degradation.

The factors supporting the candy darter having been more abundant and widely distributed within its range than indicated by post industrialization surveys include: (1) the geographical distribution and historical connectedness of known candy darter streams; (2) the diversity of habitat conditions (*e.g*., stream size, gradient, and water temperatures) associated with all known candy darter occurrences (Stauffer *et al*. 1976, p. 16; Jenkins and Kopia 1995, pp. 5–6; Dunn 2013, pp. 24–26; Dunn and Angermeier 2016, p. 1267); (3) high candy darter abundance and continuity documented in some streams with high quality habitat (Chipps *et al.* 1993, p. 52;

Leftwich *et al.* 1996, pp. 8–9); and (4) severe degradation of aquatic habitat conditions and declines in fish abundance prior to comprehensive fish surveys. Together these factors support the conclusion that the candy darter was likely more widely distributed and abundant throughout its historical range where suitable habitat (described above) existed.

Current Range and Distribution

The best available data indicate that the candy darter has been extirpated from the Bluestone and Lower New River watersheds, but that the species continues to occupy areas in the Upper Gauley, Lower Gauley, Greenbrier, Middle New, and Upper New River watersheds (figure 9 and **Appendix A**) (Jenkins and Burkhead 1994, p. 829; Welsh *et al.* 2006, p. 43; Switzer *et al*. 2007, pp. 3, 12, 22–24; Dunn 2013, p. 23; WVDNR 2016, unpublished data). These data indicate that, of 35 known candy darter populations, 17 have been extirpated, and many of the remaining populations are small and/or isolated from each other by physical barriers or long reaches of unoccupied (and possibly unsuitable) habitat (*e.g*., Stony, Walker, Wolf, and Cripple Creeks in the Middle and Upper New River watersheds and the Lower Gauley population below the Summersville Dam). The most abundant candy darter populations occur in the Upper Gauley and upper Greenbrier River watersheds, and in Stony Creek in the Middle New River watershed (Dunn 2013, p. 10; McBaine 2016, unpublished data). However, the distribution of candy darters in the Greenbrier River watershed has and is changing rapidly as variegate darters expand their range (Switzer *et al*. 2007, pp. 3–6, 22–25; Gibson 2017, p. 19), which is discussed further in the following chapters.

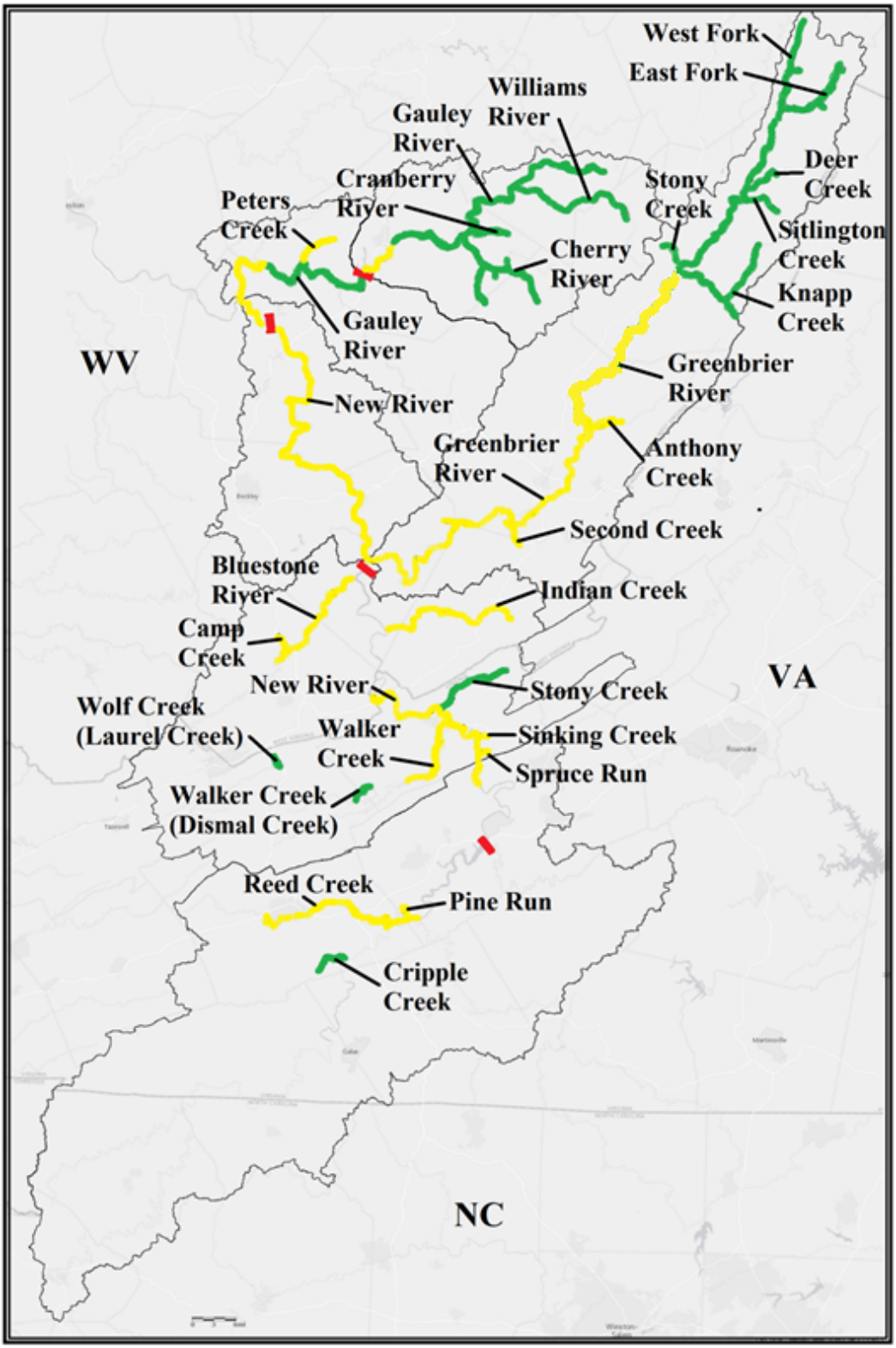

Figure 9. Current and historical distribution of the candy darter. Green indicates extant candy darter populations; Yellow indicates historical or extirpated populations. Red lines are major dams that present barriers to fish movement.

CHAPTER 3 – CURRENT CONDITIONS

Methodology

To assess the biological status of the candy darter across its range, we used the best available information, including peer reviewed scientific literature and academic reports, and survey data provided by state and federal agencies. Additionally, we consulted with several species experts who provided important information and comments on candy darter life history, genetics, and habitat. Fundamental to our analysis of the candy darter was the determination of scientifically sound analytical units at a scale useful for assessing the species. In this report, we defined candy darter analytical units (i.e., subpopulations, populations, and metapopulations) based primarily on known occurrence locations and stream connectivity. We acknowledge that specific candy darter demographic and genetic data are sparse with which to support this construct. However, the species experts generally agreed that this was a valid approach for assessing the species' condition in this SSA report. After identifying the factors (i.e., stressors) likely to affect the candy darter, we developed a semiquantitative model to estimate the condition of each candy darter population. The habitat and demographic metrics used in the model were selected because the supporting data were relatively consistent across the range of the species and at a resolution suitable for assessing the species at the population level. The model output was a condition score for each candy darter population that was then used to assess the candy darter across its range under the 3Rs. While we acknowledge there is uncertainty regarding some of the scientific data and assumptions used to assess the biological condition of the candy darter, the species experts generally agreed with the overall methodology and confirmed that the results were reflective of their observations of the candy darter and its habitat. Our approach was further validated by four independent peer reviewers.

Analytical Units

There is little information available regarding the demographic or genetic processes that define the spatial structure of candy darter populations, therefore, for purposes of analyzing the status of the candy darter in this SSA report, we defined the species' populations and metapopulations based primarily on stream and watershed connectivity (figure 10). These analytical units generally conform to a Hydrologic Unit Code (HUC) (see *https://water.usgs.gov/GIS/huc.html*, accessed Jan. 27, 2017), which are geographical units used to define watersheds at various scales. Herein, candy darter metapopulations are defined by larger watersheds, typically HUC 8 level watersheds, that maintain internal stream connectivity (i.e., no internal barriers to fish movement) but that have limited connectivity, or in cases where barriers to fish passage exist, no connectivity, with other river watersheds. Candy darter occurrences (current or historical) within an individual stream system, typically a HUC 10 watershed, are considered a population. However, we note that in some cases these candy darter populations occur (or occurred) across HUC 10 boundaries or only in a single stream within a larger HUC 10 watershed. Individual candy darter populations within a metapopulation that are separated from other populations by more than 50 skm (31 smi) are assumed to be "isolated," and have limited potential for genetic exchange. Additionally, based on the distribution of the species within some populations, we may further identify subpopulations by specific stream segments or tributary streams within a HUC 10 watershed (see **Appendix A** for a more detailed description of the analytical units).

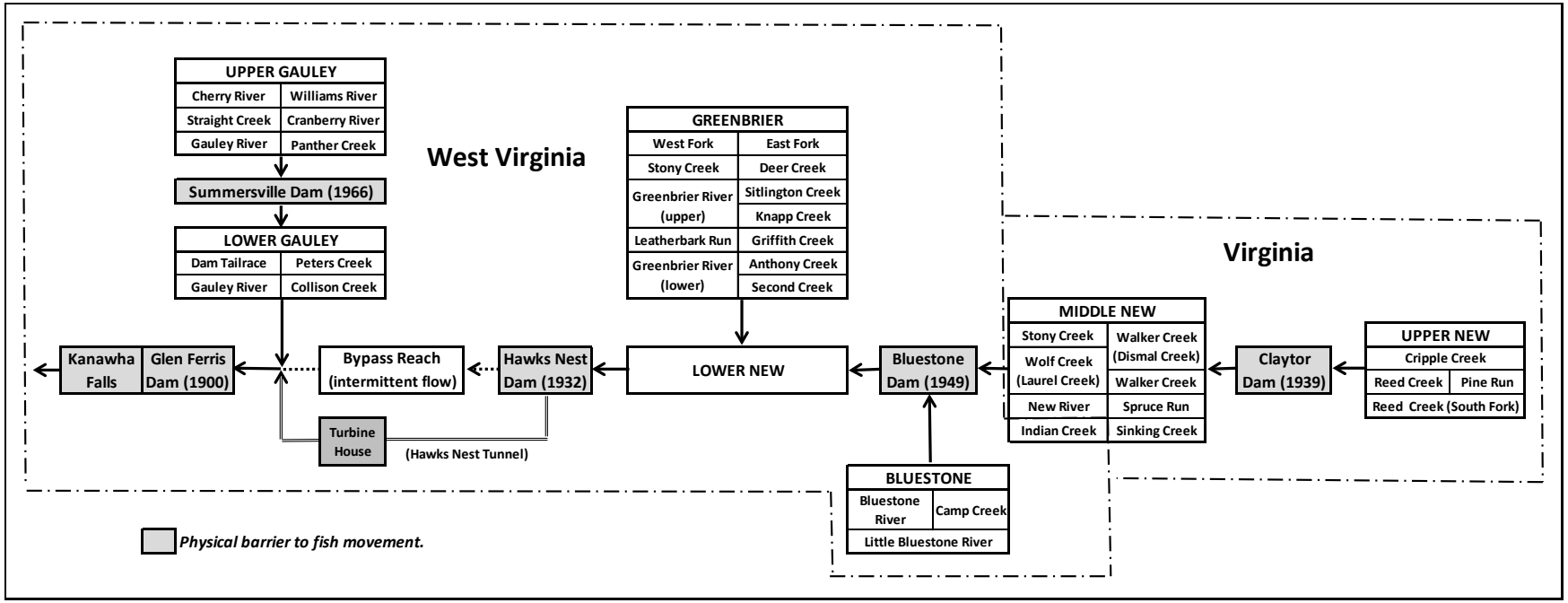

Figure 10. Relationship of candy darter analytical units used in this SSA report. Arrows indicate direction of flow.

Factors Influencing Current Condition

Based on the candy darter life history and habitat needs discussed previously and after consultation with the species' experts (Service 2016), we identified the potential stressors (negative influences) and the contributing sources of those stressors that are likely to affect the species' current condition and viability (figure 11).

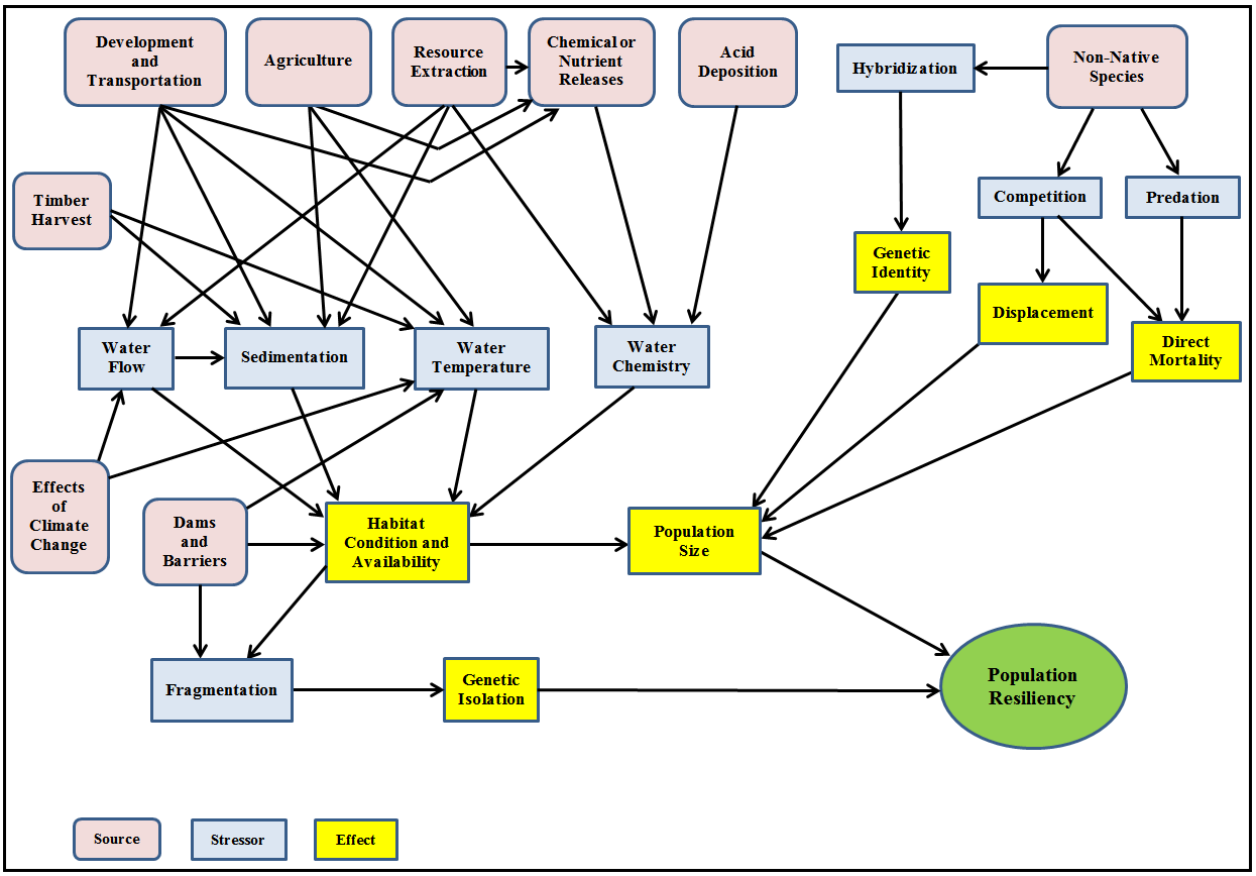

Figure 11. Influence diagram showing the relationship between potential stressors (blue), their contributing sources (pink), and their potential effect on the conditions or resources candy darter require (yellow) in support of population resiliency (green).

Hybridization—During an informational meeting of candy darter experts and land managers from the Virginia Department of Game and Inland Fisheries (VDGIF), the West Virginia Division of Natural Resources (WVDNR), the U.S. Geological Survey (USGS), the National Park Service (NPS), the U.S. Forest Service (USFS), and the Virginia Polytechnic Institute (VPI) ranked hybridization (the interbreeding of individuals from different taxa) as the primary stressor affecting the candy darter's viability (Service 2016). Hybridization is relatively common in freshwater fishes and has been documented in many darter species, under both natural conditions and following human interference (Keck and Near 2009, entire). It is important to note that hybridization is considered particularly problematic in situations where non-native species are introduced into areas outside of their natural ranges (Allendorf *et al*. 2001, entire; Todesco *et al*. 2016, entire).

As discussed previously, the variegate darter, a closely related species to the candy darter, was historically precluded from the upper Kanawha River basin by the Kanawha Falls at Glen Ferris, West Virginia. However, by the late 20th century, variegate darters were established in the upper Kanawha basin, likely as a result of human-mediated "bait-bucket" transfer (Switzer *et al*. 2007, p. 4; Service 2016). Bait bucket transfers occur when anglers or commercial bait sellers collect species of live baitfish indigenous to one watershed and transport them for use in watersheds where they may not be native. Often these baitfish escape or are intentionally released in the new watershed and, under certain conditions, can establish new reproducing populations (Ludwig and Leitch 1996, entire). Candy darters and variegate darters have similar life histories, are similarly sized, share general habitat requirements, and are not subject to any known physiological or behavioral barriers to reproduction (Hubbs and Trautman 1932, pp. 33–34; Switzer *et al*. 2007, pp. 3–6). Therefore, once variegate darters were established in the upper Kanawha basin, the interbreeding of the two species was likely inevitable (figure 12).

Figure 12. Male candy darter (top) collected from the Cherry River (Upper Gauley watershed); male hybrid specimen (middle) collected from Deer Creek (upper Greenbrier watershed); male variegate darter (bottom) collected from Twentymile Creek (Lower Gauley watershed) (photos courtesy of Dr. Stuart Welsh, USGS).

A variety of factors make hybridization between the variegate darter and the candy darter particularly problematic for the latter species. First, data indicate that variegate darter/candy darter pairings produce fertile hybrid offspring that, in turn, are capable of successfully

reproducing with other hybrids or with pure parental individuals (Switzer *et al.* 2007, pp. 22–23; Gibson 2017, pp. 16–19). This genetic "introgression" appears to result in the loss of genetically pure candy darters (and pure variegate darters) from a population (e.g., genetic swamping). This phenomenon may be exacerbated in this situation because, in West Virginia, male variegate darters (and presumably phenotypical variegate darter hybrids) are suggested to be larger than male candy darters (Stauffer *et al.* 1995, pp. 308, 315; Service 2016). Therefore, the male variegate darters (or phenotypical variegate darter hybrids) may physically outcompete male candy darters for territory or spawning opportunities, including with pure candy darter females (May 1969, 87–88; Zhou and Fuller 2016, p. 7). This reproductive asymmetry, where hybrid offspring are more likely to be the product of a variegate darter male and a candy darter female, may place the more rare candy darter at a high risk of genetic swamping (Todesco *et al*. 2016, pp. 895, 897–898).

Since their introduction, variegate darters have rapidly expanded their range in the upper Kanawha basin, including into some streams with no record of candy darter occupation. This may indicate that the variegate darter is more adaptable to certain habitat conditions than the candy darter, but we have little data with which to confirm this hypothesis. It is worth noting that this type of rapid range expansion is not unique. For example, in two separate introduction events in different watersheds, a different darter species, the greenside darter (*E. blennioides*), dramatically expanded in range and abundance within approximately 10 generations (Neely and George 2006, entire; Beneteau *et al*. 2011, entire). We summarize the general pattern of variegate darter expansion within the range of the candy darter in the paragraphs below.

The variegate darter was first documented above the Kanawha Falls in 1982, with a single specimen collected from the mainstem of the Lower New River above the Hawks Nest Dam (WVDNR 2016 unpublished data) (figures 6 and 13). Additional surveys conducted between 1988 and 2013 confirmed that the species had colonized at least 75 skm (47 smi) of the Lower New River and 10 tributary streams above the Hawks Nest Dam. This section of the Lower New River maintains unobstructed connectivity with the Greenbrier River, where the variegate darter was first discovered in 1995 (discussed below). In 2013, the variegate darter was confirmed in the Lower New River below the Hawks Nest Dam (HDR 2014, pp. 56–58). This section of the Lower New River maintains unobstructed connectivity with the Lower Gauley River, where the variegate darter was first discovered in 2002 (discussed below).

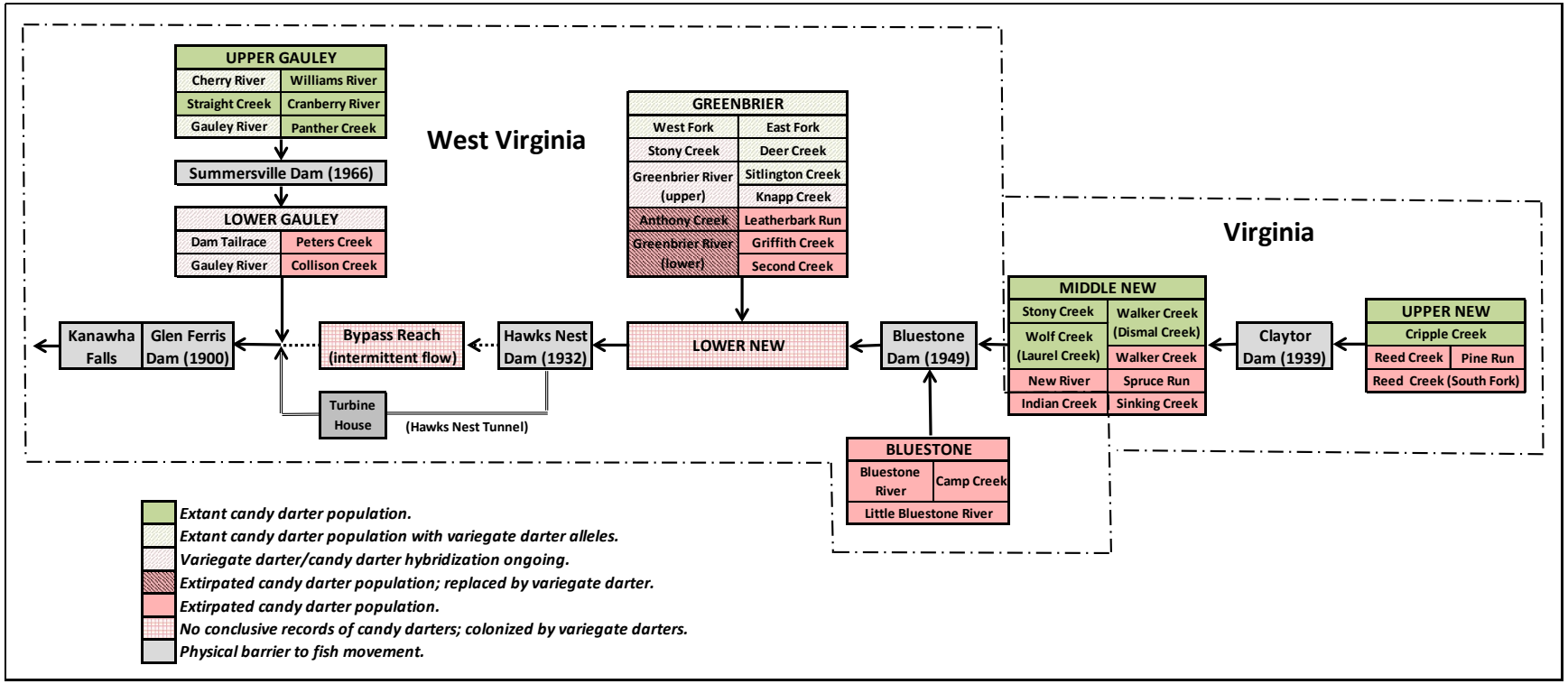

Figure 13. Conceptual model of candy darter distribution, connectivity, and hybridization status (as of April 2017). Arrows indicate direction of water flow.

In 2002, 10 variegate darter specimens (and 20 candy darters) were collected from the Gauley River near its confluence with the New River. Also in 2002, three candy darters were collected from the Gauley River approximately 13 skm (8 smi) upstream near the town of Swiss, West Virginia, but since then, no candy darters have been collected from the Lower Gauley watershed below the Summersville Dam tailwaters. Subsequent surveys between the town of Swiss and the confluence with the New River have confirmed variegate darters or hybrid specimens (n=35) in the mainstem and two tributary streams (Rich and Twentymile Creeks). In 2014, "F1" hybrids (the product of a pure candy darter and a pure variegate darter mating) were collected near the town of Swiss, indicating that at least one pure candy darter was present there during the previous reproductive cycle (Gibson 2017, pp. 14, 40). A series of large rapids and cascades preclude effective sampling between the town of Swiss and the tailwaters of the Summersville Dam (Cincotta 2016, pers. comm.); therefore, we cannot confirm the extent of variegate darter presence in this 45 skm (28 smi) reach. The only confirmed candy darter population remaining in the Lower Gauley watershed occurs in the tailwaters of the Summersville Dam, but in 2014, 6 (19 percent) of 31 specimens collected there had variegate darter alleles, indicating the presence of variegate darters in the population.

In the Upper Gauley River watershed (i.e., above the Summersville Dam), Switzer *et al*. (2007, pp. 25, 27) analyzed candy darters (n=25) collected from three different streams and found no genetic evidence of variegate darter presence in the watershed. However in 2014, specimens with variegate darter alleles were collected from two different locations (Gibson 2017, p. 40). This suggests that perhaps two variegate darter introductions have occurred in the Upper Gauley watershed since 2007. In 2016, extensive surveys of Upper Gauley (Gauley River, Cherry River, Williams River, and Cranberry River) failed to detect phenotypical variegate darters or hybrids; all of the specimens collected during this effort (n=229) appeared to be pure candy darters (Gibson 2017, pers. comm.). However, because genetic testing results of specimens from this survey were not available at the time of this SSA report, we cannot confirm the degree of variegate darter hybridization occurring in the Upper Gauley watershed.

Candy darters were known from Anthony Creek, a tributary to the lower Greenbrier River (defined here as that portion of the Greenbrier River downstream of the Knapp Creek confluence at Marlinton, West Virginia), since 1972. In 1995, a single variegate darter was collected from Anthony Creek, and until 2003, candy darters, variegate darters, and hybrid specimens were noted there. Since 2003, only variegate darters and hybrids have been confirmed in Anthony Creek; no pure candy darters have been detected (n > 63 individual darters collected). Researchers have concluded that the native candy darter population has likely been extirpated from Anthony Creek as a result of variegate darter colonization and genetic swamping (Switzer *et al*. 2007, pp. 3–6, 22–25; Gibson 2017, pp. 15–17, 40).

In 1999, 12 variegate darters were collected at a site in the mainstem of the lower Greenbrier River approximately 34 skm (21 smi) downstream of the Anthony Creek confluence. In 2009, variegate darters were confirmed in the Greenbrier River near its confluence with the Lower New River. This site is approximately 116 skm (72 smi) downstream of Anthony Creek and 17 skm (11 smi) upstream of the 1982 "first-above-the-falls" variegate darter occurrence in the Lower New River. Subsequent surveys of Anthony Creek and the lower Greenbrier River between 1999 and 2014 confirmed variegate darter colonization of the lower Greenbrier River

watershed. Since 2002, only variegate darters or hybrids have been collected from the lower Greenbrier River (n > 68 individual darters collected).

In 2006, potential hybrid specimens were noted at three locations (Knapp Creek, Sitlington Creek, and the East Fork) in the upper Greenbrier system (Switzer *et al*. 2007, p. 28). In 2014, surveys of these three creeks (and two others, Deer Creek and the West Fork) found 15 (18 percent) of 82 individual darters collected to be hybrids (Gibson 2017, p. 40). These locations are approximately 135 skm (84 smi) upstream of Anthony Creek.

As of April 2017, variegate darters have not been detected in the New River watershed above the Bluestone Dam. Therefore the three candy darter populations in the Middle New watershed and the single candy darter population in the Upper New watershed (which is also isolated by the Claytor Dam), are unaffected by variegate darter hybridization.

In summary, the data indicate that variegate darters were introduced into the upper Kanawha basin on more than one occasion within the last 35 years (Table 3) and that where variegate darter and candy darter populations are in contact the two species readily breed with each other, producing reproductively viable hybrid offspring. Over multiple darter generations (perhaps as little as 10 years), genetic introgression causes the eventual loss of pure candy darters from the population. Variegate darters were first detected in the Lower New River above the Hawks Nest Dam in 1982 and may have expanded upstream from there into the Greenbrier River, although it is also possible that a separate bait bucket introduction (or introductions) occurred further upstream in the Greenbrier River system (*e.g*., Anthony Creek) and contributed to the species expansion from there. It appears that the variegate darter was introduced into the Lower Gauley watershed around 2002 and has since spread upstream to the Summersville Dam and downstream into the Lower New River below the Hawks Nest Dam. Data from 2014 indicate that pure candy darters are present in the tailwaters of Summersville Dam and possibly the Gauley River near the town of Swiss, West Virginia, but it is unclear the degree of variegate darter hybridization in the intervening reach. The data suggests that between 2007 and 2014, variegate darters were introduced into the Upper Gauley watershed (i.e., above the Summersville Dam) at two different locations. Whatever the exact timing or sequence of events, the data indicate that variegate darters and hybrids are now well established in the Lower New, lower Greenbrier, and Lower Gauley River watersheds and that pure candy darters are no longer present in areas where variegate darters and hybrids have become established. As of April 2017, there is no information indicating variegate darters have been introduced into the Virginia portion of the candy darter range (i.e., above the Bluestone Dam).

Table 3. Summary of variegate darter presence and candy darter status in the upper Kanawha River basin.

Watershed (Metapopulation)	Stream System or Stream Segment	Candy Darter 1st Reported	Variegate Darter 1st Reported	Candy Darter Most Recent Report	Candy Darter Status
Upper Gauley	Gauley River	1933	2014	2016	Variegate Darter alleles detected
	Cherry River	1933	2014	2016	Variegate Darter alleles detected
Summersville Dam (barrier)					
Lower Gauley	Gauley River (Summersville Dam to Swiss, WV)	2002	2014	2014	Hybridization ongoing
	Gauley River (below Swiss, WV)	1976	2002	2002	Extirpated
Lower New	New River (below Hawks Nest Dam)	No Records	2013	No Records	Presumed extirpated
	Hawks Nest Dam (barrier)				
	New River (above Hawks Nest Dam)	1964 (unconfirmed)	1982	1991 (unconfirmed)	Presumed Extirpated ~1992
Greenbrier	Greenbrier River (lower)	1931	1999	1981	Extirpated after 1981
	Anthony Creek	1972	1995	2003	Extirpated ~2004
	Knapp Creek	1972	2006	2014	Hybridization ongoing
	Sitlington Creek	1972	2006	2014	Hybridization ongoing
	Deer Creek	1972	2014	2014	Hybridization ongoing
	Greenbrier River (upper)	1960	2014	2008	Hybridization ongoing
	West Fork	1970	2014	2014	Hybridization ongoing
	East Fork	1935	2006	2014	Hybridization ongoing

Sedimentation—Excessive **s**tream sedimentation (or siltation) results from soil erosion associated with upland activities (e.g., agriculture, forestry, mining, unpaved roads, road or pipeline construction, and general urbanization) as well as activities that can destabilize stream channels themselves (e.g., dredging or channelization, construction of dams, culverts, pipeline crossings, or other instream structures) (West Virginia Department of Environmental Protection (WVDEP) 2012, p.12). Excessive sediments can cover the stream bottom and fill the interstitial spaces between bottom substrate particles (i.e., sand, gravel, and cobbles) and in severe cases also cause stream bottoms to become "embedded," in which case substrate features including larger cobbles, rocks, and boulders are surrounded by, or buried in, sediment. This can affect fish species directly by limiting sheltering or breeding habitat and/or by causing shifts in the benthic community structure that alters the prey base (Berkman and Rabeni 1987, 291–293; Chambers and Messinger 2001, p. 50–51; Sutherland *et al.* 2002, entire; McGinley *et al*. 2013, pp. 223–226).

Survey results and species accounts suggest the candy darter has a strong association with clear streams with rocky bottoms (Addair 1944, p. 170; Kuehne and Barbour 2015, p. 80; Jenkins and Burkhead 1993, p. 828; Chipps *et al*. 1994, p. 179; Jenkins and Kopia 1995, p. 5). This information is consistent with the species' previously discussed life history and habitat needs, which indicate candy darters use cobbles, rocks, and boulders on the stream bottom as shelter and rely on unembedded pebble and gravel bottom substrate for egg deposition (Kuehne and Barbour 2015, p. 80; Jenkins and Kopia 1995, pp. 4–5; Leftwich *et al*. 1996, p. 6). Specific studies of candy darter habitat use indicate that candy darter presence and population "robustness" is correlated with low levels of sedimentation and stream bottom embeddedness (Chipps 1993, p. 52; Dunn and Angermeier 2016, pp. 1271–1276).

Since enactment of various state and Federal regulations (e.g., Federal Clean Water Act of 1977 (33 U.S.C. 1251 *et seq*.), Surface Mining Control and Reclamation Act of 1977 (30 U.S.C. 1234–1328), West Virginia Water Pollution Control Act (WVSC § 22–11)) and the increased implementation of forestry and construction "best management practices" (BMPs) designed to reduce erosion and sedimentation, levels of stream sedimentation have generally improved over historical conditions. However, based on the most recent state water quality reports, sedimentation remains a problem in many streams within the range of the candy darter. In the Ridge and Valley physiographic province of West Virginia, which includes the Greenbrier River watershed, an estimated 21.5 percent of the total stream miles were rated as "poor" with respect to sedimentation, 43.2 percent were rated "fair," and 35.3 percent were rated as "good." In the Appalachian Plateaus province, which includes the Gauley and Lower New watersheds, 41.5 percent of the stream miles were rated as "poor," 36.3 percent "fair," and 22.2 percent "good" (WVDEP 2012, pp. 25–26). A similar regional breakdown of stream sedimentation is not available for Virginia, but statewide estimates indicate that 39.0 percent of the stream miles were "suboptimal" with respect to sedimentation, 23.7 percent were "fair," and 37.3 percent were "optimal" VADEQ 2014, p. 182).

Although not listed as "impaired" by the WVDEP (2012, entire), the U.S. Forest Service (USFS) identified excess sedimentation as a continuing problem in portions of the upper Greenbrier River system (USFS 2011a, entire; USFS 2011b, entire). Therefore, the USFS is implementing a variety of stream restoration projects in the Monongahela National Forest specifically to reduce sedimentation in the Greenbrier watershed (USFS 2011a, entire; USFS 2011b, entire).

Future projects, such as a proposed large (107 cm (42 in) diameter) interstate natural gas pipeline, are expected to increase sediment loading in streams within the range of the candy darter (Federal Energy Regulatory Commission (FERC) 2016, pp. 4-108–4-115, 4-176–4-179). This proposed pipeline project will involve the clearing of a 23 to 38 m (75 to 125 ft) wide permanent right-of-way, trenching for the pipe, and will cross five current or historical candy darter streams (the upper Gauley River, lower Greenbrier River, Indian Creek, Stony Creek, and Sinking Creek) (FERC 2016, pp. 4-26–4-27). While project construction is not anticipated to cause direct "adverse impacts" to candy darters in Stony Creek (FERC 2016, pp. 4-187), the stream crossings and forest clearing associated with the permanent right-of-way are likely to increase sediment loading in the relevant watersheds, possibly degrading the habitat in streams potentially suitable for future candy darter reintroductions (if this is determined to be a feasible conservation tool).

Excessive sedimentation was likely a primary cause of the historical decline of the candy darter, and several species experts indicated that it continues to act as a stressor in some watersheds. However, they also expressed the view that variegate darter hybridization (discussed above) is exerting a stronger influence on candy darter distribution and population status (Service 2016).

Water Temperature—An analysis of historical water temperature data indicates a general increase in river and stream temperatures throughout the United States over about the last 90 years. These temperature increases are attributed primarily to changes in land use (e.g., urbanization and deforestation), thermal inputs (e.g., power plant discharges), and changes in climatic conditions (Kaushal *et al*. 2010, entire). Other studies demonstrate that changes in

water temperatures can lead to shifts in the range and distribution, and in some cases local extirpations, of fishes and other aquatic species (Comte and Grenouillet 2013, pp. 6–10; Isaak and Rieman 2013, pp. 747–749; Wiens, 2016, entire). Within the Kanawha River basin, extensive forest clearing in the early 20th century likely led to widespread increases in water temperatures (Swift and Messer 1971, entire; Dolloff 1994, pp.121–122), which may have been a factor in the early decline of the candy darter. Currently, deforested areas (i.e., urban areas, agricultural fields and pastures, abandoned mine lands, and timber harvests) likely continue to contribute to elevated water temperatures in some upper Kanawha streams and rivers.

Historical survey results suggest the candy darter is tolerant of warm water conditions, but that cool or cold water streams may be its preferred habitat. Empirical data on the effects of warm water temperatures on candy darter physiology or reproductive success are lacking, therefore we are uncertain about the significance of increased water temperatures on the species' viability. A "Climate Change Vulnerability Assessment" of more than 700 species in the Appalachian region ranked the candy darter "highly vulnerable" to the effects of climate change (LCC Climate Change Vulnerability Assessment, *http://applcc.org/research/applcc-funded-projects/final-narrative-climate-change-vulnerability-assessment/phase-ii-vulnerability-assessment-results/copy_of_data-access* accessed April 1, 2017). However, a vulnerability model for the native brook trout (*Salvelinus fontinalis*), a cold water-adapted species, indicated that a variety of landscape parameters such as elevation, forest cover, and ground water discharges, may buffer the effects of climate change in many cold water streams (Trumbo *et al*. 2014, pp. 182–185). Because the candy darter appears to be more tolerant of warmer temperatures than the brook trout, we conclude that candy darter populations within the higher elevation, more forested watersheds in the Upper Gauley and upper Greenbrier watersheds, which are known to hold brook trout, may be at low risk of the effects of climate change over the next 25 years. However, the isolated candy darter populations in less forested areas in the Middle and Upper New River watersheds may be increasingly stressed as warming trends continue.

While several candy darter experts indicated that increased water temperatures were likely instrumental in causing the species' historical decline and possibly limit its current distribution, they also expressed the view that variegate darter hybridization and perhaps sedimentation (discussed above) exert stronger influences on candy darter distribution and population status (Service 2016).

Water Chemistry—There is little information regarding the candy darter's tolerance of specific water quality parameters. We can infer from the available occurrence data and the scientific literature that the species is probably adapted to waters that are well oxygenated, have circumneutral pH, and are free of contaminants at levels likely to cause toxicity to native aquatic fauna. Based on data in the state water quality reports (WVDEP 2012; VADEQ 2014), it appears that the most common water quality issues across the range of the species (other than sedimentation, which is discussed separately) involve low pH levels or contamination with coliform bacteria.

In the Upper Gauley watershed, low pH (acidic) conditions are a concern in many otherwise high quality streams (McClurg *et al*. 2007, pp. 1088–1089; WVDEP 2012, 303d List). These acidic

conditions result from the combined effects of atmospheric deposition (e.g., "acid rain") and acidic runoff from abandoned mine lands within watersheds where the natural geology does not provide adequate pH buffering capacity (WVDEP 2008, pp. 26–28). However, since about 1970, the State of West Virginia has implemented various "stream liming" projects in which calcium carbonate sand or gravel is added to streams to neutralize acidic conditions. These projects appear to be generally effective, and stream water chemistry has improved in several Upper Gauley candy darter streams (McClurg *et al*. 2007, entire). Additionally, implementation of Title IV of the Clean Air Act of 1990 (40 CFR Parts 72 through 78) has reduced the contribution of atmospheric acid compounds to the watershed, slowing or reversing the rate of acidification in many streams (U.S. Environmental Protection Agency (USEPA) 2005, pp. 36–41). Therefore, it appears that stream acidification is a somewhat localized stressor that does not pose a significant risk to the species.

In the Middle and Upper New River watersheds and the Greenbrier River watershed, the most common water quality impairments are related to coliform bacteria exceedances (likely from sewage or septic releases or livestock wastes) (WVDEP 2012, 303d List; VADEP 2014, 303d List). While we have no information with which to determine if these pollutants directly affect the candy darter, they may be indicators of generally degraded conditions that make the habitat marginal for the species.

Also included in this stressor category are spills or releases of chemicals, petroleum, or other substances toxic to aquatic organisms. The risks from these types of events are difficult to predict, but we note that much of the transportation infrastructure (roads and railroads) and commercial and industrial facilities in the region are adjacent to streams and rivers, increasing the risk that a release could affect the aquatic habitat. Laurel Creek and Stony Creek in the Middle New River watershed provide relevant examples of this development pattern. The small and isolated Laurel Creek candy darter population is located immediately between and adjacent to a major interstate highway and a two-lane road, with an unidentified industrial facility located immediately upstream. The lower 5.3 skm (3.3 smi) of the Stony Creek candy darter population is adjacent to a large underground limestone mine, an associated lime plant, a railroad spur line, and a paved road. Additionally, a large (42-in diameter) interstate natural gas pipeline is proposed to cross this section of Stony Creek, along with other current or historical candy darter streams (the upper Gauley River, lower Greenbrier River, Indian Creek, and Sinking Creek), thereby increasing the spill risk in these areas.

The potential effect of a spill or release to the candy darter is dependent on the nature of the release (i.e., aquatic toxicity, quantity, etc.), the location of the release (i.e., mainstem river or tributary), and possibly other factors such as weather conditions or time of year. Because the species is currently distributed in multiple watersheds and subwatersheds it is unlikely a single spill event would pose an immediate risk to the species. However, some smaller, isolated candy darter populations or metapopulations are at an increased risk of extirpation as a result of a catastrophic event, in which case the species' redundancy and perhaps representation would be reduced.

Water Flow—As discussed in **Chapter 2** (*Habitat Needs*), candy darters inhabit streams and rivers with at least moderately flowing water. In unregulated streams and rivers in the upper

Kanawha basin, flows tend to follow a consistent seasonal pattern, with the highest flows generally occurring in March and the lowest flows occurring in September (Messinger and Hughes 2000, p. 32). Floods and droughts are naturally occurring phenomena in the region and vary widely in severity and periodicity (Messinger and Hughes 2000, pp. 39–40). Activities that can modify natural flow regimes include the construction and operation of dams, deforestation and urbanization, channelization, and water withdrawals. Additionally, changing temperature and precipitation patterns resulting from climate change are predicted to continue affecting regional flows in the 21st century (Demaria *et al*. 2016, pp. 320–321).

Given other suitable habitat conditions (e.g., water temperature, water chemistry, connectivity, and patch size), candy darter populations tolerate natural stream flow variability, including low-flow conditions in the late summer and early fall. The species also appears to tolerate some human-mediated flow variability, as demonstrated by candy darter presence below the Summersville Dam on the Gauley River. However, we note that deeper, slack water conditions created upstream of dams (i.e., reservoirs) likely make these waters unsuitable for the species. Low-flow conditions resulting from excessive water withdrawals, seasonal droughts, or hydrological changes brought about by human development or climate change (or a combination of these) could be a stressor to localized candy darter populations, especially smaller, isolated populations (e.g., Dismal Creek, in the Middle New Watershed (McBaine 2017, pers. comm.)). However, the species experts generally expressed the view that other stressors, especially variegate darter hybridization (discussed above), likely exert a stronger influence on candy darter distribution and population status (Service 2016).

Habitat Fragmentation—Little is known regarding the minimal habitat patch size or degree of habitat connectivity necessary to support persistent candy darter populations or subpopulations. However, it is generally understood in the field of conservation biology that larger and more-connected populations contribute to the long-term viability of a species and that smaller isolated populations are more at risk of decline or extirpation as a result of genetic drift, demographic or environmental stochasticity, and catastrophic events (Gilpin and Soulé 1986, pp. 32–34; Angermeier 1995, entire; Fagan 2002, p. 3248; Wiegand *et al*. 2005, entire; Letcher *et al*. 2007, 5–6; Peterson *et al*. 2014, pp. 564–565).

The Upper Gauley metapopulation currently consists of six candy darter populations that are in relatively close proximity to each other. The average length of occupied stream reaches in this metapopulation is approximately 18 skm (11 smi). The Lower Gauley metapopulation appears to currently consist of a single isolated population below the Summersville Dam. The estimated length of this population's occupied habitat is 45 skm (28 smi); however, survey data to confirm this are limited. In the Greenbrier metapopulation, the species occurs in seven populations that are generally separated by longer distances than those in the Upper Gauley metapopulation. Therefore the Greenbrier populations may be less well connected with each other than those in the Upper Gauley. The average length of occupied stream reaches in the Greenbrier is approximately 22 skm (14 smi). The Middle New metapopulation consists of three isolated populations. One of the populations occupies a stream reach approximately 19 skm (12 smi) long, while the other two occupy reaches of approximately 2.4 skm (1.5 smi) and 4.2 skm (2.6 smi). The Upper New River metapopulation consists of a single candy darter population occupying a stream reach approximately 8 skm (5 smi) long.

As discussed previously, four large dams act as barriers to fish movement within the upper Kanawha basin and limit the potential for genetic flow between the candy darter metapopulations (figure 13). It is notable here that, while barriers that fragment habitat are generally considered detriments to species viability, the Summersville and Bluestone Dams are likely limiting the upstream expansion of variegate darters into the Upper Gauley and Middle New River candy darter metapopulations, thereby helping to maintain their viability.

Nonnative Competition and Predation—The introduction of nonnative species may stress indigenous fish populations via increased predation, competitive interactions, transmission of pathogens, or hybridization (Mills *et al*. 2004, pp. 719–720; Cucherousset and Olden 2011, pp. 216–221). Research indicates the upper Kanawha River basin (above Kanawha Falls) naturally supported no more than about 45 native fish species. For comparison, the Kanawha basin below the falls supported approximately 90 native species (Messinger and Chambers 2001, p. 6). However, beginning in the late 1800s, the stocking of nonnative game species began and by the mid-20th century, improvements in transportation facilitated the movement of baitfish between watersheds. Together, these activities have resulted in the introduction of approximately 45 species not native to the upper Kanawha basin into the range of the candy darter (Messinger and Chambers 2001, pp. 6, 32). There is no information available on interactions between the vast majority of these introduced species and the indigenous candy darter, with the exception of the variegate darter hybridization issue (discussed above) and the potential for predation by stocked trout, discussed here.

Hatchery-raised rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), neither of which are native to the eastern United States, have been stocked in streams in Virginia and West Virginia since the early 1900s (Messinger and Chambers 2001, p. 6; Service 2016). Trout stocking continues in each state, and while brown trout are no longer stocked in candy darter streams in Virginia, reproducing brown trout populations have developed in some candy darter streams (Service 2016; Pinder 2016, pers. comm.; VDGIF 2017, entire; WVDNR 2017, entire; McBaine 2017, pers. comm.). This is significant in that brown trout are highly piscivorous and known to prey on smaller native fishes, including darter species (Garman and Nielsen 1982, pp. 864–866). The results of this study also found that brown trout predation appeared to cause shifts in the size structure and abundance of the native torrent sucker (*Thoburnia rhothoeca*) in a Virginia stream (Garman and Nielsen 1982, p. 866). A separate study of trout predation in Stony Creek, Virginia (Middle New watershed) found that newly released rainbow trout were unlikely to prey on native fishes, but did also confirm candy darter remains in the stomach of one brown trout (of three examined) (Leftwich *et al*. 1996, p. 6). However, because of the limited nature of the study, the authors were not able to determine any population-level effects to the indigenous candy darter population. Predation may not be the only negative effect resulting from nonnative trout introductions. Rainbow trout, while less likely to consume smaller fishes, appear to cause changes in the feeding behavior of native fishes, perhaps leading to dietary effects in these fishes (Freeman and Grossman 1992, pp. 899–901). In summary, the effects of introduced trout on the population viability of the candy darter are difficult to discern from the available information. We note that despite continued rainbow trout stocking and the presence of a reproducing brown trout population in Stony Creek, candy darters remain abundant there (Pinder 2016, pers. comm.).

In summary, the best available information indicates that at the species level, hybridization with introduced variegate darters is the most influential factor affecting the candy darter. Excessive sedimentation and increased water temperatures likely caused historical declines of the candy darter and these factors continue to affect some of the remaining populations. Additionally, the current level of habitat fragmentation isolates some populations. This reduces gene flow and limits the potential for the species to colonize or recolonize streams if habitat conditions change. Other factors such as flow alterations, water quality degradation, and the stocking of nonnative trout are not expected to cause species-level effects.

Candy Darter Current Condition

Model

Because no consistent, rangewide assessment of the candy darter is available, we developed a semiquantitative model that produced a "condition score" for each candy darter population or subpopulation (table 4). The model relies on three categories of interrelated metrics, including habitat parameters (i.e., water quality and forest cover), non-native species parameters (i.e., the presence of brown trout and/or variegate darters), and estimates of the candy darter demographic status within each unit. Because empirical data relating some of these metrics directly to candy darter life history needs are sparse, we consulted species experts who generally agreed that, for the purpose of this SSA report, the selected metrics were appropriate for assessing the viability of candy darter populations across the species' range (Cincotta 2016, pers. comm.; Dunn 2016, pers. comm.; McBaine 2016, pers. comm.; Pinder 2016, pers. comm.; Angermeier 2017, pers. comm.; Dunn 2017, pers. comm.; Gibson 2017, pers. comm.; Kirk 2017, pers. comm.; Landress 2017, pers.com.; McBaine 2017, pers. comm.; Pinder 2017, pers. comm.; Welsh 2017, pers. comm.). The individual metrics, which we ranked and scored based on criteria described in **Appendix B**, were then combined to produce a unitless condition score for each population or subpopulation.

To aid in the comparison of populations and subpopulations (with each other and under various future scenarios) and assess the species' viability under the 3Rs, we categorized the final condition scores as "high" (population generally secure), "moderate" (population marginally secure) or "low" (population generally insecure). We based these categories primarily on our understanding of candy darter habitat needs, known stressors, and the principles of conservation biology. We acknowledge that there is uncertainty associated with this model and some of the supporting data, but consider the methodology suitable for assessing the status of the candy darter across its range. In general, the species experts and peer reviewers agreed with this conclusion.

Current Condition—3Rs

The results of the candy darter population condition model provide the basis for our analyses of the species' current status using the 3Rs. The population condition scores allow us to directly assess and compare the resiliency of each candy darter population/subpopulation (table 4, figure 14), which then support our analyses of the species' redundancy (within and among the various

metapopulations) and representation (across its environmental settings). We emphasize that this portion of the assessment is a "snapshot in time" of the candy darter's current condition and does not consider future trends. **Chapter 4** assesses the species' potential condition under several future scenarios.

Table 4. Current condition of candy darter populations, including condition of physical habitat, non-native competition, and population demographic metrics. For the individual metric scores, green shading indicates the metric is conducive to the population, yellow shading indicates the metric is moderately conducive to the population, and red shading indicates the metric is unconducive to the population. For the final population condition scores, green shading indicates "good" (the population is generally secure), yellow indicates "moderate" (the population is marginally secure), and red indicates "low" (the population is generally insecure). Cross hatching indicates extirpated population. Red text indicates populations extirpated as a result of variegate darter hybridization.

			Physical Habitat Metrics				Non-native Competition Metrics			Population Demographic Metrics				
			Watershed Landcover											
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Forest Cover	Ownership	Water Quality	Total Habitat Score	Brown Trout	Variegate Darter	Total Comp Score	Space	Abundance	Connectivity	Total Demo Score	Population Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River												
		Straight Creek												
	Gauley River (upper)													
	Panther Creek													
	Williams River	Williams River												
		Tea Creek												
	Cranberry River													
	Cherry River	Cherry River												
		North Fork Cherry River												
		South Fork Cherry River												
		Laurel Creek												
		Little Laurel Creek												
Lower Gauley	Gauley River (lower)													
	Peters Creek													
	Collison Creek													
Lower New	New River													
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River												
		Little River												
	Greenbrier River (West Fork)	West Fork Greenbrier River												
		Little River												
	Greenbrier River (upper)													
	Leatherbark Run													
	Deer Creek	Deer Creek												
		North Fork												
	Stony Creek													
	Sitlington Creek													
	Knapp Creek	Knapp Creek												
		Douthat Creek												
	Anthony Creek													
	Second Creek													
	Greenbrier River (lower)													
	Griffith Creek													
Middle New	New River													
	Spruce Run													
	Sinking Creek													
	Walker Creek	Walker Creek												
		Dismal Creek												
	Stony Creek													
	Wolf Creek	Laurel Creek												
	Indian Creek	Indian Creek												
		Turkey Creek												
Bluestone	Camp Creek													
	Bluestone River													
	Little Bluestone River													
Upper New	Cripple Creek													
	Pine Run													
	Reed Creek	Reed Creek												
		South Fork Reed Creek												

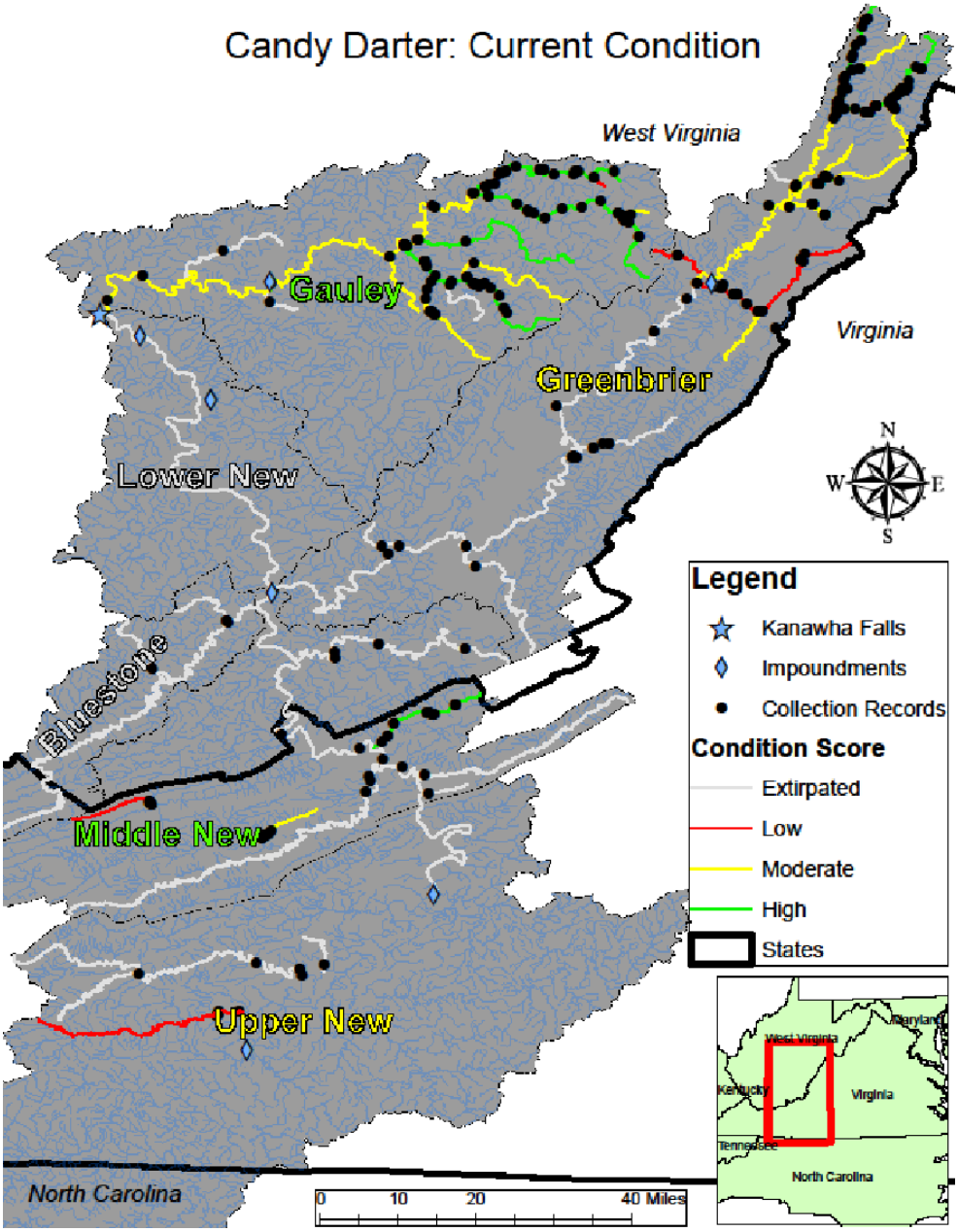

Figure 14. Current condition of candy darter populations.

Resiliency—Resiliency describes the ability of a population to withstand environmental or demographic stochastic disturbance and is positively related to population size and growth rate, patch size, and connectivity to other populations. The demographic subscores from the candy darter model, which incorporate estimates of abundance, space, and connectivity, are primarily used to assess candy darter resiliency. In cases where subpopulations are defined, we averaged those scores to derive the population score. As noted previously, our evaluation of the 3Rs here does not consider future trends or conditions, just the current status of the populations.

Upper Gauley: Six (100 percent) of six known candy darter populations in the Upper Gauley metapopulation are extant. In 2014, variegate darter alleles were determined in specimens collected from the Upper Gauley River and Cherry River populations. Two (33 percent) of the six populations have high or high to moderate resiliency and four (66 percent) have moderate resiliency (note: because of rounding, percentages may not add to 100 percent). Overall, we conclude the Upper Gauley metapopulation currently has moderate resiliency.

Lower Gauley: Two (67 percent) of the three known populations in the Lower Gauley metapopulation appear to have been extirpated in the mid- to late 20th century, likely as a result of habitat degradation. The remaining candy darter population in the mainstem of the Lower Gauley River is compromised by the presence of variegate darters. Survey data indicate that pure candy darters have been extirpated downstream of the town of Swiss, West Virginia, likely as a result of genetic swamping. In 2002, candy darters were confirmed near Swiss, but since then only variegate darters or hybrid specimens have been confirmed there. However, in 2014, F1 hybrids collected near Swiss suggested that pure candy darters were still present there at that time. A candy darter population was reported approximately 45 skm (28 smi) upstream of Swiss, immediately below the Summersville Dam (Service 2016); however, genetic analyses identified 6 (19 percent) of 31 specimens collected there in 2014 as having variegate darter alleles, indicating that hybridization is ongoing in this area (Gibson 2017, p. 40). The presence of F1 hybrids near Swiss and pure candy darters near the Summersville Dam suggests that the intervening reach of the Lower Gauley River continues to support candy darters; however, because a series of large rapids and cascades preclude effective sampling of this reach (Cincotta 2016, pers. comm.), we cannot confirm the status and extent of this population. We assume that pure candy darters occupy the reach between Swiss and the tailwaters of Summersville Dam, which leads to our assessment that this candy darter population is currently moderately resilient. We note however that if candy darters are limited only to the area immediately below the dam, where hybridization is ongoing, then this population (and therefore the metapopulation) has low resiliency.

Lower New: The Lower New metapopulation is presumed extirpated, and therefore has no resiliency.

Greenbrier: Five (41 percent) of 12 known populations in the Greenbrier metapopulation have been extirpated. Like the Lower Gauley metapopulation, the Greenbrier metapopulation is compromised by the presence of variegate darters; however, surveys conducted in the Greenbrier watershed between 2006 and 2014 indicate pure candy darters are still present in the seven extant populations. Two (28 percent) of these populations have high resiliency, four (57 percent) have moderate resiliency, and one (14 percent) has low resiliency. Overall, we conclude the Greenbrier metapopulation has moderate resiliency.

Middle New: Four (57 percent) of seven populations in the Middle New metapopulation have been extirpated. Of the three remaining populations, one candy darter population (33 percent) appears to be relatively large, but because it is isolated from any other population, it has moderate resiliency. Two other isolated populations (67 percent) in the Middle New have low resiliency; therefore, the overall resiliency of the Middle New is low.

Bluestone: The Bluestone metapopulation originally maintained 3 populations; however, all (100 percent) have been extirpated. Therefore this metapopulation has no resiliency.

Upper New: Two (67 percent) of three known populations in the Upper New metapopulation have been extirpated. The single isolated population remaining in the Upper New metapopulation has low resiliency.

In summary, the candy darter has been extirpated from almost half of its historical range; 17 (49 percent) of 35 known populations (and 2 (29 percent) of 7 known metapopulations), with the extirpations representing a complete loss of resiliency in those populations.

Of the 18 extant populations, 5 (28 percent) have a current score of high or moderate to high resiliency, 9 (50 percent) have moderate resiliency, and 4 (22 percent) have low or moderate to low resiliency. The five populations with higher resiliency scores occur in three metapopulations (the Upper Gauley in the Appalachian Plateaus physiographic province and the Greenbrier and Middle New in the Valley and Ridge physiographic province); the remaining two extant metapopulations (the Lower Gauley in the Appalachian Plateaus physiographic province and the Upper New River in the Valley and Ridge physiographic province) maintain populations with moderate and low resiliency. Therefore, we conclude the candy darter currently maintains moderate resiliency.

Redundancy—Redundancy describes the ability of a species to withstand catastrophic events by maintaining multiple, resilient populations distributed (and connected) within the species' ecological settings and across the species' range. We assessed candy darter redundancy at two scales, within the individual metapopulations and across all of the metapopulations.

Candy darters currently occur in five (71 percent) of seven known metapopulations. The Upper Gauley metapopulation maintains six (100 percent) of six presumably well-connected candy darter populations, two of which have high or high to moderate resiliency. Therefore we conclude that this metapopulation has moderate internal redundancy. The Greenbrier metapopulation maintains 7 (58 percent) of 12 moderately to well-connected populations, 2 of which are highly resilient. Therefore we conclude that the Greenbrier metapopulation has moderate internal redundancy. In the Middle New metapopulation, the species occurs in three populations that are separated from each other by considerable distances (55 to 135 skm (34 to 84 smi)), which makes it unlikely they maintain significant, if any, connectivity. One of these populations has moderate resiliency and the other two have low resiliency, therefore we conclude that the Middle New metapopulation has low internal redundancy. In the Lower Gauley and Upper New metapopulations, candy darters are limited to a single population in each; therefore these two metapopulations have no internal redundancy. Candy darters have been extirpated from the Bluestone and presumably the Lower New metapopulations, therefore they offer no redundancy to the species.

Based on the species' current distribution across its historical range (5 (71 percent) of 7 known metapopulations and 18 (51 percent) of 35 known populations) and the species' distribution and condition within each of the seven metapopulations (2 with moderate internal redundancy, 1 with

low internal redundancy, and 2 with no internal redundancy), we conclude that the candy darter's current redundancy is low

Representation—Representation describes the ability of a species to adapt to changing environmental conditions over time and is characterized by the breadth of genetic and environmental diversity within and among populations. The available genetic data for the candy darter indicate a high level of genetic differentiation between the Upper and Lower Gauley River and Greenbrier metapopulations and the loss of either metapopulation would represent a significant loss to the species' genetic diversity.

As discussed in **Chapter 2**, candy darters are known from a variety of different environmental settings in two distinct physiographic provinces. Populations have been documented in streams and rivers with varying physical characteristics (i.e., size, gradient, elevation, temperature, etc.) and from both the Valley and Ridge and Appalachian Plateaus physiographic provinces, which have distinct drainage patterns and differ in some fundamental water chemistry parameters (i.e., pH, hardness, buffering, etc.). The candy darter was once known from 35 populations, but 17 (49 percent) of those populations are now extirpated. Of the remaining 18 populations, candy darters are represented in headwater streams in the Gauley and Greenbrier River watersheds, but have been extirpated from the lower mainstem and tributary streams in these watersheds. Additionally, the species has lost representation in the mainstem of the New River (in the Lower and Middle New watersheds) and from the entire Bluestone River watershed.

When viewed by physiographic province, the candy darter currently maintains representation in both the Appalachian Plateaus and Valley and Ridge physiographic provinces. In the Appalachian Plateaus province, the Upper Gauley metapopulation has moderate to high resiliency, the Lower Gauley metapopulation has moderate to low resiliency, and the species has been extirpated from the Bluestone and presumably the Lower New River watersheds. In the Valley and Ridge province, the species is represented in the moderately resilient Greenbrier metapopulation and by four low to moderately resilient populations in the Middle New and Upper New metapopulations. Therefore, while the candy darter currently maintains representation in both the Appalachian Plateaus and Valley and Ridge physiographic provinces, these metapopulations are only moderately resilient.

As related to the species' diversity of environmental settings, candy darters have lost representation from lower mainstem rivers and tributaries, including the Lower Gauley, Lower New, lower Greenbrier, and Bluestone River watersheds. While we have no data indicating candy darters exhibit different genetic, physical, behavioral, or developmental characteristics based on their particular environmental setting, researchers have noted such differences in other stream fish species based on the species' longitudinal position in the watershed (e.g., stream size) (Neville *et al*. 2006, pp. 911–913).

Although the candy darter retains representation in both of the Appalachian Plateaus and Valley and Ridge physiographic provinces, the species has a different distribution than it had historically (e.g., its presence or absence in headwater vs. tributary streams), and likely a different ability to respond to stochastic and catastrophic events, thereby putting the species at increased risk of extinction from any such events. Therefore, because candy darter populations

are no longer found in the lower mainstem rivers and tributaries, we conclude that the species' representation is moderate to low.

Summary—There is some uncertainty regarding the genetic and biological status of the candy darter due to a general lack of rangewide genetic and life history information for the species. Because of this paucity of data, we relied on surrogate metrics to help determine the species' condition at the population level. This information, the best available, was then used to assess the condition of the species using the 3Rs.

The candy darter is currently distributed in five of the historical seven metapopulations. The populations within those metapopulations generally have moderate to low resiliency and redundancy scores. While the candy darter is present in the two physiographic provinces from which it is historically known, the species is absent from some ecological settings in which it once existed. This leads us to conclude the candy darter's representation is also moderate to low. Therefore, our analysis under the 3Rs leads us to conclude that the condition of the candy darter is currently moderate to low.

CHAPTER 4—SPECIES VIABILITY

Future Scenarios

Using the same methodology and criteria described above for assessing current condition, we modeled a total of five scenarios to assess the potential viability of the candy darter at a point up to 25 years in the future. Two scenarios (Scenarios 1 and 2) were habitat focused and three scenarios (Scenarios 3, 4, and 5) were variegate darter focused. We chose to model these scenarios out to 25 years because we have data to reasonably predict potential habitat and variegate darter changes and their effects on the candy darter within this timeframe.

Forest cover and human population trends do not suggest candy darter habitat will change sufficiently enough to affect the species' viability within the next 25 years. However, to help confirm or refute this assumption, and to assess the potential for habitat change to affect candy darter populations that might remain unaffected by variegate darter expansion, we modeled two scenarios that assume significant changes (one positive and one negative) in the species' habitat. Under these two scenarios, we left the nonnative competition metrics (i.e., variegate darter hybridization) unchanged. We concluded that because habitat change and the resultant effects to the candy darter populations are expected to occur gradually over time, the available data did not support modeling these scenarios at intervening points in time. Therefore, under these two scenarios we modeled the species' potential condition at the end of the 25-year period. We emphasize that because variegate darters are already expanding within the upper Kanawha basin and hybridization is ongoing, these two scenarios are improbable.

Based on the best available information, including the expert opinion of fish biologists with the VDGIF, WVDNR, and USGS (Service 2016), we expect the continued expansion of the variegate darter within the upper Kanawha basin to be the most likely stressor affecting the viability of the candy darter. Therefore, Scenario 3 models the condition of candy darter populations at 5-year intervals, out to 25 years, based solely on the predicted variegate darter expansion. We determined that intermediate 5-year models were supported by the available data and necessary to help assess the likely progression of variegate darter expansion and hybridization, which appear to be occurring rapidly (see **Chapter 3**, Hybridization section). Under this scenario, we left the habitat metrics unchanged.

While Scenarios 1 and 2 were independent from Scenario 3, meaning that under the habitat change scenarios, the nonnative competition metrics (i.e., variegate darter expansion) were unchanged, and under the variegate darter scenario habitat metrics were unchanged, Scenarios 4 and 5 are dependent models. These scenarios are a combination of the variegate darter expansion assumptions and the positive and negative habitat change assumptions. These scenarios help explore the significance of habitat changes on candy darter populations as variegate darter expansion continues.

Habitat Change (Scenarios 1 and 2)—For Scenarios 1 and 2, we assumed that forest cover in each candy darter population/subpopulation unit either increased or decreased 10 percent from the current condition.

A 10-percent forest cover change (positive or negative) represents a high degree of land cover alteration that would likely produce marked changes in stream habitat quality. For comparison, total forest cover in the relevant watersheds increased by an average of only 0.1 percent between 2001 and 2011 (NLCD, see Homer *et al.* 2015). In the positive habitat change scenario, we assume an increase in forest cover on all classes of landownership in all candy darter population watersheds and that this reforestation, and perhaps other habitat management or restoration efforts (e.g., enhanced erosion and sedimentation controls, stream stabilization projects), would lead to improvements in stream habitat quality (i.e., reduced sedimentation, moderated stream temperatures, etc.). Together, these habitat improvements are predicted to lead to an increase in candy darter abundance and connectivity (where possible).

In the negative habitat change scenario, we assumed that in candy darter units where the land is mostly privately owned or a mix of public and private land forest cover decreased by 10 percent from current conditions. We assumed that a similar change on public lands would not occur because the National Parks and National Forests are actively working to stabilize or improve stream and riparian habitats under existing management plans (USFS 2011a, entire; USFS 2011b, entire, National Park Service (NPS) 2011, entire). This reduction in forest cover and presumed increase in development or agriculture is assumed to lead to a degradation of stream habitat quality (e.g., increases in sedimentation and water temperature) in the affected watersheds that, in turn, would lead to a decrease in candy darter abundance and connectivity.

Variegate Darter Expansion (Scenario 3)—As discussed in previous sections, where variegate darters become established and come into contact with resident candy darter populations, hybridization between the two species results in the eventual loss of pure candy darters via genetic swamping. Based on the observed range expansion of the variegate darter in the Lower New, Lower Gauley, and Greenbrier River watersheds and the subsequent decline or loss of candy darters where the two species are in contact, we projected the likely effects of continued variegate darter expansion at 5-year intervals over the next 25 years.

Under this scenario, we assumed that the period of time from variegate darter introduction and contact with a candy darter population to candy darter population extirpation (i.e., no pure candy darters remain, only hybrid individuals) is approximately 10 years. This is based on observations from the Greenbrier River watershed, where the effects of variegate darter introduction are perhaps best documented. Survey data suggest that candy darters were extirpated from Anthony Creek (lower Greenbrier) within about 9 years after the variegate darter was first observed. Data from the upper Greenbrier, where hybrid specimens were first collected in 2006, indicate that by 2014 (a period of 8 years) the degree of hybridization in those populations had increased significantly (Gibson 2017, p. 40).

The data also indicate that after becoming established in a new river system variegate darters can rapidly expand in range. In the Greenbrier River watershed, between 1995 and 2006 (11 years), variegate darters appear to have expanded from Anthony Creek upstream to the East Fork of the Greenbrier River, a distance of approximately 170 skm (106 smi) (roughly 15 skm (9 smi) per year). In the Lower Gauley watershed, between 2002 and 2014 (12 years), variegate darters appear to have expanded from near the New River confluence upstream to the Summersville Dam, a distance of approximately 58 skm (36 smi) (roughly 5 skm (3 smi) per year). It is

notable that this section of the Gauley River includes a series of significant whitewater rapids and cascades, indicating that these features do not present barriers to darter movement.

While we have data with which to estimate the expansion of variegate darters within a candy darter metapopulation, we cannot predict with accuracy if or when a bait bucket transfer (or other anthropogenic mechanism) would result in the establishment of the species in other candy darter watersheds. We assume that if watersheds occupied by variegate darters (and hybrids) are adjacent to candy darter watersheds, the likelihood that variegate darters will be collected as bait and transported into an adjacent candy darter watershed is increased.

As discussed previously, while variegate darters have not been confirmed in the Upper Gauley watershed, in 2014, their genetic markers were detected in several specimens collected from two separate locations (Gibson 2017, p. 40). This genetic evidence suggests that some variegate darters were introduced into the watershed in recent years, perhaps on more than one occasion. The risk of additional bait-bucket introductions is likely increasing as variegate darters expand in range and displace candy darters in the adjacent Lower Gauley and Greenbrier River watersheds (figure 15).

Similarly, the Middle New River candy darter metapopulation is likely at risk of bait bucket transfers from the adjacent Big Sandy and Guyandotte River basins, both native variegate darter areas (Argentina *et al.* 2013, pp. 3, 39; Stauffer *et al.* 1989, pp. 8–10; Stauffer *et al.* 1995, p. 316), as well as variegate darters and hybrids in the Lower New and Greenbrier River watersheds. We acknowledge that we cannot predict with certainty exactly when variegate darters will become established in the remaining candy darter watersheds, but because at least four variegate darter introductions occurred within about 20 years (one in about 1982 in the Lower New, one in about 2002 in the Lower Gauley, and two in the Upper Gauley in around 2014), it appears highly likely that additional interwatershed transfers will occur within the next 25 years.

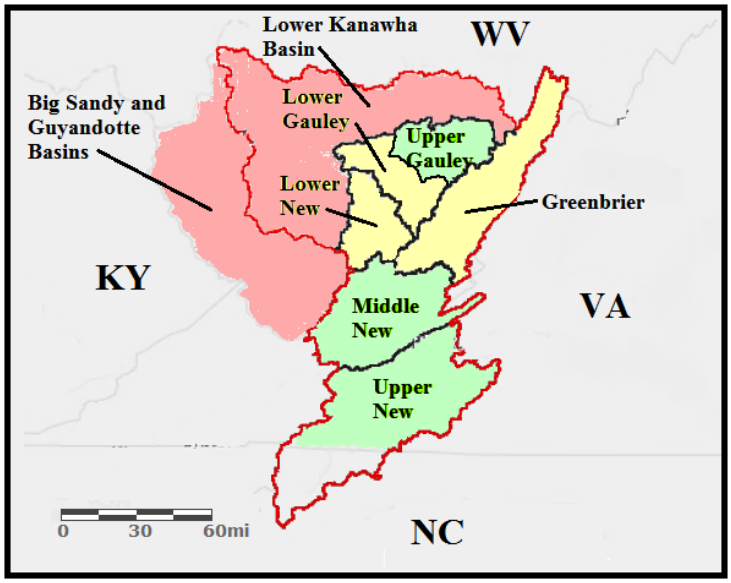

Figure 15. Proximity of variegate darter watersheds to known candy darter watersheds. Green shading indicates candy darter watersheds where variegate darters have not been confirmed (note that candy darters are not known from the North Carolina portion of the Upper New watershed); Yellow shading indicates candy darter watersheds that are now occupied by variegate darters or variegate darter/candy darter hybrids; and Red shading indicates native variegate darter watersheds.

Therefore, in our variegate darter expansion scenario, we assume that variegate darters have been introduced into the Upper Gauley watershed and will be introduced into the Middle New watershed (above the Bluestone dam) within 15 years. Because the single Upper New River candy darter population (Cripple Creek) appears to be the most geographically isolated, we assume it will remain free of variegate darter exposure for the next 25 years (in absence of data to the contrary). Regardless of exactly when variegate darters are introduced into these watersheds, we expect that once that species becomes established, the pattern of hybridization already observed in the Lower Gauley and Greenbrier River systems will continue in other candy darter metapopulations.

Variegate Darter Expansion with Positive Habitat Change (Scenario 4)—This scenario combines the variegate darter model with the positive habitat change model to explore how significant habitat improvements might affect the condition of candy darter populations in light of the variegate darter expansion and hybridization threat. We note that because the two species share many life history traits and habitat needs, improvements in habitat are also likely to benefit variegate darters too.

Variegate Darter Expansion with Negative Habitat Change (Scenario 5)—This scenario combines the variegate darter model with the negative habitat model to explore how significant habitat degradation might affect the condition of candy darter populations in light of the variegate darter expansion and hybridization threat.

Results

Scenario 1 (positive habitat changes): Based on the modeled assumptions for positive habitat changes, the condition of 11 candy darter populations are predicted to remain relatively

unchanged from the current condition, and 5 are predicted to have improved (table 5, figure 16). Under Scenario 1, the species' redundancy and representation remained unchanged (e.g., all current metapopulations remained extant), and the resiliency of the six improved populations is predicted to increase.

Table 5. Predicted condition of candy darter populations under five 25-year future condition scenarios (current condition provided for comparison purposes). Green shading indicates "high," yellow shading indicates "moderate," and red shading indicates "low." Cross hatching indicates extirpated population.

				Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Current Condition	Positive Habitat	Negative Habitat	Hybridization	Hybridization and Pos. Hab.	Hybridization and Neg. Hab.
Upper Gauley	Gauley River (headwaters)	Gauley River						
		Straight Creek						
	Gauley River (upper)							
	Panther Creek							
	Williams River	Williams River						
		Tea Creek						
	Cranberry River							
	Cherry River	Cherry River						
		North Fork Cherry River						
		South Fork Cherry River						
		Laurel Creek						
		Little Laurel Creek						
Lower Gauley	Gauley River (lower)							
	Peters Creek							
	Collison Creek							
Lower New	New River							
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River						
		Little River						
	Greenbrier River (West Fork)	West Fork Greenbrier River						
		Little River						
	Greenbrier River (upper)							
	Leatherbark Run							
	Deer Creek	Deer Creek						
		North Fork						
	Stony Creek							
	Sitlington Creek							
	Knapp Creek	Knapp Creek						
		Douthat Creek						
	Anthony Creek							
	Second Creek							
	Greenbrier River (lower)							
	Griffith Creek							
Middle New	New River							
	Spruce Run							
	Sinking Creek							
	Walker Creek	Walker Creek						
		Dismal Creek						
	Stony Creek							
	Wolf Creek	Laurel Creek						
	Indian Creek	Indian Creek						
		Turkey Creek						
Bluestone	Camp Creek							
	Bluestone River							
	Little Bluestone River							
Upper New	Cripple Creek							
	Pine Run							
	Reed Creek	Reed Creek						
		South Fork Reed Creek						

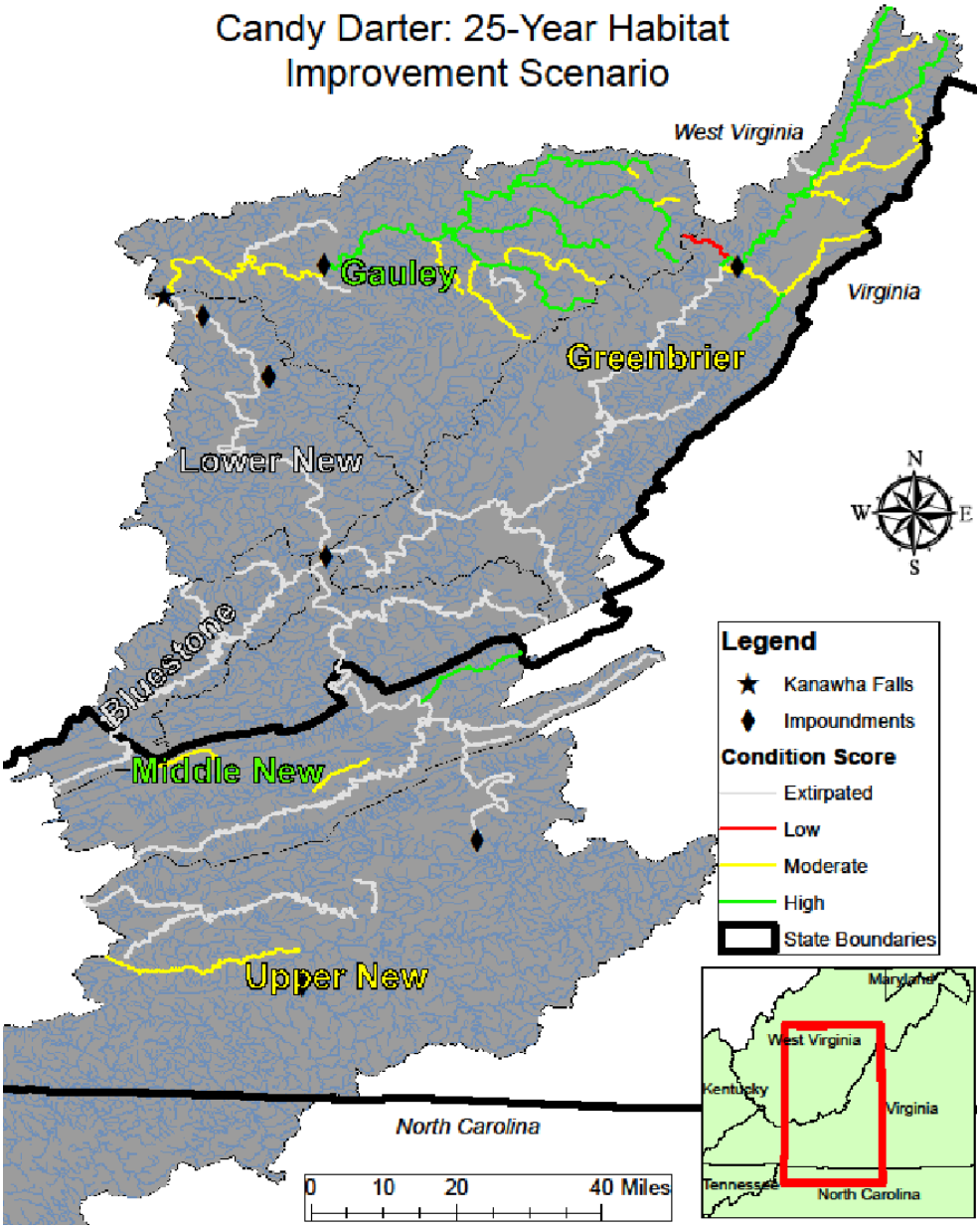

Figure 16. Spatial distribution and predicted condition of candy darter populations/subpopulations under Scenario 1 (25 years of positive habitat change).

Scenario 2 (negative habitat change):
Under this scenario, 10 populations are predicted to remain relatively unchanged, and 8 are predicted to decrease in condition (table 5, figure 17). Under this scenario, five populations (Panther Creek, Lower Gauley, upper Greenbrier River, Deer Creek, and Sitlington Creek) are predicted to fall into the low condition category and three are predicted to remain in the low category. It should be noted that two of these populations (Lower Gauley and Cripple Creek) are isolated and at an increased risk of extirpation due to environmental or demographic stochasticity. The loss of either population would also represent the loss of their respective metapopulation, thereby reducing the redundancy and representation of the species and increasing the candy darter's risk of extinction. Therefore, under the negative habitat change scenario, while the candy darter would maintain extant populations over the next 25 years, the species would lose resiliency in three (60 percent) of the five current metapopulations, with two of these being at a high risk of extirpation.

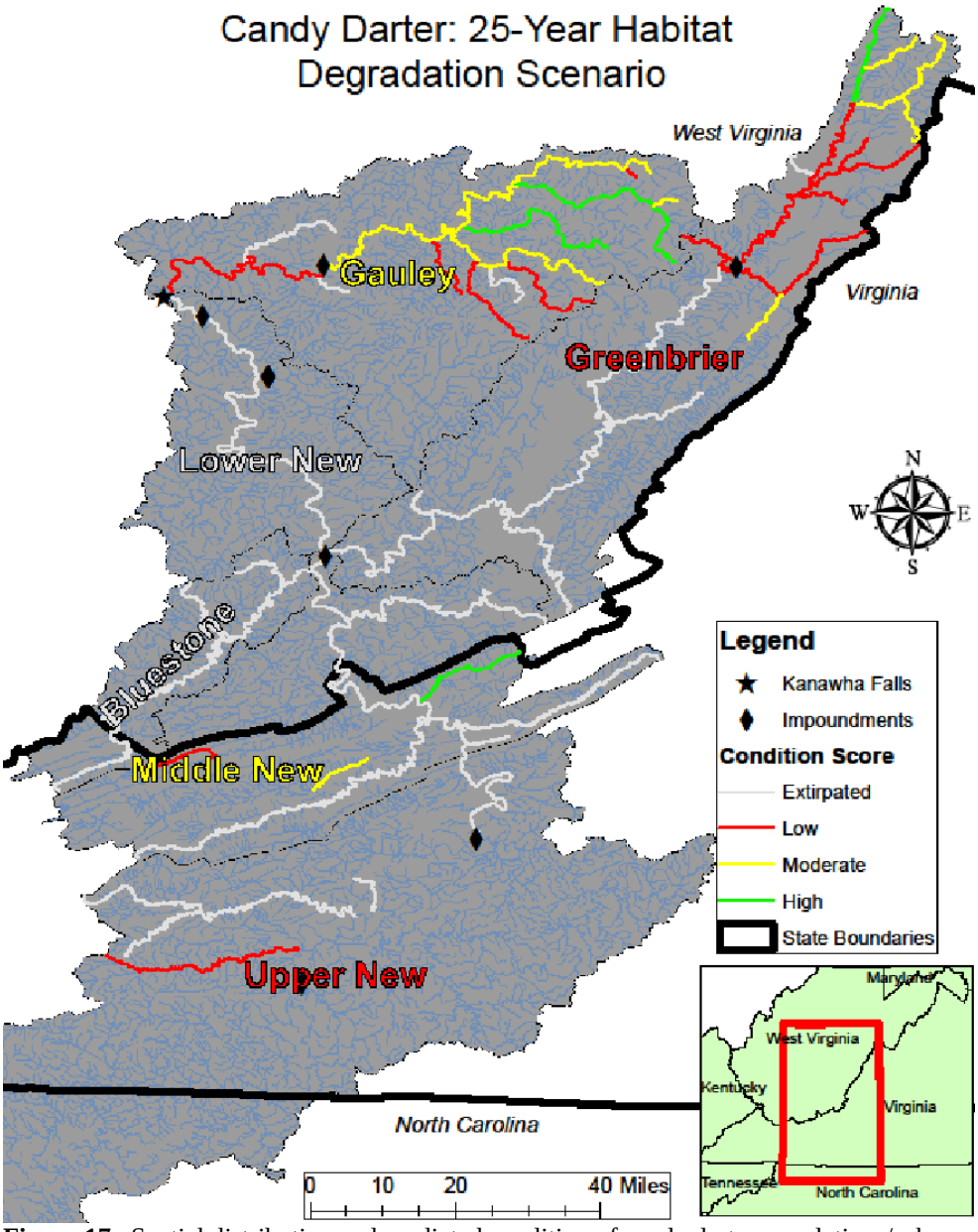

Figure 17. Spatial distribution and predicted condition of candy darter populations/subpopulations under Scenario 2 (25 years of negative habitat change).

Scenario 3 (variegate darter expansion):
Under our assumptions for continued variegate darter expansion, within the next 10 years, ongoing hybridization and genetic swamping is expected to cause the decline and loss of pure candy darters in the Greenbrier and Lower Gauley watersheds (table 6). The data suggest variegate darters have been introduced into the Upper Gauley watershed and that additional bait bucket transfers will result in the pattern of candy darter hybridization and loss previously observed in the Lower Gauley and Greenbrier to be repeated. Within 15 years, pure candy darters will be lost from the Upper Gauley watershed. Within 20 years, variegate darters will have been introduced into the Middle New watershed where we anticipate the pattern of hybridization with candy darters will continue. Therefore, based on the modeled assumptions for variegate darter expansion, the redundancy of the candy darter will decline from five to two metapopulations within the next 25 years. Notably, two of the extirpated metapopulations, the Greenbrier and the Upper Gauley, include perhaps the best connected and abundant candy darter populations known. Additionally, the data indicate that there is a high level of genetic differentiation between the Gauley River candy darters those in the Greenbrier, therefore the loss of the Gauley River metapopulations reduces the species' genetic representation as well as eliminating the species' representation in the Appalachian Plateaus physiographic province. Therefore, variegate darter range expansion strongly influences the viability of the candy darter over the next 25 years. By the year 2042, four candy darter populations are predicted to remain, one with a moderate condition score and three with low condition scores (figure 18). Because the remaining populations are also isolated, they will be at an increased risk of extirpation from catastrophic or stochastic events. Therefore variegate darter expansion and hybridization significantly increases the candy darter's risk of extinction over the next 25 years.

Table 6. Scenario 3: Predicted condition of candy darter populations/subpopulations under the variegate darter expansion scenario at 5-year intervals (current condition is included for comparison purposes). Green shading indicates "high," yellow shading indicates "moderate," and red shading indicates "low." Cross hatching indicates extirpated population.

Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Current	+ 5 Yr	+10 Yr	+15 Yr	+20 Yr	+ 25 Yr
Upper Gauley	Gauley River (headwaters)	Gauley River						
		Straight Creek						
	Gauley River (upper)							
	Panther Creek							
	Williams River	Williams River						
		Tea Creek						
	Cranberry River							
	Cherry River	Cherry River						
		North Fork Cherry River						
		South Fork Cherry River						
		Laurel Creek						
		Little Laurel Creek						
Lower Gauley	Gauley River (lower)							
	Peters Creek							
	Collison Creek							
Lower New	New River							
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River						
		Little River						
	Greenbrier River (West Fork)	West Fork Greenbrier River						
		Little River						
	Greenbrier River (upper)							
	Leatherbark Run							
	Deer Creek	Deer Creek						
		North Fork						
	Stony Creek							
	Sitlington Creek							
	Knapp Creek	Knapp Creek						
		Douthat Creek						
	Anthony Creek							
	Second Creek							
	Greenbrier River (lower)							
	Griffith Creek							
Middle New	New River							
	Spruce Run							
	Sinking Creek							
	Walker Creek	Walker Creek						
		Dismal Creek						
	Stony Creek							
	Wolf Creek	Laurel Creek						
	Indian Creek	Indian Creek						
		Turkey Creek						
Bluestone	Camp Creek							
	Bluestone River							
	Little Bluestone River							
Upper New	Cripple Creek							
	Pine Run							
	Reed Creek	Reed Creek						
		South Fork Reed Creek						

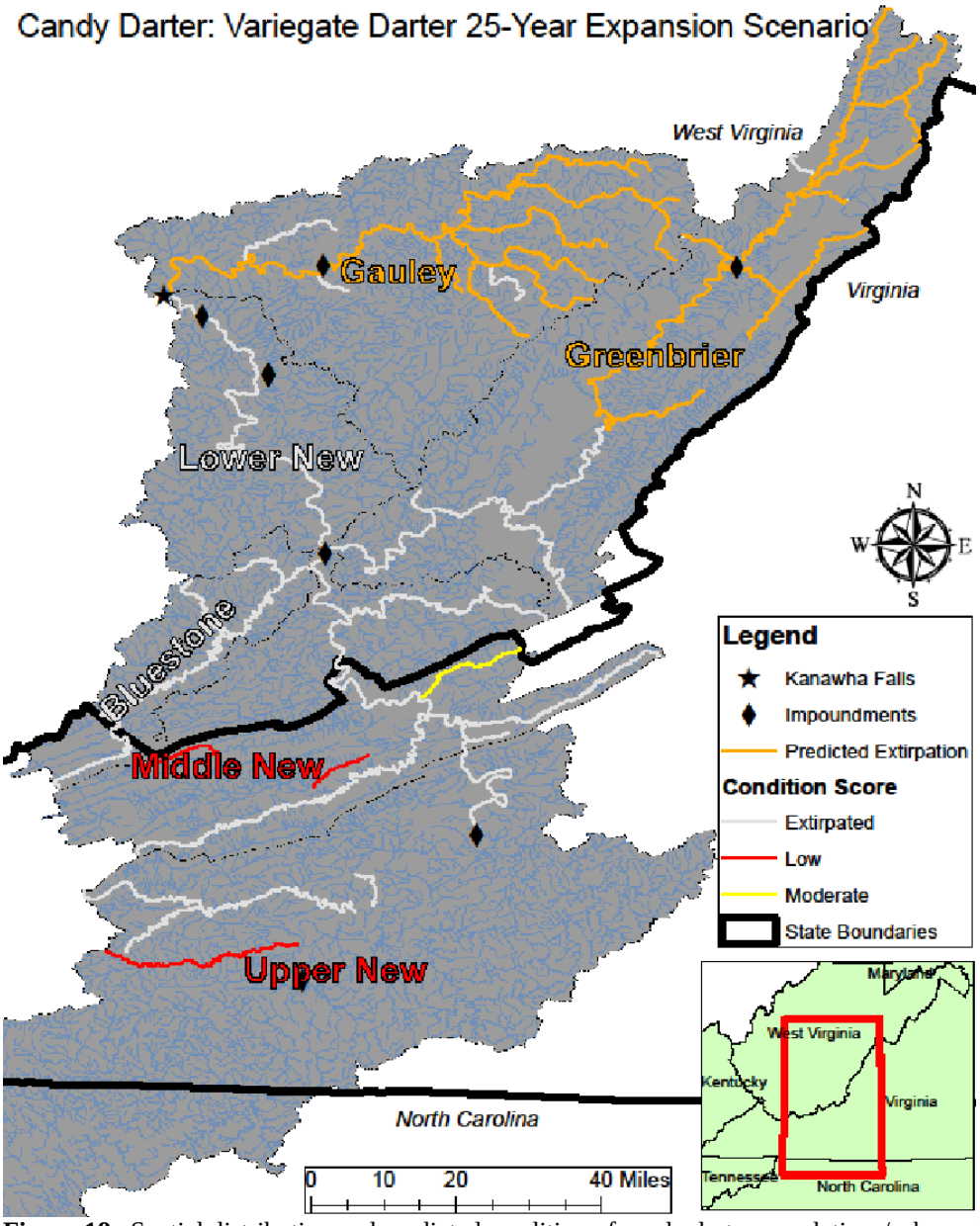

Figure 18. Spatial distribution and predicted condition of candy darter populations/subpopulations under Scenario 3 (25 years of variegate darter expansion).

Scenario 4 (variegate darter expansion with the positive habitat change):
Under this scenario (table 5, figure 19), the pattern of candy darter metapopulation extirpations is predicted to remain the same as described under Scenario 3. This is because the two species share general habitat requirements (see previous discussion in the **Habitat Needs** section and Argentina *et al.* 2013, pp. 5–8), therefore, improvements in habitat quality that benefit the candy darter will also likely benefit the variegate darter. Significant positive habitat changes may increase the resiliency of two of the four candy darter populations that are expected to remain extant following 25 years of variegate darter expansion and hybridization. But because of its expected contact with variegate darters, one candy darter population is expected to decrease in condition even with positive habitat changes. Therefore, habitat improvements alone do not significantly reduce the risk of extinction resulting from variegate darter expansion.

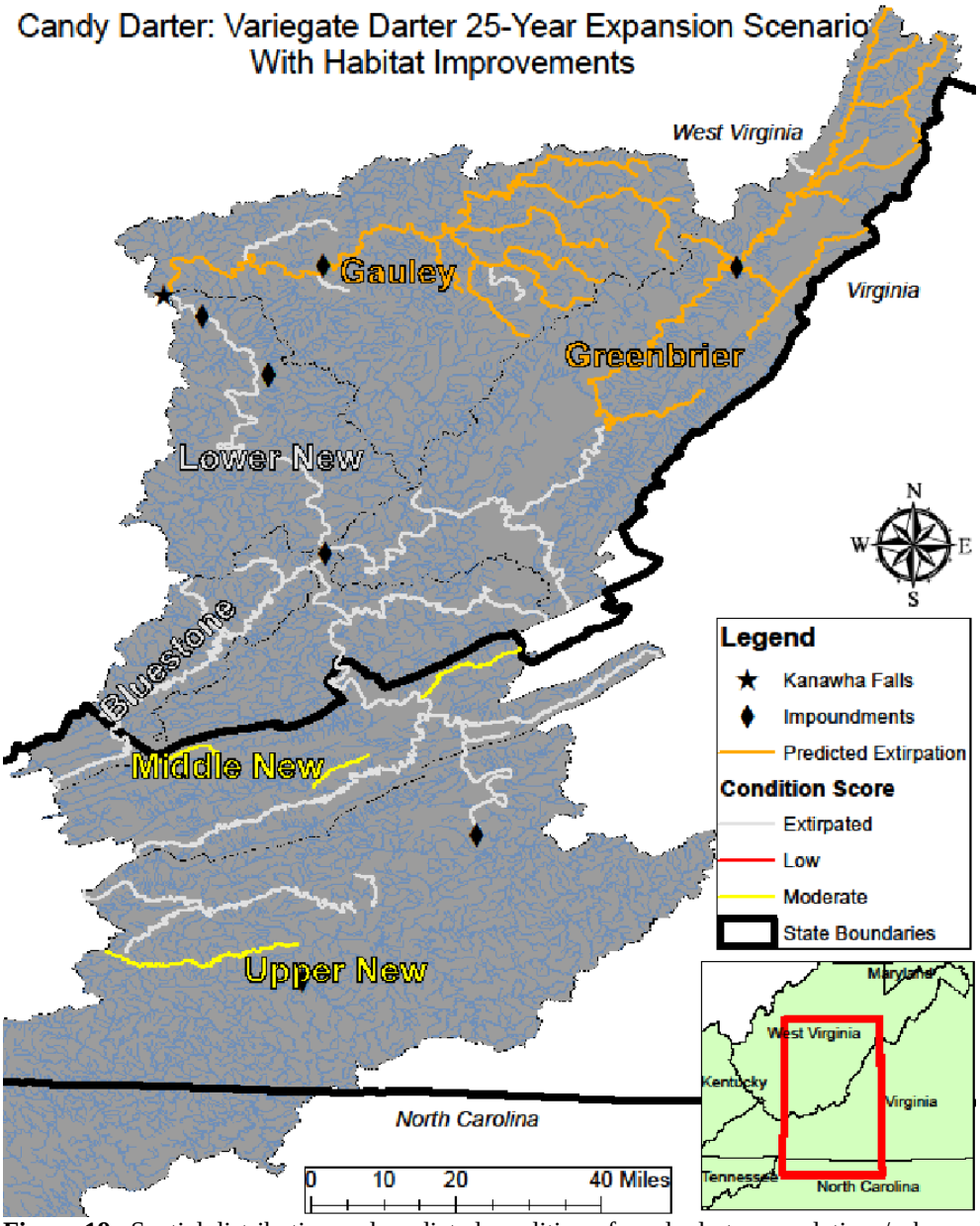

Figure 19. Spatial distribution and predicted condition of candy darter populations/subpopulations under Scenario 4 (25 years of variegate darter expansion and positive habitat change).

Scenario 5 (variegate darter expansion with negative habitat change):
Under this scenario (table 5, figure 20), the pattern of candy darter metapopulation extirpations again remain the same as described under Scenario 3. The negative habitat changes are expected to reduce the resiliency of all four candy darter populations that are expected to remain extant following 25 years of variegate darter expansion and hybridization. Three of these isolated populations will have low condition scores and one will be moderate, placing them at risk of extirpation from stochastic events. Furthermore, it is possible that variegate darters are tolerant of a wider range of habitat conditions, as is suggested by that species wide range in the greater Ohio River basin. Therefore degraded habitat conditions might selectively favor the variegate darter over the candy darter, placing the candy darter at additional risk of extinction.

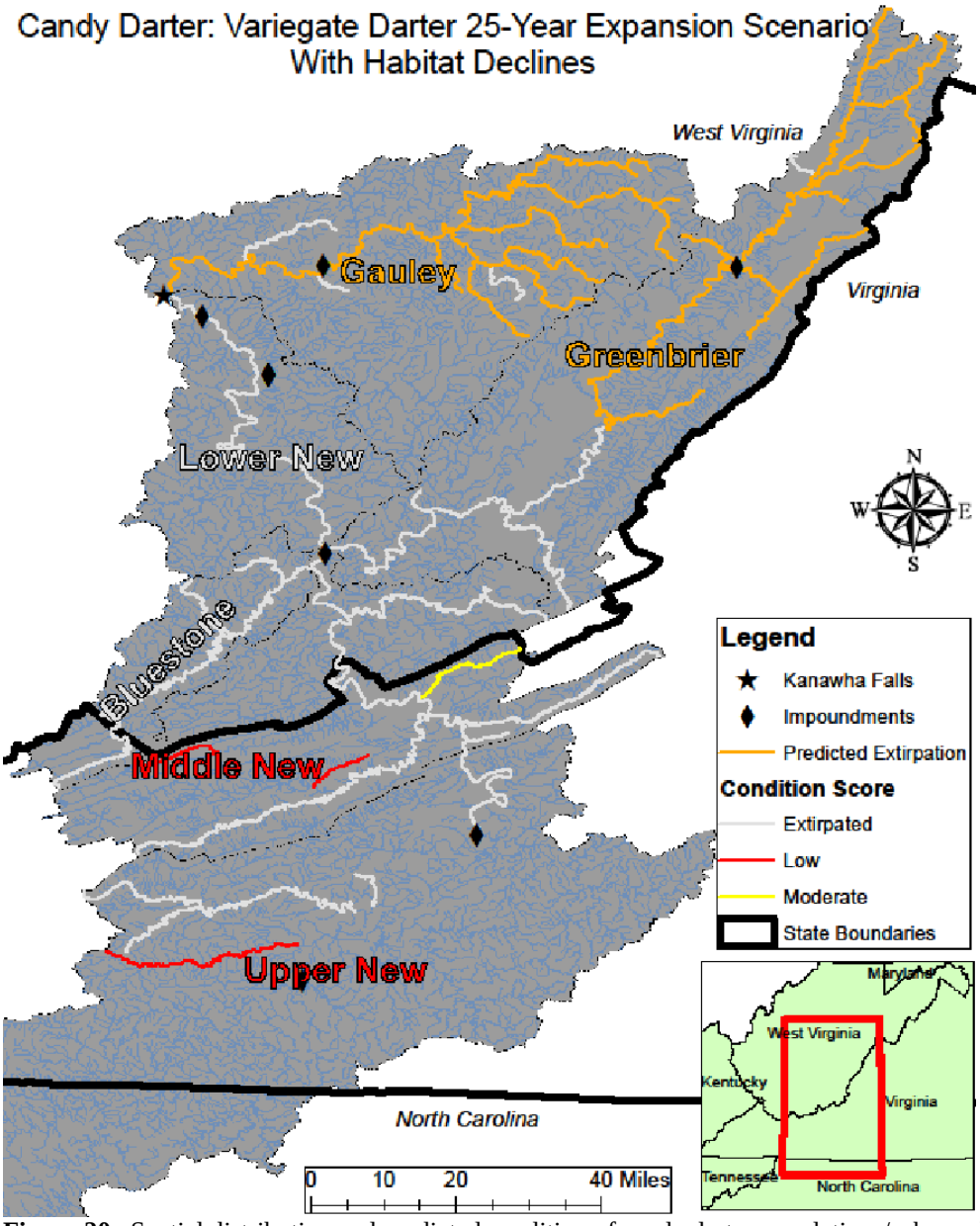

Figure 20. Spatial distribution and predicted condition of candy darter populations/subpopulations under Scenario 5 (25 years of variegate darter expansion and negative habitat change).

Summary

The best available data indicate that, of the 35 known candy darter populations, 17 have been extirpated and many of the remaining populations are small and/or isolated. The most abundant candy darter populations occur in the Upper Gauley and upper Greenbrier River watersheds, and in Stony Creek in the Middle New River watershed. However, the distribution of candy darters in the Greenbrier River watershed is changing rapidly as introduced variegate darters expand their range. We expect that the Upper Gauley metapopulation will experience similar patterns of decline based on the current presence of variegate darter alleles in this metapopulation.

We considered what the candy darter needs to maintain viability by characterizing the status of the species in terms of its resiliency, redundancy, and representation. For the purpose of this assessment, we generally define viability as the ability of the species to sustain populations in natural stream ecosystems within a biologically meaningful timeframe: in this case, 25 years. We chose 25 years because we have data to reasonably predict the potential significant effects of stressors within the range of the candy darter within this timeframe.

Based on the candy darter life history and habitat needs, and in consultation with the species' experts, we identified the potential stressors (negative influences), and the contributing sources of those stressors, that are likely to affect the species' current condition and viability. We evaluated how these stressors may be currently affecting the species and whether, and to what extent, they would affect the species in the future. While water temperature, sedimentation, habitat fragmentation, water chemistry, water flow, and nonnative competition likely influenced the species' current condition and may affect some individual populations in the future, the hybridization with variegate darters appears to be having, and will continue to have, the greatest influence on candy darter populations and its overall viability within the next 25 years.

Under the three plausible scenarios (Scenarios 3, 4, and 5), the predicted rate of variegate darter expansion and hybridization remains the same and at the end of 25 years, the candy darter will occur in four isolated populations and maintain little resilience, redundancy, or representation. At this point, the candy darter will be functionally extinct. The effects of significant positive or negative habitat changes (Scenarios 4 and 5) do not alter this outcome, although it is possible negative habitat changes could selectively benefit variegate darters and increase the rate at which pure candy darters are extirpated.

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APPENDIX A

Candy darter analytical units based on known occurrence data. Asterisks indicate extirpated metapopulation, population, or subpopulation.

Watershed (Metapopulation)	Physiographic Province	Stream System (Population)	Stream Position/Size	Stream (Subpopulation)	Hydrologic Unit Code (HUC)	Description
Upper Gauley	Appalachian Plateaus	Gauley River (headwaters)	Upper Trib	Gauley River	0505000503	Includes the Gauley River and tributaries above the confluence of the Gauley River and Williams River.
				Straight Creek		
		Gauley River (upper)	Upper Main		0505000503, 0505000508	Includes the Gauley River from the confluence of the Gauley River and Williams River downstream to the Summersville Reservior.
		Panther Creek	Upper Trib		0505000508	Includes Panther Creek
		Williams River	Upper Trib	Williams River	0505000501	Includes the Williams River and tributaries.
				Tea Creek		
		Cranberry River	Upper Trib		0505000502	Includes the Cranberry River.
		Cherry River	Upper Trib	Cherry River	0505000504	Includes the Cherry River and tributaries.
				North Fork Cherry River		
				South Fork Cherry River		
				Laurel Creek		
				Little Laurel Creek		
Lower Gauley	Appalachian Plateaus	Gauley River (lower)	Lower Main		0505000508	Includes the Gauley River from the Summersville Dam downstream to the confluence of the Gauley River and the New River.
		Peters Creek*	Lower Trib		0505000508	Includes Peters Creek.
		Collison Creek*	Lower Trib		0505000508	Includes Collison Creek.
Lower New*	App. Plateaus	Lower New River*	Lower Main		05050004	Includes the Lower New and tributaries.
Greenbrier	Valley and Ridge	Greenbrier River (East Fork)	Upper Trib	East Fork Greenbrier River	0505000301	Includes the East Fork of the Greenbrier River and tributaries above the confluence of the East Fork and West Fork of the Greenbrier
				Little River		
		Greenbrier River (West Fork)	Upper Trib	West Fork Greenbrier River	0505000301	Includes the West Fork of the Greenbrier River and tributaries above the confluence of the East Fork and West Fork of the Greenbrier
				Little River		
		Greenbrier River (upper)	Upper Main		0505000301, 0505000304	Includes the Greenbrier River from the confluence of the East and West Forks of the Greenbrier River downstream to the confluence of Knapp Creek and the Greenbrier River.
		Leatherbark Run*	Upper Trib		0505000301	Includes Leatherbark Run.
		Deer Creek	Upper Trib	Deer Creek	0505000301	Includes Deer Creek and tributaries.
				North Fork		
		Stony Creek	Upper Trib		0505000304	Includes Stony Creek.
		Sitlington Creek	Upper Trib		0505000304	Includes Sitlington Creek.
		Knapp Creek	Upper Trib	Knapp Creek	0505000302	Includes Knapp Creek and tributaries.
				Douthat Creek		
		Anthony Creek*	Lower Trib		0505000305	Includes Anthony Creek.
		Second Creek*	Lower Trib		0505000307	Includes Second Creek.
		Greenbrier River (lower)*	Lower Main		0505000309	Includes the Greenbrier River from the Knapp Creek confluence downstream to the New River confluence.
		Griffith Creek*	Lower Trib		0505000309	Includes Griffith Creek.
Middle New	Appalachian Plateaus	New River*	Lower Main		0505000203, 0505000206, 0505000210	Includes the New River from the Claytor Dam downstream to the Bluestone Reservoir.
		Spruce Run*	Lower Trib		0505000203	Includes Spruce Run.
		Sinking Creek*	Lower Trib		0505000203	Includes Sinking Creek.
		Walker Creek	Lower Trib	Walker Creek*	0505000202	Includes Walker Creek.
				Dismal Creek	0505000201	Includes Dismal Creek.
		Stony Creek	Lower Trib		0505000203	Includes Stony Creek.
		Wolf Creek	Lower Trib	Laurel Creek	0505000205	Includes Laurel Creek.
		Indian Creek*	Lower Trib	Indian Creek	0505000207	Includes Indian Creek and tributaries.
				Turkey Creek		
Bluestone*	Appalachian Plateaus	Camp Creek*	Upper Trib		0505000209	Includes Camp Creek.
		Bluestone River*	Lower Main		0505000209	Includes the Bluestone River (candy darter occurrence site now affected by the Bluestone Reservoir).
		Little Bluestone River*	Lower Trib		0505000209	Includes the Little Bluestone River (candy darter occurrence site now affected by the Bluestone Reservoir).
Upper New	Valley and Ridge	Cripple Creek	Lower Trib		0505000108	Includes Cripple Creek.
		Pine Run*	Lower Trib		0505000111	Includes Pine Run.
		Reed Creek*	Lower Trib	Reed Creek	0505000110	Includes Reed Creek and tributaries.
				South Fork Reed Creek	0505000109	

APPENDIX B

Model

Because no consistent, rangewide assessment of the candy darter is available, we developed a semiquantitative model that produced a "condition score" for each candy darter population or subpopulation. The model relies on three categories of interrelated metrics, including habitat parameters (i.e., water quality and forest cover), non-native species parameters (i.e., the presence of brown trout and/or variegate darters), and estimates of the candy darter demographic status within each unit. Because empirical data relating some of these metrics directly to candy darter life history needs are sparse, we consulted species experts who generally agreed that, for the purpose of this SSA report, the selected metrics were appropriate for assessing the viability of candy darter populations across the species' range (Cincotta 2016, pers. comm.; Dunn 2016, pers. comm.; McBaine 2016, pers. comm.; Pinder 2016, pers. comm.; Angermeier 2017, pers. comm.; Dunn 2017, pers. comm.; Gibson 2017, pers. comm.; Kirk 2017, pers. comm.; Landress 2017, pers.com.; McBaine 2017, pers. comm.; Pinder 2017, pers. comm.; Welsh 2017, pers. comm.). The individual metrics, which we ranked and scored based on criteria described below, were then combined to produce a unitless condition score for each population or subpopulation.

To aid in the comparison of populations and subpopulations (with each other and under various future scenarios) and assess the species viability under the 3Rs, we categorized the final condition scores as "high" (population generally secure), "moderate" (population marginally secure) or "low" (population generally insecure). We based these categories primarily on our understanding of candy darter habitat needs, known stressors, and the principles of conservation biology. We acknowledge that there is uncertainty associated with this model and some of the supporting data, but consider the methodology suitable for assessing the status of the candy darter across its range. In general, the species experts and independent peer reviewers agreed with this conclusion.

Metrics

To inform the model, we identified eight metrics meeting the following criteria: (1) the metric is relevant for assessing candy darter population or subpopulation condition; (2) the data supporting the metric are generally complete and comparable across the range of the species; and (3) the data supporting the metric are at a resolution sufficient to allow for comparisons between individual candy darter populations or subpopulations. Species experts were consulted on these metrics and generally agreed that, for the purpose of this SSA report, the selected metrics were appropriate for assessing candy darter condition (Cincotta 2016, pers. comm.; Dunn 2016, pers. comm.; McBaine 2016, pers. comm.; Pinder 2016, pers. comm.). The following is a brief discussion of each metric.

<u>Forest Cover</u>—Total percent forest cover is used to infer instream habitat conditions (specifically sedimentation, stream bottom embeddedness, and water temperature), which are associated with the distribution and abundance of the candy darter (Addair 1944, p.170; Chipps, *et al*., 1993, p.52; Jenkins and Kopia 1995, pp. 5–6; Dunn 2013, pp. 16, 25–29; Dunn and Angermeier 2016, pp. 1271–1275). Riparian forest cover can mediate stream temperature due to shading. Removal

of forest cover, depending on method and adherence to best management practices, can influence the amount of sedimentation entering a stream, as well as the resultant level of stream bottom embeddedness. A study of the closely related variegate darter in Virginia found that when forest cover in a watershed was less than about 82 percent the likelihood of variegate darter presence was very low (Argentina 2013, p. 7). Other studies relating forest cover to aquatic habitat condition include Hudy *et al*. (2008, entire), who found that most intact brook trout subwatersheds maintained greater than 68-percent forest cover, and Burcher *et al*. (2008, entire), who found lower fish assemblage structural and functional diversity/richness in streams where less than 75 percent of the proximal watershed was forested (Midway *et al*. (2014)).

For candy darter populations and subpopulations defined by a hydrologic unit code (HUC) 10 watershed, the corresponding National Land Cover Dataset (NLCD) (Homer *et. al.* 2015) data were used to calculate percent forest cover (we include shrub/scrub) in the watershed. For candy darter populations or subpopulations that do not occur within a single defined HUC watershed, total forest cover was estimated from U.S. Geological Survey (USGS) topographic maps and ESRI aerial imagery.

Land Ownership—Public landownership is used as an indirect measure to infer the current condition and future trends in instream habitat conditions. Public land management plans generally include specific measures designed to protect and improve aquatic habitat conditions (U.S. Forest Service (USFS) 2011a, entire; USFS 2001b, entire; National Park Service (NPS) 2011, entire), especially stream sedimentation. Private lands may also contribute to healthy or improving aquatic habitat conditions, but we lack consistent, rangewide information for that class of landownership. Therefore, we relied on the public land dataset for this metric. Percent landownership was estimated for each candy darter HUC watershed using boundary lines from the Service's protected lands dataset and USGS topographic maps.

Water Quality—Water quality is a direct metric used to describe the relative health of a stream and its presumed habitability for candy darters. Three parameters from the most recent water quality reports available were used to derive the score: (1) whether the stream segment is designated as a "cold" or "trout" stream; (2) whether the stream segment meets the relevant aquatic life criteria (e.g., trout water, warm water fishery); and (3) whether the stream segment meets other designated use criteria (e.g., public water supply, swimming and fishing) (*https://iaspub.epa.gov/tmdl_waters10/attains_index.home*; accessed Nov 16, 2016). These three components are considered in aggregate to derive a water quality score for each candy darter population or subpopulation.

Space—Space is an estimate of the occupied (or historically occupied) stream habitat within each candy darter population. Stream distance is based on known candy darter occurrence locations and includes the potential candy darter habitat between the upstream and downstream occurrences where no barriers to fish movement are known.

Abundance—Abundance is a fundamental metric for assessing the viability of candy darter populations or subpopulations. However, because details of the various survey methodologies used to collect candy darters are not available, we are unable to develop precise estimates of the species' abundance or catch per unit effort. Therefore, we categorized relative abundance based

on the raw number of specimens reported during individual sampling events or relied on the professional opinion of species experts with direct knowledge of specific candy darter populations.

Connectivity—Connectivity is also considered a fundamental metric for assessing candy darter viability. It is generally understood in the field of conservation biology that larger and more-connected populations contribute to the long-term viability of a species and that smaller isolated populations are more at risk of decline or extirpation as a result of genetic drift, demographic or environmental stochasticity, or catastrophic events (Gilpin and Soulé 1986, pp. 32–34; Angermeier 1995, entire; Fagan 2002, p. 3248; Wiegand *et al*. 2005, entire; Letcher *et al*. 2007, 5–6; Peterson *et al*. 2014, pp. 564–565).

Nonnative Trout—Nonnative rainbow trout (*Oncorhynchus mykiss*) and/or brown trout (*Salmo trutta*) are regularly stocked in some candy darter streams in Virginia and West Virginia (Messinger and Chambers 2001, p. 6; Service 2016; VDGIF 2017, entire; WVDNR 2017, entire). Brown trout are highly piscivorous and are known to prey on smaller native fishes, including darter species (Garman and Nielsen 1982, pp. 864–866). The results of this study also found that brown trout predation appeared to cause shifts in the size structure and abundance of another native fish species (Garman and Nielsen 1982, p. 866). A separate study of trout predation in Stony Creek, Virginia (Middle New watershed) found that newly released rainbow trout were unlikely to prey on native fishes, but did confirm candy darter remains in the stomach of one brown trout (of three examined) (Leftwich *et al*. 1996, p. 6). Predation may not be the only negative effect resulting from nonnative trout introductions. Rainbow trout, while less likely to consume smaller fishes, appear to cause changes in the feeding behavior of native fishes, perhaps leading to dietary effects in these fishes (Freeman and Grossman 1992, pp. 899–901). We note that, despite continued rainbow trout stocking and the presence of a reproducing brown trout population in Stony Creek (Middle New watershed), candy darters remain abundant there (Pinder 2016, pers. comm.). Although data on the effects of nonnative trout on candy darter populations are sparse, the potential for negative interactions is generally supported by the literature and the candy darter species experts.

Hybridization—Variegate darters are introduced species that are known to interbreed with resident candy darters (Switzer *et al*. 2007; Gibson 2017, pp. 6–20). While native to Virginia, we use the term “introduced” to mean locations outside of the variegate darter’s known historical range. Where populations of variegate and candy darters come into contact, variegate darters appear to physically and/or genetically dominate candy darters, which results in the eventual extirpation of the affected candy darter populations.

Metric Scoring

For each candy darter population or subpopulation, the metrics are ranked as “high,” “moderate,” or “low” (with corresponding numerical ranking scores of 1, 0, or -1, respectively). These ranks and corresponding scores are estimates of the conduciveness of the metric condition to the security of the population (table 1). Note that under the Abundance metric, extirpation is scored -5 so that the final population condition score clearly reflects the severity of a population extirpation.

Table 1. Metric ranking and criteria.

FOREST COVER

HIGH (+1)	MODERATE (0)	LOW (-1)
Greater than 90 percent forested.	Between 75 and 90 percent forested.	Less than 75 percent forested.

LAND OWNERSHIP

HIGH (+1)	MODERATE (0)	LOW (-1)
Mostly managed public land.	Mix of public and private land.	Mostly private land.

WATER QUALITY

HIGH (+1)	MODERATE (0)	LOW (-1)
Cool/cold water stream that meets all designated use standards.	Warm water stream that meets all designated use standards ***or*** cool/cold water stream with impairments.	Warm water stream not meeting aquatic life standard.

SPACE

HIGH (+1)	MODERATE (0)	LOW (-1)
Greater than 10 stream miles of occupied or potential habitat.	5 to 10 miles of occupied or potential habitat.	Less than 5 stream miles of occupied or potential habitat.

ABUNDANCE

HIGH (+1)	MODERATE (0)	LOW (-1)	EXTIRPATED (-5)
More than 10 candy darters collected on more than one occasion ***or*** recent characterization by species expert.	More than 10 candy darters collected on a single occasion with fewer numbers collected on multiple occassions ***or*** recent characterization by species expert.	Fewer than 5 candy darters collected on any occassion ***or*** recent characterization by species expert.	Population Extirpated

CONNECTIVITY

HIGH (+1)	MODERATE (0)	LOW (-1)
Population is proximate with others in the metapopulation and movement of individuals between populations is probable.	Population is distant from others in the metapopulation and movement of individuals between populations is less likely.	Population is isolated from others within the metapopulation and movement of individuals between populations is highly unlikely.

NON-NATIVE TROUT

HIGH (+1)	MODERATE (0)	LOW (-1)
Brown trout not reported.	Stocked with brown trout ***or*** no data available.	Reproducing brown trout population.

HYBRIDIZATION

HIGH (+1)	MODERATE (0)	LOW (-1)
Variegate darters (or their genetic markers) not known in the candy darter population.	Variegate darters (or their genetic markers) present in the candy darter population for 5 years or less.	Variegate darters (or their genetic markers) present in the candy darter population for more than 5 years.

To account for potential differences in the data or our assumptions regarding their relationship to the metrics, we multiplied each individual metric ranking score by the sum of three weighting factors (figure 1). The weighting factors are intended to help account for variations in the quality or consistency of the supporting data ("quality"), our confidence that the underlying data are reasonably tied to candy darter condition ("confidence"), and the significance of the metric score to candy darter condition ("significance"). Each weighting factor is scored individually on a scale of 1 to 5 based on the criteria indicated in figure 1.

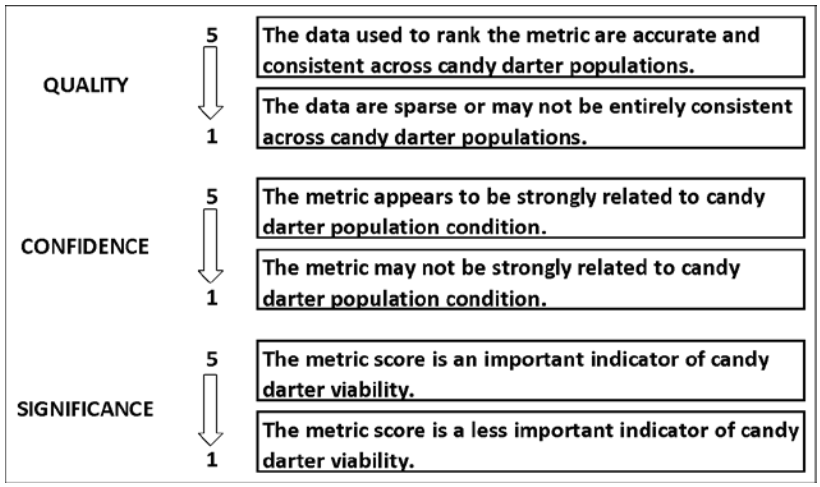

Figure 1. Metric weighting factors.

The summed weighting factors are then multiplied by the individual metric ranking scores for each population/subpopulation to produce a final metric score for each population/subpopulation (figure 2).

						Physical Habitat Metrics		
		Watershed Landcover				EPA/Stat		
Stream	Percent	Forest Cover		Ownership		Trout or	Designate	
(Subpopulation)	Forested	Rank	Score	Rank	Score	Cold Stream	Aquatic Life	O
Gauley River	92%	1	13	0	0	Yes	"good"	"goo
Straight Creek	90%	0	0	-1	-8	Yes	ND	ND
	90%	0	0	0	0	Yes	"good"	"good"
	88%	0	0	-1	-8	Yes	mpaired (Al, Fe	mpaired (Al, F
Williams River	97%	1	13	1	8	Yes	impaired (Al)	"good"
Tea Creek		1	13	1	8	Yes	impaired (pH)	impaired (pH)
	97%	1	13	1	8	Yes	impaired (Al)	"good"
Cherry River	93%	1	13	0	0	Yes	impaired (Fe)	"good"
North Fork Cherry River	96%	1	13	1	8	Yes	impaired (Al)	"good"
South Fork Cherry River	99%	1	13	-1	-8	Yes	"good"	"good"
Laurel Creek	96%	1	13	-1	-8	Yes	"good"	"good"
Little Laurel Creek	90%	1	13	-1	-8	Yes	"good"	"goo

Quality	5	5
Confidence	4	2
Significance	4	1
Sum	13	8

Figure 2. Modified excerpt of the current condition model for the Upper Gauley watershed. Note color-coded metric ranks (green for "high," yellow for "moderate," and red for "low") and corresponding numerical metric ranking scores (1, 0, -1) for each population/subpopulation. Individual and summed weighting factors for each metric are shown in the cells below each metric column. The final metric score for each population/subpopulation is produced by multiplying the individual metric ranking scores by the summed weighting factors for each metric.

Condition Scoring

The final metric ranking scores for each metric are then summed to derive: (1) intermediate condition scores for each of the three metric categories, "Physical Habitat," "Nonnative Competition," and "Population Demographics;" and (2) the final population condition score for

each candy darter population/subpopulation (figure 3). The intermediate and final condition scores are categorized “high,” “moderate,” and “low” (and color-coded appropriately) based on the range of potential scores for each metric and calibrated to be reflective of known conditions, based on the scientific literature, the principles of conservation biology, and input from the species experts.

Stream (Subpopulation)	Total Habitat Score	Total Comp Score	Total Demo Score	Population Condition Score
Gauley River	23	8	34	65
Straight Creek	-8	8	-10	-10
	10	-6	34	38
	-8	20	-10	2
Williams River	21	8	34	63
Tea Creek	21	8	-10	19
	21	20	12	53
Cherry River	13	0	34	47
North Fork Cherry River	21	-6	0	15
South Fork Cherry River	15	6	22	43
Laurel Creek	15	-6	22	31
Little Laurel Creek	15	20	-50	-15
	13	5	15	33
	>15	>10	>15	>40
	0 - 15	0 - 10	0 - 15	0 - 40
	<0	<0	<0	<0

Figure 3. Condensed excerpt of the current condition model showing the intermediate and final condition scores for candy darter populations in the Upper Gauley watershed. The range of scores and corresponding color codes are indicated in the cells beneath each metric category. Note also the average condition scores for each candy darter metapopulation provided at the bottom of each column.

Results

The following tables present the results of the candy darter current condition model as discussed in **Chapter 3** and the future condition scenarios discussed in **Chapter 4.** Note that the current condition model is presented in full, but that the future condition models are condensed for clarity.

Table 2. Current condition model for assessing and comparing the relative condition of known candy darter populations. For the individual metric scores, green shading indicates the metric is conducive to the population, yellow shading indicates the metric is moderately conducive to the population, and red shading indicates the metric is unconducive to the population. For the final population condition scores, green shading indicates "good" (the population is generally secure), yellow indicates "moderate" (the population is marginally secure), and red indicates "low" (the population is generally insecure). Cross hatching indicates extirpated population. Red text indicates population extirpated as a result of variegate darter hybridization. "ND" indicates no data.

			Physical Habitat Metrics											Non-native Competition Metrics					Population Demographic Metrics									
				Watershed Landcover				EPA/State Water Quality Standards																				
				Forest Cover		Ownership		Designated Use			Water Quality			Brown Trout		Variegate Darter			Estimated Length of Potential Habitat		Space		Abundance		Connectivity			
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Percent Forested	Rank	Score	Rank	Score	Trout or Cold Stream	Aquatic Life	Others	Rank	Score	Total Habitat Score	Rank	Score	Rank	Score	Total Comp Score	(km)	(mi)	Rank	Score	Rank	Score	Rank	Score	Total Demo Score	Population Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River	92%	1	13	0	0	Yes	"good"	"good"	1	10	23	-1	-6	1	14	8	33	20	1	12	1	10	1	12	34	65
		Straight Creek	90%	0	0	-1	-8	Yes	ND	ND	0	0	-8	-1	-6	1	14	8	1	1	-1	-12	-1	-10	1	12	-10	-10
	Gauley River (upper)		90%	0	0	0	0	Yes	"good"	"good"	1	10	10	-1	-6	0	0	-6	58	36	1	12	1	10	1	12	34	38
	Panther Creek		88%	0	0	-1	-8	Yes	impaired (Al, Fe	impaired (Al, Fe	0	0	-8	1	6	1	14	20	1	1	-1	-12	-1	-10	1	12	-10	2
	Williams River	Williams River	97%	1	13	1	8	Yes	impaired (Al)	"good"	0	0	21	-1	-6	1	14	8	44	27	1	12	1	10	1	12	34	63
		Tea Creek		1	13	1	8	Yes	impaired (pH)	impaired (pH)	0	0	21	-1	-6	1	14	8	1	1	-1	-12	-1	-10	1	12	-10	19
	Cranberry River		97%	1	13	1	8	Yes	impaired (Al)	"good"	0	0	21	1	6	1	14	20	10	6	0	0	0	0	1	12	12	53
	Cherry River	Cherry River	93%	1	13	0	0	Yes	impaired (Fe)	"good"	0	0	13	0	0	0	0	0	18	11	1	12	1	10	1	12	34	47
		North Fork Cherry River	96%	1	13	1	8	Yes	impaired (Al)	"good"	0	0	21	-1	-6	0	0	-6	4	2	-1	-12	0	0	1	12	0	15
		South Fork Cherry River	99%	1	13	-1	-8	Yes	"good"	"good"	1	10	15	1	6	0	0	6	15	9	0	0	1	10	1	12	22	43
		Laurel Creek	96%	1	13	-1	-8	Yes	"good"	"good"	1	10	15	-1	-6	0	0	-6	12	7	0	0	1	10	1	12	22	31
		Little Laurel Creek	90%	1	13	1	8	Yes	"good"	"good"	1	10	15	1	6	1	14	20	1	1	-1	-12	-5	-50	1	12	-50	-15
													13					5								15	33
Lower Gauley	Gauley River (lower)		79%	0	0	0	0	No	"good"	"good"	0	0	0	0	0	0	0	0	45	28	1	12	1	10	-1	-12	10	10
	Peters Creek		86%	0	0	1	-8	Yes	impaired (Fe)	npaired (colifor	0	0	-8	0	0	-1	-14	-14	15	9	0	0	-5	-50	1	-12	-62	-84
	Collison Creek		80%	0	0	1	-8	Yes	"good"	"good"	1	10	2	0	0	-1	-14	-14	4	2	-1	-12	-5	-50	0	0	-62	-74
													0					0								10	10
Lower New	New River		78%	0	0	0	0	No	"good"	npaired (colifor	0	0	0	0	0	-1	-14	-14	114	71	1	12	-5	-50	1	12	-26	-40
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River	93%	1	13	0	0	Yes	"good"	"good"	1	10	23	-1	-6	0	0	-6	29	18	1	12	1	10	1	12	34	51
		Little River		1	13	1	8	Yes	ND	"good"	1	10	31	-1	-6	0	0	-6	3	2	-1	-12	0	0	1	12	0	25
	Greenbrier River (West Fork)	West Fork Greenbrier River	96%	1	13	1	8	Yes	"good"	"good"	1	10	31	0	0	0	0	0	37	23	1	12	1	10	1	12	34	65
		Little River		1	13	1	8	Yes	"good"	"good"	1	10	31	-1	-6	0	0	-6	2	1	-1	-12	-1	-10	1	12	-10	15
	Greenbrier River (upper)		93%	1	13	0	0	Yes	ND	npaired (colifor	0	0	13	0	0	0	0	0	77	48	1	12	-1	-10	1	12	14	27
	Leatherbark Run		90%	0	0	1	8	Yes	ND	"good"	1	10	18	0	0	0	0	0	1	1	-1	-12	-5	-50	0	0	-62	-44
	Deer Creek	Deer Creek	80%	0	0	-1	-8	Yes	"good"	npaired (colifor	0	0	-8	0	0	0	0	0	18	11	1	12	0	0	1	12	24	16
		North Fork	85%	0	0	-1	-8	Yes	"good"	npaired (colifor	0	0	-8	0	0	0	0	0	10	6	0	0	0	0	1	12	12	4
	Stony Creek		70%	-1	-13	-1	-8	Yes	"good"	paired (coliform	0	0	-21	0	0	-1	-14	-14	7	4	-1	-12	-1	-10	0	0	-22	-57
	Sitlington Creek		87%	0	0	-1	-8	Yes	"good"	"good"	1	10	2	1	6	-1	-14	-8	10	6	0	0	0	0	1	12	12	6
	Knapp Creek	Knapp Creek	89%	0	0	-1	-8	Yes	"good"	npaired (colifor	0	0	-8	0	0	-1	-14	-14	39	24	1	12	0	0	0	0	12	-10
		Douthat Creek	95%	1	13	1	8	Yes	ND	npaired (colifor	0	0	21	1	6	-1	-14	-8	9	6	0	0	0	0	0	0	0	13
	Anthony Creek		95%	1	13	0	0	Yes	"good"	"good"	1	10	23	0	0	-1	-14	-14	11	7	0	0	-5	-50	0	0	-50	-41
	Second Creek		66%	-1	-13	-1	-8	No	"good"	npaired (colifor	0	0	-21	0	0	-1	-14	-14	8	5	0	0	-5	-50	0	0	-50	-85
	Greenbrier River (lower)		81%	0	0	0	0	No	impaired (algal	npaired (colifor	-1	-10	-10	0	0	-1	-14	-14	182	113	1	12	-5	-50	1	12	-26	-50
	Griffith Creek		84%	0	0	-1	-8	No	"good"	"good"	0	0	-8	0	0	-1	-14	-14	1	1	-1	-12	-5	-50	0	0	-62	-84
													10					-6								10	14
Middle New	New River		76%	0	0	1	-8	No	"good"	impaired (PCBs)	0	0	-8	1	6	1	14	20	50	31	1	12	-5	-50	0	0	-38	-26
	Spruce Run		60%	-1	-13	-1	-8	No	ND	ND	0	0	-21	1	6	1	14	20	1	1	-1	-12	-5	-50	0	0	-62	-63
	Sinking Creek		66%	-1	-13	-1	-8	Yes	impaired (temp	npaired (colifor	0	0	-21	1	6	1	14	20	4	2	-1	-12	-5	-50	0	0	-62	-63
	Walker Creek	Walker Creek	79%	0	0	-1	-8	No	"good"	npaired (colifor	0	0	-8	1	6	1	14	20	17	11	1	12	-5	-50	0	0	-38	-26
		Dismal Creek	84%	0	0	1	8	Yes	"good"	ND	1	10	18	1	6	1	14	20	2	1	-1	-12	-1	-10	-1	-12	-34	4
	Stony Creek		97%	1	13	1	8	Yes	"good"	mpaired (PCBs)	1	10	31	-1	-6	1	14	8	19	12	1	12	1	10	-1	-12	10	49
	Wolf Creek	Laurel Creek	89%	0	0	-1	-8	Yes	"good"	npaired (colifor	0	0	-8	1	6	1	14	20	4	3	-1	-12	1	10	-1	-12	-14	-2
	Indian Creek	Indian Creek	71%	-1	-13	-1	-8	No	ND	ND	0	0	-21	0	0	1	14	14	49	30	1	12	-5	-50	0	0	38	-45
		Turkey Creek	80%	0	0	-1	-8	No	ND	ND	0	0	-8	0	0	1	14	14	8	5	-1	-12	-5	0	0	0	-12	-6
													14					16								-13	17
Bluestone	Camp Creek		83%	0	0	0	0	ND	ND	ND	0	0	0	0	0	1	14	14	5	3	-1	-12	-5	-50	1	-12	-74	-60
	Bluestone River		79%	0	0	0	0	No	ND	red (coliform,	0	0	0	1	6	1	14	20	27	17	1	12	-5	-50	1	-12	-50	-30
	Little Bluestone River		78%	0	0	-1	-8	ND	ND	ND	0	0	-8	0	0	1	14	14	6	4	-1	-12	-5	-50	1	-12	-74	-68
Upper New	Cripple Creek		62%	-1	-13	-1	-8	Yes	"good"	npaired (colifor	0	0	-21	1	6	1	14	20	8	5	0	0	0	0	-1	-12	-12	-13
	Pine Run		38%	-1	-13	1	-8	No	ND	ND	0	0	-21	1	6	1	14	20	2	1	-1	-12	-5	-50	1	-12	-74	-75
	Reed Creek	Reed Creek	51%	-1	-13	1	-8	No	"good"	red (coliform,	0	0	-21	1	6	1	14	20	22	14	1	12	-5	-50	1	-12	-50	-51
		South Fork Reed Creek	27%	-1	-13	-1	-8	No	"good"	npaired (colifor	0	0	-21	1	6	1	14	20	1	1	-1	-12	-5	-50	1	-12	-74	-75
													-21					20								-12	-13
			Quality	5		5					4		>15	4		4		>10			2		1		4		>15	>40
			Confidence	4		2					3		0 - 15	1		5		0 - 10			5		4		4		0 - 15	0 - 40
			Significance	4		1					3		<0	1		5		<0			5		5		4		<0	<0
			Sum	13		8					10			6		14					12		10		12			

Table 3. Scenario 1 (25-year positive habitat change). Assumes a ten percent increase in forest cover in all candy darter watersheds with a corresponding improvement in candy darter abundance and connectivity, where possible. Cross hatching indicates extirpated populations. Red text indicates populations extirpated as a result of variegate darter hybridization.

			Physical Habitat Metrics				Non-native Competition			Population Demographic Metrics				
			Watershed Landcove		Water Quali	Total	Brown	Variegat	Total		Abundan	Connecti	Total	Population
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Forest Rank	Land Rank	Water Rank	Habitat Score	Trout Rank	e Darter Rank	Comp Score	Space Rank	ce Rank	vity Rank	Demo Score	Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River	1	0	1	23	-1	1	8	1	1	1	34	65
		Straight Creek	1	-1	1	15	-1	1	8	-1	0	1	0	23
	Gauley River (upper)		1	0	1	23	-1	0	-6	1	1	1	34	51
	Panther Creek		1	-1	1	15	1	1	20	-1	0	1	0	35
	Williams River	Williams River	1	1	1	31	-1	1	8	1	1	1	34	73
		Tea Creek	1	1	1	31	-1	1	8	-1	0	1	0	39
	Cranberry River		1	1	1	31	1	1	20	0	1	1	22	73
	Cherry River	Cherry River	1	0	1	23	0	0	0	1	1	1	34	57
		North Fork Cherry River	1	1	1	31	-1	0	-6	-1	1	1	10	35
		South Fork Cherry River	1	-1	1	15	1	0	6	0	1	1	22	43
		Laurel Creek	1	-1	1	15	-1	0	-6	0	1	1	22	31
		Little Laurel Creek	1	-1	1	15	1	1	20	-1	-5	1	-50	-15
						23			5				19	48
Lower Gauley	Gauley River (lower)		0	0	1	10	0	0	0	1	1	-1	10	20
	Peters Creek		1	-1	1	15	0	-1	-14	0	-5	-1	-62	-61
	Collison Creek		0	-1	1	2	0	-1	-14	-1	-5	0	-62	-74
						10			0				10	20
Lower New	New River		0	0	1	10	0	-1	-14	1	-5	1	-26	-30
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River	1	0	1	23	-1	0	-6	1	1	1	34	51
		Little River	1	1	1	31	-1	0	-6	-1	1	1	10	35
	Greenbrier River (West Fork)	West Fork Greenbrier River	1	1	1	31	0	0	0	1	1	1	34	65
		Little River	1	1	1	31	-1	0	-6	-1	0	1	0	25
	Greenbrier River (upper)		1	0	1	23	0	0	0	1	0	1	24	47
	Leatherbark Run		1	1	1	31	0	0	0	-1	-5	1	-50	-19
	Deer Creek	Deer Creek	0	-1	1	2	0	0	0	1	1	1	34	36
		North Fork	1	-1	1	15	0	0	0	0	1	1	22	37
	Stony Creek		0	-1	1	2	0	-1	-14	-1	0	1	0	-12
	Sitlington Creek		1	-1	1	15	1	-1	-8	0	1	1	22	29
	Knapp Creek	Knapp Creek	1	-1	1	15	0	-1	-14	1	1	1	34	35
		Douthat Creek	1	1	1	31	1	-1	-8	0	1	1	22	45
	Anthony Creek		1	0	1	23	0	-1	-14	0	-5	1	-38	-29
	Second Creek		0	-1	1	2	0	-1	-14	0	-5	1	-38	-50
	Greenbrier River (lower)		1	0	0	13	0	-1	-14	1	-5	1	-26	-27
	Griffith Creek		1	-1	1	15	0	-1	-14	-1	-5	1	-50	-49
						20			-6				21	36
Middle New	New River		0	-1	1	2	1	1	20	1	-5	1	-26	-4
	Spruce Run		-1	-1	1	-11	1	1	20	-1	-5	1	-50	-41
	Sinking Creek		0	-1	1	2	1	1	20	-1	-5	1	-50	-28
	Walker Creek	Walker Creek	0	-1	1	2	1	1	20	1	-5	1	-26	-4
		Dismal Creek	1	1	1	31	1	1	20	-1	0	0	-12	39
	Stony Creek		1	1	1	31	-1	1	8	1	1	0	22	61
	Wolf Creek	Laurel Creek	1	-1	1	15	1	1	20	-1	1	0	-2	33
	Indian Creek	Indian Creek	0	-1	1	2	0	1	14	1	-5	1	-26	-10
		Turkey Creek	0	-1	1	2	0	1	14	-1	-5	1	0	16
						26			16				3	44
Bluestone	Camp Creek		1	0	1	23	0	1	14	-1	-5	0	-62	-25
	Bluestone River		0	0	1	10	1	1	20	1	-5	0	-38	-8
	Little Bluestone River		0	-1	1	2	0	1	14	-1	-5	0	-62	-46
Upper New	Cripple Creek		-1	-1	1	-11	1	1	20	0	1	-1	-2	7
	Pine Run		-1	-1	1	-11	1	1	20	-1	-5	-1	-74	-65
	Reed Creek	Reed Creek	-1	-1	1	-11	1	1	20	1	-5	-1	-50	-41
		South Fork Reed Creek	-1	-1	1	-11	1	1	20	-1	-5	-1	-74	-65
						-11			20				-2	7
		Data Quality	5	5	4	>15	4	4	>10	2	1	4	>15	>40
		Confidence	4	2	3	0 - 15	1	5	0 - 10	5	4	4	0 - 15	0 - 40
		Significance	4	1	3	<0	1	5	<0	5	5	4	<0	<0
		Weighting Factor (sum)	13	8	10		6	14		12	10	12		

Table 4. Scenario 2 (25-year negative habitat change). Assumes a 10-percent decrease in forest cover in watersheds where the land is mostly privately owned or a mix of public and private land with a corresponding decrease in candy darter abundance and connectivity. Cross hatching indicates extirpated populations. Red text indicates populations extirpated as a result of variegate darter hybridization.

			Physical Habitat Metrics				Non-native Competition			Population Demographic Metrics				
			Watershed Landcove		Water Quali	Total	Brown	Variegat	Total		Abundan	Connecti	Total	Population
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Forest Rank	Land Rank	Water Rank	Habitat Score	Trout Rank	e Darter Rank	Comp Score	Space Rank	ce Rank	vity Rank	Demo Score	Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River	0	0	0	0	-1	1	8	1	0	0	12	20
		Straight Creek	0	-1	-1	-18	-1	1	8	-1	-1	0	-22	-32
	Gauley River (upper)		0	0	0	0	-1	0	-6	1	0	0	12	6
	Panther Creek		0	-1	-1	-18	1	1	20	-1	-1	0	-22	-20
	Williams River	Williams River	1	1	0	21	-1	1	8	1	1	1	34	63
		Tea Creek	1	1	0	21	-1	1	8	-1	-1	1	-10	19
	Cranberry River		1	1	0	21	1	1	20	0	0	1	12	53
	Cherry River	Cherry River	0	0	-1	-10	0	0	0	1	0	0	12	2
		North Fork Cherry River	1	1	0	21	-1	0	-6	-1	0	1	0	15
		South Fork Cherry River	0	-1	0	-8	1	0	6	0	0	0	0	-2
		Laurel Creek	0	-1	0	-8	-1	0	-6	0	0	0	0	-14
		Little Laurel Creek	0	-1	0	-8	1	1	20	-1	-5	0	-62	-50
						2			5				3	10
Lower Gauley	Gauley River (lower)		-1	0	-1	-23	0	0	0	1	0	-1	0	-23
	Peters Creek		0	-1	-1	-18	0	-1	-14	0	-5	-1	-62	-94
	Collison Creek		-1	-1	0	-21	0	-1	-14	-1	-5	0	-62	-97
						-23			0				0	-23
Lower New	New River		-1	0	0	-13	0	-1	-14	1	-5	1	-26	-53
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River	0	0	0	0	-1	0	-6	1	0	0	12	6
		Little River	1	1	1	31	-1	0	-6	-1	0	1	0	25
	Greenbrier River (West Fork)	West Fork Greenbrier River	1	1	1	31	0	0	0	1	1	1	34	65
		Little River	1	1	1	31	-1	0	-6	-1	-1	1	-10	15
	Greenbrier River (upper)		0	0	-1	-10	0	0	0	1	-1	0	2	-8
	Leatherbark Run		0	-1	-1	-2	0	0	0	-1	-5	0	-62	-64
	Deer Creek	Deer Creek	-1	-1	-1	-31	0	0	0	1	-1	0	2	-29
		North Fork	0	-1	-1	-18	0	0	0	0	-1	0	-10	-28
	Stony Creek		-1	-1	-1	-31	0	-1	-14	-1	-1	0	-22	-67
	Sitlington Creek		0	-1	0	-8	1	-1	-8	0	-1	0	-10	-26
	Knapp Creek	Knapp Creek	0	-1	-1	-18	0	-1	-14	1	-1	0	2	-30
		Douthat Creek	1	1	0	21	1	-1	-8	0	0	0	0	13
	Anthony Creek		0	0	0	0	0	-1	-14	0	-5	0	-50	-64
	Second Creek		-1	-1	-1	-31	0	-1	-14	0	-5	0	-50	-95
	Greenbrier River (lower)		-1	0	-1	-23	0	-1	-14	1	-5	0	-38	-75
	Griffith Creek		-1	-1	-1	-31	0	-1	-14	-1	-5	0	-62	-107
						0			-6				0	-6
Middle New	New River		-1	-1	-1	-31	1	1	20	1	-5	0	-38	-49
	Spruce Run		-1	-1	-1	-31	1	1	20	-1	-5	0	-62	-73
	Sinking Creek		-1	-1	-1	-31	1	1	20	-1	-5	0	-62	-73
	Walker Creek	Walker Creek	-1	-1	-1	-31	1	1	20	1	-5	0	-38	-49
		Dismal Creek	0	1	1	18	1	1	20	-1	-1	-1	-34	4
	Stony Creek		1	1	1	31	-1	1	8	1	1	-1	10	49
	Wolf Creek	Laurel Creek	0	-1	-1	-18	1	1	20	-1	-1	-1	-34	-32
	Indian Creek	Indian Creek	-1	-1	-1	-31	0	1	14	1	-5	-1	-50	-67
		Turkey Creek	-1	-1	-1	-31	0	1	14	-1	-5	-1	-24	-41
						10			16				-19	7
Bluestone	Camp Creek		-1	0	0	-13	0	1	14	-1	-5	-1	-74	-73
	Bluestone River		-1	0	0	-13	1	1	20	1	-5	-1	-50	-43
	Little Bluestone River		-1	-1	0	-21	0	1	14	-1	-5	-1	-74	-81
Upper New	Cripple Creek		-1	-1	0	-21	1	1	20	0	-1	-1	-22	-23
	Pine Run		-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
	Reed Creek	Reed Creek	-1	-1	0	-21	1	1	20	1	-5	-1	-50	-51
		South Fork Reed Creek	-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
						-21			20				-22	-23
		Data Quality	5	5	4	>15	4	4	>10	2	1	4	>15	>40
		Confidence	4	2	3	0 - 15	1	5	0 - 10	5	4	4	0 - 15	0 - 40
		Significance	4	1	3	<0	1	5	<0	5	5	4	<0	<0
		Weighting Factor (sum)	13	8	10		6	14		12	10	12		

Table 5. Scenario 3a (5-year variegate darter expansion). Variegate darters expand in the Upper Gauley watershed. Variegate darter hybridization ongoing in the Lower Gauley watershed. Variegate darter hybridization in the Greenbrier watershed results in the extirpation of candy darter populations that have been exposed to variegate darters for more than 10 years. Cross hatching indicates extirpated populations. Red text indicates populations extirpated as a result of variegate darter hybridization.

			Physical Habitat Metrics				Non-native Competition			Population Demographic Metrics				
			Watershed Landcover/Water Quali											
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Forest Rank	Land Rank	Water Rank	Total Habitat Score	Brown Trout Rank	Variegate Darter Rank	Total Comp Score	Space Rank	Abundance Rank	Connectivity Rank	Total Demo Score	Population Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River	1	0	1	23	-1	0	-6	1	1	1	34	51
		Straight Creek	0	-1	0	-8	-1	0	-6	-1	-1	1	-10	-24
	Gauley River (upper)		0	0	1	10	-1	-1	-20	1	1	1	34	24
	Panther Creek		0	-1	0	-8	1	0	6	-1	-1	1	-10	-12
	Williams River	Williams River	1	1	0	21	-1	0	-6	1	1	1	34	49
		Tea Creek	1	1	0	21	-1	0	-6	-1	-1	1	-10	5
	Cranberry River		1	1	0	21	1	0	6	0	0	1	12	39
	Cherry River	Cherry River	1	0	0	13	0	-1	-14	1	1	1	34	33
		North Fork Cherry River	1	1	0	21	-1	-1	-20	-1	0	1	0	1
		South Fork Cherry River	1	-1	1	15	1	-1	-8	0	1	1	22	29
		Laurel Creek	1	-1	1	15	-1	-1	-20	0	1	1	22	17
		Little Laurel Creek	1	-1	1	15	1	1	20	-1	-5	1	-50	-15
						13			-9				15	19
Lower Gauley	Gauley River (lower)		0	0	0	0	0	-1	-14	1	1	-1	10	-4
	Peters Creek		0	-1	0	-8	0	-1	-14	0	-5	-1	-62	-84
	Collison Creek		0	-1	1	2	0	-1	-14	-1	-5	0	-62	-74
						0			-14				10	-4
Lower New	New River		0	0	0	0	0	-1	-14	1	-5	1	-26	-40
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River	1	0	1	23	-1	-1	-20	1	1	1	34	37
		Little River	1	1	1	31	-1	-1	-20	-1	0	1	0	11
	Greenbrier River (West Fork)	West Fork Greenbrier River	1	1	1	31	0	-1	-14	1	1	1	34	51
		Little River	1	1	1	31	-1	-1	-20	-1	-1	1	-10	1
	Greenbrier River (upper)		1	0	0	13	0	-1	-14	1	-1	1	14	13
	Leatherbark Run		0	1	1	18	0	-1	-14	-1	-5	0	-62	-58
	Deer Creek	Deer Creek	0	-1	0	-8	0	-1	-14	1	0	1	24	2
		North Fork	0	-1	0	-8	0	-1	-14	0	0	1	12	-10
	Stony Creek		-1	-1	0	-21	0	-1	-14	-1	-5	0	-62	-97
	Sitlington Creek		0	-1	1	2	1	-1	-8	0	-5	1	-38	-44
	Knapp Creek	Knapp Creek	0	-1	0	-8	0	-1	-14	1	-5	0	-38	-60
		Douthat Creek	1	1	0	21	1	-1	-8	0	-5	0	-50	-37
	Anthony Creek		1	0	1	23	0	-1	-14	0	-5	0	-50	-41
	Second Creek		-1	-1	0	-21	0	-1	-14	0	-5	0	-50	-85
	Greenbrier River (lower)		0	0	-1	-10	0	-1	-14	1	-5	1	-26	-50
	Griffith Creek		0	-1	0	-8	0	-1	-14	-1	-5	0	-62	-84
						10			-15				-7	-12
Middle New	New River		0	-1	0	-8	1	1	20	1	-5	0	-38	-26
	Spruce Run		-1	-1	0	-21	1	1	20	-1	-5	0	-62	-63
	Sinking Creek		-1	-1	0	-21	1	1	20	-1	-5	0	-62	-63
	Walker Creek	Walker Creek	0	-1	0	-8	1	1	20	1	-5	0	-38	-26
		Dismal Creek	0	1	1	18	1	1	20	-1	-1	-1	-34	4
	Stony Creek		1	1	1	31	-1	1	8	1	1	-1	10	49
	Wolf Creek	Laurel Creek	0	-1	0	-8	1	1	20	-1	1	-1	-14	-2
	Indian Creek	Indian Creek	-1	-1	0	-21	0	1	14	1	-5	0	-38	-45
		Turkey Creek	0	-1	0	-8	0	1	14	-1	-5	0	-12	-6
						14			16				-13	17
Bluestone	Camp Creek		0	0	0	0	0	1	14	-1	-5	-1	-74	-60
	Bluestone River		0	0	0	0	1	1	20	1	-5	-1	-50	-30
	Little Bluestone River		0	-1	0	-8	0	1	14	-1	-5	-1	-74	-68
Upper New	Cripple Creek		-1	-1	0	-21	1	1	20	0	0	-1	-12	-13
	Pine Run		-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
	Reed Creek	Reed Creek	-1	-1	0	-21	1	1	20	1	-5	-1	-50	-51
		South Fork Reed Creek	-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
						-21			20				-12	-13
		Data Quality	5	5	4	>15	4	4	>10	2	1	4	>15	>40
		Confidence	4	2	3	0 - 15	1	5	0 - 10	5	4	4	0 - 15	0 - 40
		Significance	4	1	3	<0	1	5	<0	5	5	4	<0	<0
		Weighting Factor (sum)	13	8	10		6	14		12	10	12		

Table 6. Scenario 3b (10-year variegate darter expansion). Variegate darter hybridization in the Upper Gauley watershed results in the extirpation of candy darter populations that have been exposed to variegate darters for more than 10 years. Variegate darter competition results in the extirpation of the Lower Gauley candy darter population. Variegate darter hybridization in the Greenbrier watershed results in the extirpation of all candy darter populations there. Cross hatching indicates extirpated populations. Red text indicates populations extirpated as a result of variegate darter hybridization.

			Physical Habitat Metrics				Non-native Competition			Population Demographic Metrics				
			Watershed Landcover/Water Quali			Total	Brown	Variegat	Total		Abundan	Connecti	Total	Population
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Forest Rank	Land Rank	Water Rank	Habitat Score	Trout Rank	e Darter Rank	Comp Score	Space Rank	ce Rank	vity Rank	Demo Score	Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River	1	0	1	23	-1	-1	-20	1	1	1	34	37
		Straight Creek	0	-1	0	-8	-1	-1	-20	-1	-1	1	-10	-38
	Gauley River (upper)		0	0	1	10	-1	-1	-20	1	-5	1	-26	-36
	Panther Creek		0	-1	0	-8	1	-1	-8	-1	-1	1	-10	-26
	Williams River	Williams River	1	1	0	21	-1	-1	-20	1	1	1	34	35
		Tea Creek	1	1	0	21	-1	-1	-20	-1	-1	1	-10	-9
	Cranberry River		1	1	0	21	1	-1	-8	0	0	1	12	25
	Cherry River	Cherry River	1	0	0	13	0	-1	-14	1	-5	1	-26	-27
		North Fork Cherry River	1	1	0	21	-1	-1	-20	-1	-5	1	-50	-49
		South Fork Cherry River	1	-1	1	15	1	-1	-8	0	-5	1	-38	-31
		Laurel Creek	1	-1	1	15	-1	-1	-20	0	-5	1	-38	-43
		Little Laurel Creek	1	-1	1	15	1	0	6	-1	-5	1	-50	-29
						13			-16				-12	-15
Lower Gauley	Gauley River (lower)		0	0	0	0	0	-1	-14	1	-5	-1	-50	-64
	Peters Creek		0	-1	0	-8	0	-1	-14	0	-5	-1	-62	-84
	Collison Creek		0	-1	1	2	0	-1	-14	-1	-5	0	-62	-74
Lower New	New River		0	0	0	0	0	-1	-14	1	-5	1	-26	-40
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River	1	0	1	23	-1	-1	-20	1	-5	1	-26	-23
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (West Fork)	West Fork Greenbrier River	1	1	1	31	0	-1	-14	1	-5	1	-26	-9
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (upper)		1	0	0	13	0	-1	-14	1	-5	1	-26	-27
	Leatherbark Run		0	1	1	18	0	-1	-14	-1	-5	0	-62	-58
	Deer Creek	Deer Creek	0	-1	0	-8	0	-1	-14	1	-5	1	-26	-48
		North Fork	0	-1	0	-8	0	-1	-14	0	-5	1	-38	-60
	Stony Creek		-1	-1	0	-21	0	-1	-14	-1	-5	0	-62	-97
	Sittington Creek		0	-1	1	2	1	-1	-8	0	-5	1	-38	-44
	Knapp Creek	Knapp Creek	0	-1	0	-8	0	-1	-14	1	-5	0	-38	-60
		Douthat Creek	1	1	0	21	1	-1	-8	0	-5	0	-50	-37
	Anthony Creek		1	0	1	23	0	-1	-14	0	-5	0	-50	-41
	Second Creek		-1	-1	0	-21	0	-1	-14	0	-5	0	-50	-85
	Greenbrier River (lower)		0	0	-1	-10	0	-1	-14	1	-5	1	-26	-50
	Griffith Creek		0	-1	0	-8	0	-1	-14	-1	-5	0	-62	-84
Middle New	New River		0	-1	0	-8	1	1	20	1	-5	0	-38	-26
	Spruce Run		-1	-1	0	-21	1	1	20	-1	-5	0	-62	-63
	Sinking Creek		-1	-1	0	-21	1	1	20	-1	-5	0	-62	-63
	Walker Creek	Walker Creek	0	-1	0	-8	1	1	20	1	-5	0	-38	-26
		Dismal Creek	0	1	1	18	1	1	20	-1	-1	-1	-34	4
	Stony Creek		1	1	1	31	-1	1	8	1	1	-1	10	49
	Wolf Creek	Laurel Creek	0	-1	0	-8	1	1	20	-1	1	-1	-14	-2
	Indian Creek	Indian Creek	-1	-1	0	-21	0	1	14	1	-5	0	-38	-45
		Turkey Creek	0	-1	0	-8	0	1	14	-1	-5	0	-12	-6
						14			16				-13	17
Bluestone	Camp Creek		0	0	0	0	0	1	14	-1	-5	-1	-74	-60
	Bluestone River		0	0	0	0	1	1	20	1	-5	-1	-50	-30
	Little Bluestone River		0	-1	0	-8	0	1	14	-1	-5	-1	-74	-68
Upper New	Cripple Creek		-1	-1	0	-21	1	1	20	0	0	-1	-12	-13
	Pine Run		-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
	Reed Creek	Reed Creek	-1	-1	0	-21	1	1	20	1	-5	-1	-50	-51
		South Fork Reed Creek	-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
						-21			20				-12	-13
		Data Quality	5	5	4	>15	4	4	>10	2	1	4	>15	>40
		Confidence	4	2	3	0 - 15	1	5	0 - 10	5	4	4	0 - 15	0 - 40
		Significance	4	1	3	<0	1	5	<0	5	5	4	<0	<0
		Weighting Factor (sum)	13	8	10		6	14		12	10	12		

Table 7. Scenario 3c (15-year variegate darter expansion). Variegate darter hybridization results in the extirpation of the Upper Gauley candy darter populations. Cross hatching indicates extirpated populations. Red text indicates populations extirpated as a result of variegate darter hybridization.

			Physical Habitat Metrics				Non-native Competition			Population Demographic Metrics				
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Watershed Forest Rank	Landcover Rank	Water Quali Water Rank	Total Habitat Score	Brown Trout Rank	Variegate Darter Rank	Total Comp Score	Space Rank	Abundance Rank	Connectivity Rank	Total Demo Score	Population Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River	1	0	1	23	-1	-1	-20	1	-5	1	-26	-23
		Straight Creek	0	-1	0	-8	-1	-1	-20	-1	-5	1	-50	-78
	Gauley River (upper)		0	0	1	10	-1	-1	-20	1	-5	1	-26	-36
	Panther Creek		0	-1	0	-8	1	-1	-8	-1	-5	1	-50	-66
	Williams River	Williams River	1	1	0	21	-1	-1	-20	1	-5	1	-26	-25
		Tea Creek	1	1	0	21	-1	-1	-20	-1	-5	1	-50	-49
	Cranberry River		1	1	0	21	1	-1	-8	0	-5	1	-38	-25
	Cherry River	Cherry River	1	0	0	13	0	-1	-14	1	-5	1	-26	-27
		North Fork Cherry River	1	1	0	21	-1	-1	-20	-1	-5	1	-50	-49
		South Fork Cherry River	1	-1	1	15	1	-1	-8	0	-5	1	-38	-31
		Laurel Creek	1	-1	1	15	-1	-1	-20	0	-5	1	-38	-43
		Little Laurel Creek	1	-1	1	15	1	-1	-8	-1	-5	1	-50	-43
Lower Gauley	Gauley River (lower)		0	0	0	0	0	-1	-14	1	-5	-1	-50	-64
	Peters Creek		0	-1	0	-8	0	-1	-14	0	-5	-1	-62	-84
	Collison Creek		0	-1	1	2	0	-1	-14	-1	-5	0	-62	-74
Lower New	New River		0	0	0	0	0	-1	-14	1	-5	1	-26	-40
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River	1	0	1	23	-1	-1	-20	1	-5	1	-26	-23
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (West Fork)	West Fork Greenbrier River	1	1	1	31	0	-1	-14	1	-5	1	-26	-9
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (upper)		1	0	0	13	0	-1	-14	1	-5	1	-26	-27
	Leatherbark Run		0	1	1	18	0	-1	-14	-1	-5	0	-62	-58
	Deer Creek	Deer Creek	0	-1	0	-8	0	-1	-14	1	-5	1	-26	-48
		North Fork	0	-1	0	-8	0	-1	-14	0	-5	1	-38	-60
	Stony Creek		-1	-1	0	-21	0	-1	-14	-1	-5	0	-62	-97
	Sittington Creek		0	-1	1	2	1	-1	-8	0	-5	1	-38	-44
	Knapp Creek	Knapp Creek	0	-1	0	-8	0	-1	-14	1	-5	0	-38	-60
		Douthat Creek	1	1	0	21	1	-1	-8	0	-5	0	-50	-37
	Anthony Creek		1	0	1	23	0	-1	-14	0	-5	0	-50	-41
	Second Creek		-1	-1	0	-21	0	-1	-14	0	-5	0	-50	-85
	Greenbrier River (lower)		0	0	-1	-10	0	-1	-14	1	-5	1	-26	-50
	Griffith Creek		0	-1	0	-8	0	-1	-14	-1	-5	0	-62	-84
Middle New	New River		0	-1	0	-8	1	1	20	1	-5	0	-38	-26
	Spruce Run		-1	-1	0	-21	1	1	20	-1	-5	0	-62	-63
	Sinking Creek		-1	-1	0	-21	1	1	20	-1	-5	0	-62	-63
	Walker Creek	Walker Creek	0	-1	0	-8	1	1	20	1	-5	0	-38	-26
		Dismal Creek	0	1	1	18	1	1	20	-1	-1	-1	-34	4
	Stony Creek		1	1	1	31	-1	1	8	1	1	-1	10	49
	Wolf Creek	Laurel Creek	0	-1	0	-8	1	1	20	-1	1	-1	-14	-2
	Indian Creek	Indian Creek	-1	-1	0	-21	0	1	14	1	-5	0	-38	-45
		Turkey Creek	0	-1	0	-8	0	1	14	-1	-5	0	-12	-6
						14			16				-13	17
Bluestone	Camp Creek		0	0	0	0	0	1	14	-1	-5	-1	-74	-60
	Bluestone River		0	0	0	0	1	1	20	1	-5	-1	-50	-30
	Little Bluestone River		0	-1	0	-8	0	1	14	-1	-5	-1	-74	-68
Upper New	Cripple Creek		-1	-1	0	-21	1	1	20	0	0	-1	-12	-13
	Pine Run		-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
	Reed Creek	Reed Creek	-1	-1	0	-21	1	1	20	1	-5	-1	-50	-51
		South Fork Reed Creek	-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
						-21			20				-12	-13
		Data Quality	5	5	4	>15	4	4	>10	2	1	4	>15	>40
		Confidence	4	2	3	0 - 15	1	5	0 - 10	5	4	4	0 - 15	0 - 40
		Significance	4	1	3	<0	1	5	<0	5	5	4	<0	<0
		Weighting Factor (sum)	13	8	10		6	14		12	10	12		

Table 8. Scenario 3d (20-year variegate darter expansion). Variegate darters become established in the Middle New watershed. Cross hatching indicates extirpated populations. Red text indicates populations extirpated as a result of variegate darter hybridization.

			Physical Habitat Metrics				Non-native Competition			Population Demographic Metrics				
			Watershed	Landcover	Water Quali	Total	Brown	Variegat	Total		Abundan	Connecti	Total	Population
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Forest Rank	Land Rank	Water Rank	Habitat Score	Trout Rank	e Darter Rank	Comp Score	Space Rank	ce Rank	vity Rank	Demo Score	Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River	1	0	1	23	-1	-1	-20	1	-5	1	-26	-23
		Straight Creek	0	-1	0	-8	-1	-1	-20	-1	-5	1	-50	-78
	Gauley River (upper)		0	0	1	10	-1	-1	-20	1	-5	1	-26	-36
	Panther Creek		0	-1	0	-8	1	-1	-8	-1	-5	1	-50	-66
	Williams River	Williams River	1	1	0	21	-1	-1	-20	1	-5	1	-26	-25
		Tea Creek	1	1	0	21	-1	-1	-20	-1	-5	1	-50	-49
	Cranberry River		1	1	0	21	1	-1	-8	0	-5	1	-38	-25
	Cherry River	Cherry River	1	0	0	13	0	-1	-14	1	-5	1	-26	-27
		North Fork Cherry River	1	1	0	21	-1	-1	-20	-1	-5	1	-50	-49
		South Fork Cherry River	1	-1	1	15	1	-1	-8	0	-5	1	-38	-31
		Laurel Creek	1	-1	1	15	-1	-1	-20	0	-5	1	-38	-43
		Little Laurel Creek	1	-1	1	15	1	-1	-8	-1	-5	1	-50	-43
Lower Gauley	Gauley River (lower)		0	0	0	0	0	-1	-14	1	-5	-1	-50	-64
	Peters Creek		0	-1	0	-8	0	-1	-14	0	-5	-1	-62	-84
	Collison Creek		0	-1	1	2	0	-1	-14	-1	-5	0	-62	-74
Lower New	New River		0	0	0	0	0	-1	-14	1	-5	1	-26	-40
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River	1	0	1	23	-1	-1	-20	1	-5	1	-26	-23
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (West Fork)	West Fork Greenbrier River	1	1	1	31	0	-1	-14	1	-5	1	-26	-9
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (upper)		1	0	0	13	0	-1	-14	1	-5	1	-26	-27
	Leatherbark Run		0	1	1	18	0	-1	-14	-1	-5	0	-62	-58
	Deer Creek	Deer Creek	0	-1	0	-8	0	-1	-14	1	-5	1	-26	-48
		North Fork	0	-1	0	-8	0	-1	-14	0	-5	1	-38	-60
	Stony Creek		-1	-1	0	-21	0	-1	-14	-1	-5	0	-62	-97
	Sittington Creek		0	-1	1	2	1	-1	-8	0	-5	1	-38	-44
	Knapp Creek	Knapp Creek	0	-1	0	-8	0	-1	-14	1	-5	0	-38	-60
		Douthat Creek	1	1	0	21	1	-1	-8	0	-5	0	-50	-37
	Anthony Creek		1	0	1	23	0	-1	-14	0	-5	0	-50	-41
	Second Creek		-1	-1	0	-21	0	-1	-14	0	-5	0	-50	-85
	Greenbrier River (lower)		0	0	-1	-10	0	-1	-14	1	-5	1	-26	-50
	Griffith Creek		0	-1	0	-8	0	-1	-14	-1	-5	0	-62	-84
Middle New	New River		0	-1	0	-8	1	-1	-8	1	-5	0	-38	-54
	Spruce Run		-1	-1	0	-21	1	-1	-8	-1	-5	0	-62	-91
	Sinking Creek		-1	-1	0	-21	1	-1	-8	-1	-5	0	-62	-91
	Walker Creek	Walker Creek	0	-1	0	-8	1	-1	-8	1	-5	0	-38	-54
		Dismal Creek	0	1	1	18	1	0	6	-1	-1	-1	-34	-10
	Stony Creek		1	1	1	31	-1	0	-6	1	1	-1	10	35
	Wolf Creek	Laurel Creek	0	-1	0	-8	1	0	6	-1	1	-1	-14	-16
	Indian Creek	Indian Creek	-1	-1	0	-21	0	-1	-14	1	-5	0	-38	-73
		Turkey Creek	0	-1	0	-8	0	-1	-14	-1	-5	0	-12	-34
						14			2				-13	3
Bluestone	Camp Creek		0	0	0	0	0	1	14	-1	-5	1	-74	-60
	Bluestone River		0	0	0	0	1	1	20	1	-5	-1	-50	-30
	Little Bluestone River		0	-1	0	-8	0	1	14	-1	-5	-1	-74	-68
Upper New	Cripple Creek		-1	-1	0	-21	1	1	20	0	0	-1	-12	-13
	Pine Run		-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
	Reed Creek	Reed Creek	-1	-1	0	-21	1	1	20	1	-5	-1	-50	-51
		South Fork Reed Creek	-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
						-21			20				-12	-13
		Data Quality	5	5	4	>15	4	4	>10	2	1	4	>15	>40
		Confidence	4	2	3	0 - 15	1	5	0 - 10	5	4	4	0 - 15	0 - 40
		Significance	4	1	3	<0	1	5	<0	5	5	4	<0	<0
		Weighting Factor (sum)	13	8	10		6	14		12	10	12		

Table 9. Scenario 3e (25-year variegate darter expansion). Variegate darter hybridization in the Middle New watershed continues. Cross hatching indicates extirpated populations. Red text indicates populations extirpated as a result of variegate darter hybridization.

			Physical Habitat Metrics				Non-native Competition			Population Demographic Metrics				
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Watershed Forest Rank	Landcover Land Rank	Water Quali Water Rank	Total Habitat Score	Brown Trout Rank	Variegate Darter Rank	Total Comp Score	Space Rank	Abundance Rank	Connectivity Rank	Total Demo Score	Population Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River	1	0	1	23	-1	-1	-20	1	-5	1	-26	-23
		Straight Creek	0	-1	0	-8	-1	1	-20	-1	-5	1	-50	-78
	Gauley River (upper)		0	0	1	10	-1	-1	-20	1	-5	1	-26	-36
	Panther Creek		0	-1	0	-8	1	-1	-8	-1	-5	1	-50	-66
	Williams River	Williams River	1	1	0	21	-1	-1	-20	1	-5	1	-26	-25
		Tea Creek	1	1	0	21	-1	-1	-20	-1	-5	1	-50	-49
	Cranberry River		1	1	0	21	1	-1	-8	0	-5	1	-38	-25
	Cherry River	Cherry River	1	0	0	13	0	-1	-14	1	-5	1	-26	-27
		North Fork Cherry River	1	1	0	21	-1	-1	-20	-1	-5	1	-50	-49
		South Fork Cherry River	1	-1	1	15	1	-1	-8	0	-5	1	-38	-31
		Laurel Creek	1	-1	1	15	-1	-1	-20	0	-5	1	-38	-43
		Little Laurel Creek	1	-1	1	15	1	-1	-8	-1	-5	1	-50	-43
Lower Gauley	Gauley River (lower)		0	0	0	0	0	-1	-14	1	-5	-1	-50	-64
	Peters Creek		0	-1	0	-8	0	-1	-14	0	-5	-1	-62	-84
	Collison Creek		0	-1	1	2	0	-1	-14	-1	-5	0	-62	-74
Lower New	New River		0	0	0	0	0	-1	-14	1	-5	1	-26	-40
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River	1	0	1	23	-1	-1	-20	1	-5	1	-26	-23
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (West Fork)	West Fork Greenbrier River	1	1	1	31	0	-1	-14	1	-5	1	-26	-9
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (upper)		1	0	0	13	0	-1	-14	1	-5	1	-26	-27
	Leatherbark Run		0	1	1	18	0	-1	-14	-1	-5	0	-62	-58
	Deer Creek	Deer Creek	0	-1	0	-8	0	-1	-14	1	-5	1	-26	-48
		North Fork	0	-1	0	-8	0	-1	-14	0	-5	1	-38	-60
	Stony Creek		-1	-1	0	-21	0	-1	-14	-1	-5	0	-62	-97
	Sittington Creek		0	-1	1	2	1	-1	-8	0	-5	1	-38	-44
	Knapp Creek	Knapp Creek	0	-1	0	-8	0	-1	-14	1	-5	0	-38	-60
		Douthat Creek	1	1	0	21	1	-1	-8	0	-5	0	-50	-37
	Anthony Creek		1	0	1	23	0	-1	-14	0	-5	0	-50	-41
	Second Creek		-1	-1	0	-21	0	-1	-14	0	-5	0	-50	-85
	Greenbrier River (lower)		0	0	-1	-10	0	-1	-14	1	-5	1	-26	-50
	Griffith Creek		0	-1	0	-8	0	-1	-14	-1	-5	0	-62	-84
Middle New	New River		0	-1	0	-8	1	-1	-8	1	-5	0	-38	-54
	Spruce Run		-1	-1	0	-21	1	-1	-8	-1	-5	0	-62	-91
	Sinking Creek		-1	-1	0	-21	1	-1	-8	-1	-5	0	-62	-91
	Walker Creek	Walker Creek	0	-1	0	-8	1	-1	-8	1	-5	0	-38	-54
		Dismal Creek	0	1	1	18	1	-1	-8	-1	-1	-1	-34	-24
	Stony Creek		1	1	1	31	-1	-1	-20	1	-1	-1	-10	1
	Wolf Creek	Laurel Creek	0	-1	0	-8	1	-1	-8	-1	0	-1	-24	-40
	Indian Creek	Indian Creek	-1	-1	0	-21	0	-1	-14	1	-5	0	-38	-73
		Turkey Creek	0	-1	0	-8	0	-1	-14	-1	-5	0	-12	-34
						14			-12				-23	-21
Bluestone	Camp Creek		0	0	0	0	0	1	14	-1	-5	-1	-74	-60
	Bluestone River		0	0	0	0	1	1	20	1	-5	-1	-50	-30
	Little Bluestone River		0	-1	0	-8	0	1	14	-1	-5	-1	-74	-68
Upper New	Cripple Creek		-1	-1	0	-21	1	1	20	0	0	-1	-12	-13
	Pine Run		-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
	Reed Creek	Reed Creek	-1	-1	0	-21	1	1	20	1	-5	-1	-50	-51
		South Fork Reed Creek	-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
						-21			20				-12	-13
		Data Quality	5	5	4	>15	4	4	>10	2	1	4	>15	>40
		Confidence	4	2	3	0 - 15	1	5	0 - 10	5	4	4	0 - 15	0 - 40
		Significance	4	1	3	<0	1	5	<0	5	5	4	<0	<0
		Weighting Factor (sum)	13	8	10		6	14		12	10	12		

Table 10. Scenario 4 (25-year positive habitat change coupled with 25-year variegate darter expansion). Cross hatching indicates extirpated populations. Red text indicates populations extirpated as a result of variegate darter hybridization.

			Physical Habitat Metrics				Non-native Competition			Population Demographic Metrics				
			Watershed	Landcover	Water Quali	Total	Brown	Variegat	Total		Abundan	Connecti	Total	Population
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Forest Rank	Land Rank	Water Rank	Habitat Score	Trout Rank	e Darter Rank	Comp Score	Space Rank	ce Rank	vity Rank	Demo Score	Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River	1	0	1	23	-1	-1	-20	1	-5	1	-26	-23
		Straight Creek	1	-1	1	15	-1	-1	-20	-1	-5	1	-50	-55
	Gauley River (upper)		1	0	1	23	-1	-1	-20	1	-5	1	-26	-23
	Panther Creek		1	-1	1	15	1	-1	-8	-1	-5	1	-50	-43
	Williams River	Williams River	1	1	1	31	-1	-1	-20	1	-5	1	-26	-15
		Tea Creek	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Cranberry River		1	1	1	31	1	-1	-8	0	-5	1	-38	-15
	Cherry River	Cherry River	1	0	1	23	0	-1	-14	1	-5	1	-26	-17
		North Fork Cherry River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
		South Fork Cherry River	1	-1	1	15	1	-1	-8	0	-5	1	-38	-31
		Laurel Creek	1	-1	1	15	-1	-1	-20	0	-5	1	-38	-43
		Little Laurel Creek	1	-1	1	15	1	-1	-8	-1	-5	1	-50	-43
Lower Gauley	Gauley River (lower)		0	0	1	10	0	-1	-14	1	-5	-1	-50	-54
	Peters Creek		1	-1	1	15	0	-1	-14	0	-5	-1	-62	-61
	Collison Creek		0	-1	1	2	0	-1	-14	-1	-5	0	-62	-74
Lower New	New River		0	0	1	10	0	-1	-14	1	-5	1	-26	-30
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River	1	0	1	23	-1	-1	-20	1	-5	1	-26	-23
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (West Fork)	West Fork Greenbrier River	1	1	1	31	0	-1	-14	1	-5	1	-26	-9
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (upper)		1	0	1	23	0	-1	-14	1	-5	1	-26	-17
	Leatherbark Run		1	1	1	31	0	-1	-14	-1	-5	0	-62	-45
	Deer Creek	Deer Creek	0	-1	1	2	0	-1	-14	1	-5	1	-26	-38
		North Fork	1	-1	1	15	0	-1	-14	0	-5	1	-38	-37
	Stony Creek		0	-1	1	2	0	-1	-14	-1	-5	0	-62	-74
	Sittington Creek		1	-1	1	15	1	-1	-8	0	-5	1	-38	-31
	Knapp Creek	Knapp Creek	1	-1	1	15	0	-1	-14	1	-5	0	-38	-37
		Douthat Creek	1	1	1	31	1	-1	-8	0	-5	0	-50	-27
	Anthony Creek		1	0	1	23	0	-1	-14	0	-5	0	-50	-41
	Second Creek		0	-1	1	2	0	-1	-14	0	-5	0	-50	-62
	Greenbrier River (lower)		1	0	0	13	0	-1	-14	1	-5	1	-26	-27
	Griffith Creek		1	-1	1	15	0	-1	-14	-1	-5	0	-62	-61
Middle New	New River		0	-1	1	2	1	-1	-8	1	-5	0	-38	-44
	Spruce Run		-1	-1	1	-11	1	-1	-8	-1	-5	0	-62	-81
	Sinking Creek		0	-1	1	2	1	-1	-8	-1	-5	0	-62	-68
	Walker Creek	Walker Creek	0	-1	1	2	1	-1	-8	1	-5	0	-38	-44
		Dismal Creek	1	1	1	31	1	-1	-8	-1	0	0	-12	11
	Stony Creek		1	1	1	31	-1	-1	-20	1	1	0	22	33
	Wolf Creek	Laurel Creek	1	-1	1	15	1	-1	-8	-1	1	0	-2	5
	Indian Creek	Indian Creek	0	-1	1	2	0	-1	-14	1	-5	0	-38	-50
		Turkey Creek	0	-1	1	2	0	-1	-14	-1	-5	0	-12	-24
						26			-12				3	16
Bluestone	Camp Creek		1	0	1	23	0	1	14	-1	-5	1	-74	-37
	Bluestone River		0	0	1	10	1	1	20	1	-5	-1	-50	-20
	Little Bluestone River		0	-1	1	2	0	1	14	-1	-5	-1	-74	-58
Upper New	Cripple Creek		-1	-1	1	-11	1	1	20	0	1	-1	-2	7
	Pine Run		-1	-1	1	-11	1	1	20	-1	-5	1	-74	-65
	Reed Creek	Reed Creek	-1	-1	1	-11	1	1	20	1	-5	-1	-50	-41
		South Fork Reed Creek	-1	-1	1	-11	1	1	20	-1	-5	-1	-74	-65
						-11			20				-2	7
		Data Quality	5	5	4	>15	4	4	>10	2	1	4	>15	>40
		Confidence	4	2	3	0 - 15	1	5	0 - 10	5	4	4	0 - 15	0 - 40
		Significance	4	1	3	<0	1	5	<0	5	5	4	<0	<0
		Weighting Factor (sum)	13	8	10		6	14		12	10	12		

Table 11. Scenario 5 (25-year negative habitat change coupled with 25-year variegate darter expansion). Cross hatching indicates extirpated populations. Red text indicates populations extirpated as a result of variegate darter hybridization.

			Physical Habitat Metrics				Non-native Competition			Population Demographic Metrics				
			Watershed	Landcover	Water Quali	Total	Brown	Variegat	Total		Abundan	Connecti	Total	Population
Watershed (Metapopulation)	Stream System (Population)	Stream (Subpopulation)	Forest Rank	Land Rank	Water Rank	Habitat Score	Trout Rank	e Darter Rank	Comp Score	Space Rank	ce Rank	vity Rank	Demo Score	Condition Score
Upper Gauley	Gauley River (headwaters)	Gauley River	0	0	0	0	-1	-1	-20	1	-5	0	-38	-58
		Straight Creek	0	-1	-1	-18	-1	-1	-20	-1	-5	0	-62	-100
	Gauley River (upper)		0	0	0	0	-1	-1	-20	1	-5	0	-38	-58
	Panther Creek		0	-1	-1	-18	1	-1	-8	-1	-5	0	-62	-88
	Williams River	Williams River	1	1	0	21	-1	-1	-20	1	-5	1	-26	-25
		Tea Creek	1	1	0	21	-1	-1	-20	-1	-5	1	-50	-49
	Cranberry River		1	1	0	21	1	-1	-8	0	-5	1	-38	-25
	Cherry River	Cherry River	0	0	-1	-10	0	-1	-14	1	-5	0	-38	-62
		North Fork Cherry River	1	1	0	21	-1	-1	-20	-1	-5	1	-50	-49
		South Fork Cherry River	0	-1	0	-8	1	-1	-8	0	-5	0	-50	-66
		Laurel Creek	0	-1	0	-8	-1	-1	-20	0	-5	0	-50	-78
		Little Laurel Creek	0	-1	0	-8	1	-1	-8	-1	-5	0	-62	-78
Lower Gauley	Gauley River (lower)		-1	0	-1	-23	0	-1	-14	1	-5	-1	-50	-87
	Peters Creek		0	-1	-1	-18	0	-1	-14	0	-5	-1	-62	-94
	Collison Creek		-1	-1	0	-21	0	-1	-14	-1	-5	0	-62	-97
Lower New	New River		-1	0	0	-13	0	-1	-14	1	-5	1	-26	-53
Greenbrier	Greenbrier River (East Fork)	East Fork Greenbrier River	0	0	0	0	-1	-1	-20	1	-5	0	-38	-58
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (West Fork)	West Fork Greenbrier River	1	1	1	31	0	-1	-14	1	-5	1	-26	-9
		Little River	1	1	1	31	-1	-1	-20	-1	-5	1	-50	-39
	Greenbrier River (upper)		0	0	-1	-10	0	-1	-14	1	-5	0	-38	-62
	Leatherbark Run		0	1	-1	-2	0	-1	-14	-1	-5	0	-62	-78
	Deer Creek	Deer Creek	-1	-1	-1	-31	0	-1	-14	1	-5	0	-38	-83
		North Fork	0	-1	-1	-18	0	-1	-14	0	-5	0	-50	-82
	Stony Creek		-1	-1	-1	-31	0	-1	-14	-1	-5	0	-62	-107
	Sittington Creek		0	-1	0	-8	1	-1	-8	0	-5	0	-50	-66
	Knapp Creek	Knapp Creek	0	-1	-1	-18	0	-1	-14	1	-5	0	-38	-70
		Douthat Creek	1	1	0	21	1	-1	-8	0	-5	0	-50	-37
	Anthony Creek		0	0	0	0	0	-1	-14	0	-5	0	-50	-64
	Second Creek		-1	-1	-1	-31	0	-1	-14	0	-5	0	-50	-95
	Greenbrier River (lower)		-1	0	-1	-23	0	-1	-14	1	-5	0	-38	-75
	Griffith Creek		-1	-1	-1	-31	0	-1	-14	-1	-5	0	-62	-107
Middle New	New River		-1	-1	-1	-31	1	-1	-8	1	-5	0	-38	-77
	Spruce Run		-1	-1	-1	-31	1	-1	-8	-1	-5	0	-62	-101
	Sinking Creek		-1	-1	-1	-31	1	-1	-8	-1	-5	0	-62	-101
	Walker Creek	Walker Creek	-1	-1	-1	-31	1	-1	-8	1	-5	0	-38	-77
		Dismal Creek	0	1	1	18	1	-1	-8	-1	-1	-1	-34	-24
	Stony Creek		1	1	1	31	-1	-1	-20	1	-1	-1	-10	1
	Wolf Creek	Laurel Creek	0	-1	-1	-18	1	-1	-8	-1	0	0	-12	-38
	Indian Creek	Indian Creek	-1	-1	-1	-31	0	-1	-14	1	-5	-1	-50	-95
		Turkey Creek	-1	-1	-1	-31	0	-1	-14	-1	-5	-1	-24	-69
Bluestone	Camp Creek		-1	0	0	-13	0	1	14	-1	-5	-1	-74	-73
	Bluestone River		-1	0	0	-13	1	1	20	1	-5	-1	-50	-43
	Little Bluestone River		-1	-1	0	-21	0	1	14	-1	-5	-1	-74	-81
Upper New	Cripple Creek		-1	-1	0	-21	1	1	20	0	-1	-1	-22	-23
	Pine Run		-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
	Reed Creek	Reed Creek	-1	-1	0	-21	1	1	20	1	-5	-1	-50	-51
		South Fork Reed Creek	-1	-1	0	-21	1	1	20	-1	-5	-1	-74	-75
						-21			20				-22	-23
		Data Quality	5	5	4	>15	4	4	>10	2	1	4	>15	>40
		Confidence	4	2	3	0 - 15	1	5	0 - 10	5	4	4	0 - 15	0 - 40
		Significance	4	1	3	<0	1	5	<0	5	5	4	<0	<0
		Weighting Factor (sum)	13	8	10		6	14		12	10	12		