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“Mining” for a Reference Condition in Southern West Virginia Streams

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
at Virginia Commonwealth University

by
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B.A. Virginia Polytechnic Institute and State University, 2007

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Preface

This thesis was organized in a format suitable for publication in the peer-reviewed journal North American Journal of Fisheries Management with minor modifications to comply with Virginia Commonwealth University guidelines for thesis submission. Chapter 2 is the manuscript prepared for submission, while chapters 1, 3, and 4 were prepared independently of journal format and will not be submitted for publication.

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Abstract

“MINING” FOR A REFERENCE CONDITION IN SOUTHERN WEST VIRGINIA STREAMS

By: Matthew G. Rouch, M.S. Environmental Science

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

Virginia Commonwealth University, 2014

Thesis Director: Dr. Daniel McGarvey, Center for Environmental Studies

Quarterly samples were used to estimate assemblage-level (all species combined) fish production within three minimally-impacted, southern West Virginia streams. The total annual fish production estimate was highest in Slaunch Fork ($37.52 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$), a tributary of the Tug Fork River, and lowest in Cabin Creek ($10.59 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$), a Guyandotte River tributary. Creek Chub *Semotilus atromaculatus*, Mottled Sculpin *Cottus bairdii* and Blacknose Dace *Rhinichthys atratulus* were the most abundant species among sites, accounting for >90% of all sampled individuals. Reference condition criteria were also selected and metrics calculated for each of the three stream sites using a variety of established metrics. According to established criteria, all three of our sites scored high enough to be listed as “reference” sites. Third, a comprehensive GIS analysis was conducted in order to determine land use patterns and predict where similar assemblages would be present using various climatological and physical characteristics of our stream sites. These analyses revealed rapid expansion of surface mining activities putting many stream systems at risk.

CHAPTER 1. OVERVIEW AND NATURE OF STUDY

Zoogeography and History

The southern Appalachians escaped glaciation during the Pleistocene and are among the oldest mountainous ecosystems on earth (Hocutt et al. 1978). As a result, they support some of the highest levels of biodiversity on the planet. This region supports over 2,000 species of plants, several endemic salamander species, diverse and abundant invertebrate populations, and fish species, such as brook trout, that are only found in headwater streams (Ross and Matthews 2014).

Zoogeography is the study of the present, and past, distributions of animal species on the planet. Alfred Russell Wallace originally defined seven zoogeographic regions or realms based on the flora and fauna found there

(Matthews 1998). North America falls in the Nearctic region which contains an estimated 1,061 fish species (Ross and Matthews 2014). The major divide in fish assemblages in North America occurs along the continental divide. According to Figure 1 by Ross and Matthews (2014), there is a clear distinction between diversity of fishes in the eastern U.S. as compared to the west. Diversity is also higher in the southeastern part of the U.S., especially in the Appalachian region.

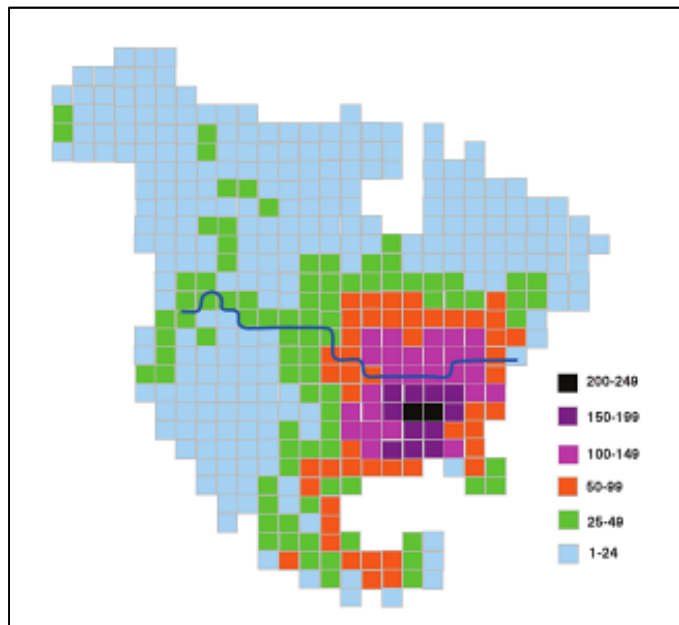


Figure 1. Biodiversity of fish fauna in North America

In West Virginia, the unique geologic history of this region has played a significant role in the formation of fish assemblages in the northern and southern parts of the state. The glacial period of the Pleistocene helped to shape the current major drainages of West Virginia (Hocutt et al. 1978). Before the Pleistocene, during the early Pliocene, the two major rivers flowing through the state were the Pittsburgh River in the north and the Teays River in the south (Stauffer et al. 1995). It is believed the Pittsburgh River flowed north into the Great Lakes and was an important dispersal route for fish moving south into West Virginia (Stauffer et al. 1995). The ancient Teays River (Figure 2, West Virginia Encyclopedia) system flowed northwest from North Carolina to Indiana eventually leading into the Mississippi River and was the primary route of dispersal of fishes east towards the Atlantic Slope (Hocutt et al. 1978). During glaciation, glaciers carved the

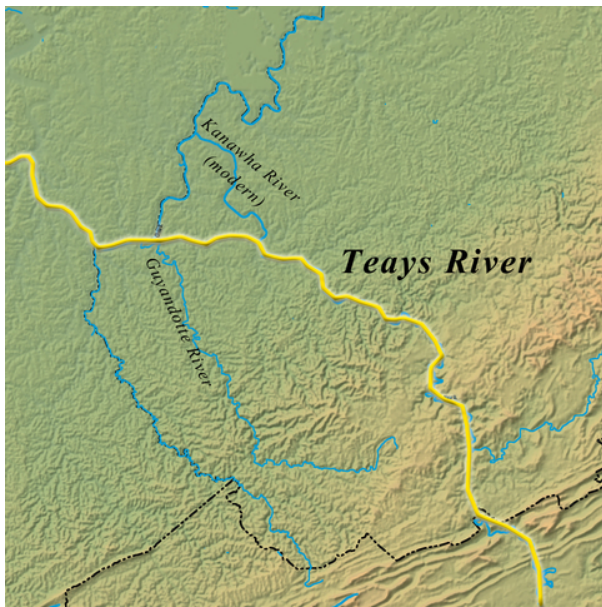


Figure 2. The ancient Teays River System

(Hocutt et al. 1978).

landscape in the northern U.S. directly altering the Pittsburgh River drainage. The part of the Teays River in West Virginia was affected indirectly by the glaciers in Illinois and Indiana which cut-off the Teays from its connection to the Mississippi (Stauffer et al. 1995). The Teays eventually found its way into the Ohio River and today the New-Kanawha River systems are considered to be remnants of the Teays River

These two ancient systems brought a plethora of fish fauna to West Virginia streams. Some of the most abundant families of fishes in West Virginia are Cyprinidae (minnows),

Catostomidae (suckers), Ictaluridae (catfishes), Centrarchidae (sunfishes), and Percidae (perches and darters) (Stauffer et al. 1995). (Matthews 1998).

Drainage basins and the connectivity of stream networks within those basins are the primary factors determining the diversity of freshwater fish assemblages (Matthews 1998). In West Virginia, one major distinction in fish assemblages occurs in the Potomac and James River Basins. These basins drain towards the Atlantic Slope and contain different fish assemblages than those found in the rest of the state. The remaining drainages are located within the Greater Ohio River Basin which drains >75% of the total land area of the state (Stauffer et al. 1995). These drainages contain fish assemblages that are closely related given their similar geologic history. The Upper Kanawha/New River system has a unique and diverse fauna with several endemic species. The New River drainage is considered to have four endemic fish species, the Kanawha darter (*Etheostoma kanawhae*), bigmouth chub (*Nocomis platyrhynchus*), New River shiner (*Notropis scabriceps*), and Kanawha minnow (*Phenacobius teretulus*). The candy darter (*Etheostoma obsburni*) and diamond darter (*Crystallaria cincotta*) are considered to be endemic to the Kanawha River system.

Anthropogenic Disturbances

The southern Coalfield region of West Virginia is undervalued and understudied with regards to freshwater fish ecology largely due to its long history of logging and surface mining. These anthropogenic disturbances have had profound effects on the biotic integrity of the region's streams, in addition to terrestrial ecosystems. It is well-documented that surface mining activities and subsequent burning of fossil fuels contribute to greenhouse gas emissions and climate change (Fox and Campbell 2010, Whitaker et al. 2012). Additionally, surface mining

operations often decrease the amount of available stream habitat through disturbances such as valley-fills and acid mine drainage (Bernhardt and Palmer 2011).

West Virginia is a juxtaposition of a wealth of natural resources and a long history of anthropogenic disturbances to those resources. It began with the Winchester and Potomac Railroad first coming through the state in 1836, this allowed West Virginias’ logging industry to further expand operations (Johnston II 1961). Coal, while discovered in the region in 1742, did not become a booming industry until the 1890’s when railroads connected coalfields across the state (Clarke 2003). In the 1960’s coal companies began surface mining operations, including mountaintop mining, which were harmful in two major ways. First, this method of mining is highly profitable in the sense that coal companies found a way to increase the amount of coal they produce while decreasing the number of jobs (Figure 3; Appalachian Voices 2013).

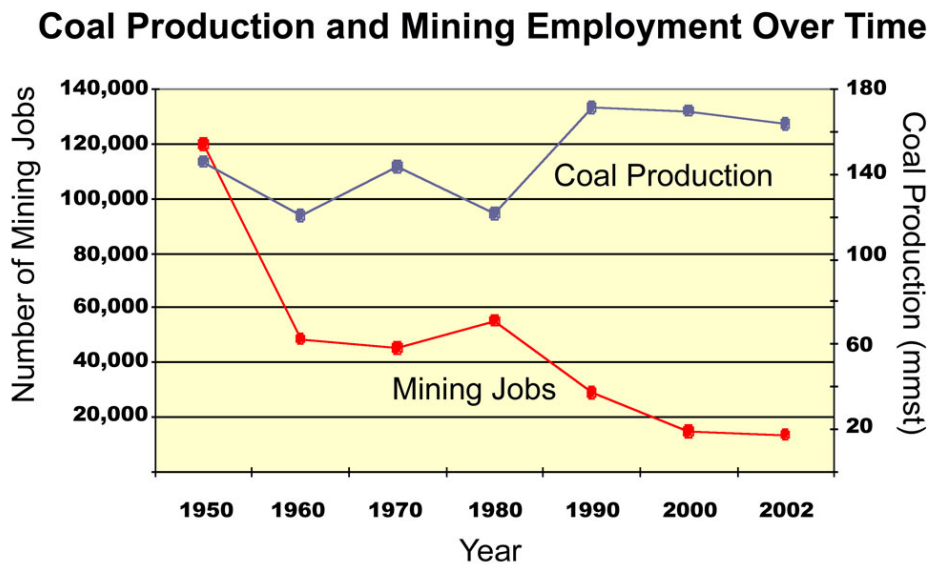


Figure 3. Coal production compared to mining employment (Appalachian Voices 2013)

Second, this method has had devastating consequences for the environment and public support is at-best wavering. This method of mining uses over 2,500 tons of explosives daily, which is the equivalent of a Hiroshima-strength atomic bomb being dropped in this region every week (Appalachian Voices 2013). The impacts of this method have been largely felt by aquatic ecosystems through the pollution of streams and the toxification of watersheds. This has not only lead to significant risks for human health concerns, but also for the aquatic organisms living in these streams and rivers. Surface mining lowers pH and elevates the concentration of heavy metals such as aluminum and iron, in some cases preventing an aquatic life from surviving (Cravotta 2010). A dramatic example of the consequences of this method of mining can be seen in the picture below of Kayford Mountain, West Virginia.



Kayford Mountain, WV, photo by Vivian Stockman, Oct. 2003

An Understudied Region

Fish assemblage studies have been conducted in the eastern portion of West Virginia in the upper Kanawha River drainage (Chipps et al. 1994). Darter communities have been studied in the western portion of the state in the Elk River drainage (Welsh and Perry 1998). In the northern region of the state the focus of most studies has been on brook trout populations (Carline and McCullough 2003, Freund and Petty 2007, Hakala and Hartman 2004).

Conversely, relatively little is known about stream fish assemblages in southern West Virginia. Quantitative records on natural fish assemblage characteristics, including composition, population sizes, biomass and production rates are rare or nonexistent for most southern streams. One of the only comprehensive studies of the fish assemblages in this region was conducted by renowned naturalist Edward D. Cope who surveyed two tributaries of the Kanawha/New River in September of 1867. Staying in a cabin near Walker's Creek and Strouble's Creek, Cope spent weeks surveying these streams with a fine mesh seine (Cope 1869). With each draw of the net he recorded catching 100-200 individuals and noted which species were highly abundant, abundant/common, or rare. This was the last major survey of streams in this general region before it was subjected to large-scale anthropogenic disturbances.

Biomonitoring

From a biomonitoring and conservation perspective, the need for knowledge on the types of streams fishes present in the southern region of the state in *minimally-impacted* streams is of vital importance. These baseline data or reference conditions can then be compared to impacted streams in the region to quantify the level of impairment using a multi-metric index or IBI (Karr 1981, Barbour et al. 1999). Reference conditions are rare and difficult to find (Davis and Simon

1995), particularly in an area with such large-scale anthropogenic disturbances like southern West Virginia. Additionally, the window of opportunity to collect these data is shrinking due to the rapid expansion of surface mining activities in this region which continue to threaten aquatic and terrestrial ecosystems.

However, one major challenge to this process is that most bioassessment protocols rely on fish assemblages to determine the level of impairment in a stream. This becomes an issue in small headwater streams, including streams in southern West Virginia, where biodiversity is typically lower, usually only supporting a handful of species (Davis and Simon 1995).

Fish Production

Fish production, particularly of small stream fishes, is a comprehensive indicator of overall stream health (Dolbeth 2005). Production is defined here as the total quantity of fish tissue generated over time (Hayes et al. 2007). Fish production provides a rate or flow that enables effective monitoring and management through time making it one of the best indicators of population health (Randall and Minns 2000). Because secondary production represents an aggregate of energy flow through multiple trophic levels, it is also one of the most comprehensive indicators of overall stream health (Dolbeth 2005). Production estimates based on seasonal data can also help describe fluxes in biomass at different times of the year and provide clues about factors limiting production (Gerking 1978). Therefore, data on annual fish production could be a very useful tool in assessing stream health and establishing reference conditions.

Fish production studies have been largely concentrated on sport fishes, such as brook trout (*Salvelinus fontinalis*) and smallmouth bass (*Micropterus dolomieu*; Goodnight and Bjorn

1971, Neves 1981, Waters 1982, Roell and Orth 1993, Eggleton and Morgan 2000). While the role of lower trophic-level stream fishes has been overlooked, these fishes can constitute a significant portion of the biomass in streams (Mann 1971, Small 1975, Mahon et al. 1979, Neves and Pardue 1983, Chipps et al. 1994).

Objectives

The primary objective of this study was to estimate annual production for stream fishes in three sites in southern West Virginia. This study consisted of four seasonal sampling events starting in July of 2013 and ending in May of 2014. Study sites were chosen to represent minimally-impacted conditions in a region known for large-scale anthropogenic disturbances, such as mountaintop mining. Once production was known, our next objective was to compare our results with other studies, either in a similar location or with similar species and stream characteristics. Secondary objectives included performing an ad-hoc biological assessment of these streams using established criteria, as well as a comprehensive GIS analysis to determine land use patterns and predict where similar assemblages could occur given certain stream characteristics. This study will begin to fill a critical knowledge-gap for fish biology in southern West Virginia streams.

CHAPTER 2. MANUSCRIPT SUBMISSION

Assemblage-Level Fish Production in Three Minimally-Impacted, Southern West Virginia Streams

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Abstract – Quarterly samples were used to estimate assemblage-level (all species combined) fish production within three minimally-impacted, southern West Virginia streams. Zippin multiple-pass depletion surveys were first conducted to estimate fish population sizes. Length-frequency data were then used to identify species' cohorts and annual production was estimated with the increment summation method. The total annual fish production estimate was highest in Slaunch Fork ($37.52 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$), a tributary of the Tug Fork River, and lowest in Cabin Creek ($10.59 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$), a Guyandotte River tributary. Creek Chub *Semotilus atromaculatus*, Mottled Sculpin *Cottus bairdii* and Blacknose Dace *Rhinichthys atratulus* were the most abundant species among sites, accounting for >90% of all sampled individuals. Notably, our fish assemblage production estimates were comparable to assemblage-level production rates from other, more intensively studied eastern U.S. streams (e.g., Coweeta Creek, NC and Steeles Run, KY). We therefore suggest that streams within this understudied and imperiled region may warrant further study and protection.

Introduction

Annual production studies have been conducted for many fishes of management concern, particularly for sport fishes (e.g., Goodnight and Bjorn 1971; Waters 1982; Eggleton and Morgan 2000). However, production studies that include multiple species or non-game fishes are comparatively rare, with notable examples from rivers in eastern North American including Lotrich (1973), Small (1975), Mahon et al. (1979), Neves and Pardue (1983), Freeman et al. (1988), and Storck and Momot (1989). This lack of empirical information on assemblage-level production is unfortunate for at least two reasons. First, it is difficult to understand aquatic systems at the ecosystem level when production rates are known for only one or several of the constituent consumer groups. Odum's (1957) classic study of Silver Springs, Florida illustrated this point; without data on primary, secondary, and tertiary producers, the energy budget of Silver Springs would be incomplete.

Second, assemblage-level production may be more responsive to anthropogenic disturbance than production of one or several sport fishes. For instance, Penczak et al. (1984) found that assemblage-level fish production in the Speed River, Ontario changed significantly following the construction of Guelph dam. But sport fishes (Smallmouth Bass *Micropterus dolomieu* and Rock Bass *Ambloplites rupestris*) accounted for < 25% of the total annual fish production in the Speed River (see also Mahon et al. 1979). Thus, sport fish production may not be a sensitive indicator of overall fish assemblage health.

In this study, we estimated assemblage-level fish production in three southern West Virginia streams. To our knowledge, this is the first such study in southern West Virginia. We focused on streams in the southern part of the state because relatively little work has been performed in them (but see Austen and Orth 1988; Roell and Orth 1993); streams in the northern

half of the state have been studied more intensively (e.g., Carline and McCullough 2003; Hakala and Hartman 2004; Martin and Petty 2009; Utz and Hartman 2009; Petty et al. 2012). We also constrained our study to minimally-impacted streams, with the intent of estimating natural or historical rates of fish assemblage production. This was important because many of the streams in southern West Virginia have been degraded by anthropogenic disturbances such as industrial logging and mountaintop removal surface mining (Stewart Burns 2007; Bernhardt and Palmer 2011), and the effects of these disturbances on assemblage-level fish production are largely unknown.

Specific objectives for our study were to: (1) estimate seasonal fish population densities in each study stream; (2) estimate seasonal biomass densities at each site; (3) use the seasonal biomass data to estimate annual production for each species; and (4) compare summed, assemblage-level production estimates with multi-species or assemblage-level production results from other eastern U.S. streams. In future work, we hope to expand our sampling network in southern West Virginia to include more heavily impacted streams and to build a comparative framework for assessing the impacts of different types of anthropogenic disturbance on assemblage-level fish production.

Study sites

We surveyed a single headwater stream in each of the three major river basins in southern West Virginia: the Tug Fork River basin, the Guyandotte River basin, and the Bluestone River (tributary to the New River) basin (Figure 4). Because a core objective of our study was to characterize fish production within minimally-impacted streams, we used the Critical Forest Map of Maxwell et al. (2012) to screen potential sampling sites. The Critical Forest Map is a digital

(raster) representation of ecosystem health throughout the Southern Coal Fields region of West Virginia. Similar to a multimetric index of biotic integrity (e.g., Karr 1981; Fausch et al. 1984), the Critical Forest Map uses multiple indicators of landscape structure and ‘health’, including land use/cover type, geomorphology, and degree of forest fragmentation, to calculate an integrated, categorical index of ecosystem integrity. Specifically, forest plots (i.e., grid cells) were ranked on an ordinal scale ranging from 0–3, with 3 being the least-disturbed forest habitat. By overlaying the Critical Forest Map on the 1:100,000 scale NHDPlus (Version 2) digital stream network (McKay et al. 2014) within a Geographic Information System (ArcMap 10.2), we were able to identify stream catchments that were mostly populated by plots with Critical Forest scores of 2 or 3. Final sites were then selected from this subset, with the additional requirement that each site was located on public land to ensure access.

The selected site in the Tug Fork River basin was Slaunch Fork, a 4th order stream (tributary to Panther Creek) located in the Panther Wildlife Management Area (McDowell County; 37.395° latitude, -81.890° longitude; Figure 4). Slaunch Fork was characterized by alternating riffles, runs and pools, with the lowest average channel gradient (2.4%) of the three study sites. Substrate consisted primarily of large, flat cobbles with sand and silt in alternating pools. Specific conductivity ranged (among sampling events) from 77.6–304.6 $\mu\text{S/s}$, dissolved oxygen (DO) ranged from 8.61–11.6 mg/L, and mean channel width and depth were 7.0 m and 0.33 m, respectively.

Cabin Creek was selected as our Guyandotte River site. It is a 3rd order stream located at the southern margin of Twin Falls State Park (Wyoming County; 37.616° latitude, -81.453° longitude; Figure 4). Cabin Creek had the steepest average channel gradient (6.0%) of the three sites and was characterized by a series of pools, riffles and runs flowing through a deeply incised

gorge. Specific conductivity ranged from 77.9–117.3 $\mu\text{S/s}$, DO ranged from 6.72–11.83 mg/L, and mean channel width and depth were 4.4 m and 0.35 m, respectively. Substrate consisted mostly of large boulders with small amounts of gravel distributed in riffles and the tails of pools.

Camp Creek was selected as our Bluestone River site. It is a 4th order stream in Camp Creek State Park (Mercer County; 37.502° latitude, -81.144° longitude, Figure 4). The surveyed reach consisted of a deep pool and alternating riffles and runs, with an average channel gradient of 3.4%. Specific conductivity ranged from 84.5–169.5 $\mu\text{S/s}$, DO ranged from 7.64–11.6 mg/L, and mean channel width and depth were 6.5 m and 0.35 m, respectively. Substrate was a mix of sand and silt in pools and large, flat cobbles in riffles.

Methods

Fish surveys

Fishes were collected with a Halltech HT-2000 backpack electrofisher. Block nets (6.35 mm mesh seine) were secured at the upstream and downstream end of each survey reach to ensure that fishes were sampled from closed populations. The length of each survey reach was approximately 20× the mean channel width; logistical constraints (i.e., daylight hours and among-site travel times) prevented us from surveying longer reaches. Three successive electrofishing passes were made during each sampling event, removing stunned fishes with dip nets, and all collected fishes were maintained in live-wells. After each sampling pass, all fishes were identified (species-level), weighed (g wet-weight), measured (mm total length), then released downstream of the sample reach.

Next, cohorts were identified using modal-progression analysis (MPA) in the program FiSAT II (Gayanilo et al. 2005). MPA uses among-sampling interval shifts in the relative

positions of peaks (i.e., modal body sizes) in length-frequency histograms to identify and track cohorts (Weatherly et al. 1987). MPA analyses were performed using Hasselblad's NORMSEP routine (Hasselblad 1966). The NORMSEP routine uses a maximum-likelihood algorithm to separate component distributions in length-frequency data and assumes that length is normally distributed. NORMSEP requires an initial estimate for the expected mean of each cohort as an input (i.e., the 'seed'). A best-guess was made manually for each cohort by examining where the clusters of "peaks" occurred in the length-frequency data. Once seeded, NORMSEP calculates the mean and variance of each group that are most probable given the data (Gayaniilo et al. 1997). An example of the NORMSEP routine is shown in Figure 5 for Creek Chub at the Slauch Fork site.

Population abundances were estimated for each cohort using the Zippin multiple-depletion method (Zippin 1958), which is effective in small streams where population sizes are relatively small (e.g., < 2,000 individuals) and successive samples are separated by short time intervals (e.g., hourly intervals; Lockwood and Schneider 2000). Specifically, we used a maximum-likelihood procedure to estimate population size (N_c) for each cohort of each fish species (Carle and Strub 1978). The procedure began by calculating an intermediate statistic X as:

$$X = \sum_{i=1}^k (k - i)C_i , \quad (1)$$

where i is the i^{th} electrofishing pass ($i = 1, 2, \text{ or } 3$), k is the total number of passes conducted during a sampling event ($k = 3$), and C_i is the total number of fish caught (of a cohort of a given

species) in the i^{th} pass. The maximum-likelihood estimate of N_c , denoted as n (i.e., $N_c \approx n$), was then calculated through iteration by substituting potential values for n until:

$$\left[\frac{n+1}{n-T+1} \right] \prod_{i=1}^k \left[\frac{kn-X-T+1+(k-i)}{kn-X+2+(k-i)} \right]_i \leq 1.0, \quad (2)$$

where n is the smallest integer that satisfies equation (2), T is the total number of individuals (of a cohort of a given species) caught in all three passes, and all other variables are as defined above for equation (1).

Species' N_c estimates were divided by the surface area of sampled stream habitat at each study site (survey reach length \times mean channel width) to obtain initial population density estimates, then normalized to per-hectare densities (D_c). Total observed abundances (summed counts among three passes) were used as our N estimates for rare species, when cohorts could not be identified and equations (1) and (2) could not be solved. For each sampling interval, wet-weights of sampled individuals were averaged by cohort for each species to obtain mean individual, cohort-level weight-wets (w_c). The D_c estimates were then multiplied by the mean wet-weights for each cohort to obtain population biomass estimates (B_c , in g/ha).

Annual production

When species could be partitioned into cohorts, we used the cohort-based, increment summation method to calculate annual production estimates for each species (Waters 1977; Dolbeth et al. 2005). Increment summation is most often used to estimate secondary production of benthic macroinvertebrates (Benke and Huryn 2011), but it has also been adapted for use with fish and other aquatic organisms (Hayes et al. 2007; Cob et al. 2009). Growth increments

between sampling intervals were calculated for each cohort and summed to estimate annual production (P , in $\text{kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$ wet-weight) following Winberg (1971):

$$P = \sum_{t=0}^{t=n} \left(\frac{N_i + N_{t+i}}{2} \right) \times (\bar{w}_{t+i} - \bar{w}_t), \quad (3)$$

where t is the sampling date or interval, n is the total number of intervals ($n=4$), and N and \bar{w} are as defined above. The start interval ($t = 0$) for each species was determined using life history descriptions (i.e., timing of the primary spawning season) in Jenkins and Burkhead (1994) and Stauffer et al. (1995); start intervals were not arbitrarily associated with our earliest chronological sampling event. In this way, we were able to track growth increments among species with differing life histories, using a common set of four seasonal samples. Production rates for intervals with negative growth or observed weight losses were assumed to be zero.

For species that could not be partitioned into cohorts, we used a non-cohort-based method, Simple Increment Summation (Dolbeth 2005), to estimate production. This method simply sums the increases in biomass after each sampling period. This method assumes all data represent a single cohort and therefore introduces some error. Although this method is less accurate than Increment Summation, it is still useful for showing the contribution of additional species to biomass and production.

Results and discussion

The types of fishes found at these sites were consistent with predicted findings (Stauffer et al. 1995) and consisted of smaller lower trophic-level stream fishes with a lack of large piscivorous fishes. All three sites were primarily dominated by Blacknose Dace *Rhinichthys*

atratus and Creek Chub *Semotilus atromaculatus*, with the Slaunch Fork site having a large population of Mottled Sculpin *Cottus bairdii*. The Slaunch Fork site contained the highest observed species richness. A full list of species that were sampled is contained in Appendix 1.

Densities varied greatly between sites (Table 1 and 2). Highest seasonal densities were observed in May and October across all sites. In general, the greatest increases in densities were observed between the July and October samples, and the greatest decreases in densities were observed between the October and May samples. Biomass also varied significantly between sites. Seasonally, the lowest observed biomass occurred in March and July, with the highest observed biomass generally occurring in October and May. Overall, population densities and biomass remained fairly stable throughout the year at the Cabin Creek site. The Slaunch Fork site had strong seasonal variation in density and biomass of Creek Chub, Blacknose Dace and Mottled Sculpin. At the Camp Creek site, Blacknose Dace density and biomass greatly increased throughout the year.

The highest annual fish production was observed at Slaunch Fork, $37.52 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$. Annual production at Cabin Creek and Camp Creek were estimated to be 10.59 and $11.02 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$, respectively (Table 3). Creek Chub accounted for 84% of production at Camp Creek, and 74% and 65% at Slaunch Fork and Cabin Creek, respectively.

Our estimates of annual production for Mottled Sculpin and Blacknose Dace were equal to or greater than estimates in another small headwater Appalachian stream (Neves and Pardue 1983; see Table 4). Our estimates of production bracketed estimates found at Coweeta Creek (Freeman et al. 1988) which ranged from 13.6 to $35.9 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$. Mean annual production in four 2nd and 3rd order stream sites in Kentucky ranged from 2.0 to $11.4 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$ (Small 1975) which was lower than the estimates for our sites. A study involving Creek Chub in Indian Creek,

Ohio (Storck and Momot 1989) estimated annual production to be $136 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$, which greatly exceeded estimates for Creek Chub at our sites. Our production estimates for Blacknose Dace, Creek Chub and White Sucker *Catostomus commersonii* were higher than those found in the Speed River in Ontario, Canada (Mahon et al. 1979), although our estimates for Fantail Darter *Etheostoma flabellare* were lower. While Mahon's study was not in a similar region to our sites, we believed it was an interesting comparison for two reasons: (i) it is one of best documented studies of assemblage-level production and (ii) there was a strong overlap in terms of species composition. Our production estimates bracketed Mahon's estimates both at the species-level and assemblage-level.

None of these estimates are directly comparable due to species composition, water chemistry, stream size, method of fish sampling and production estimation. However, there is a lack of data on assemblage-level fish production and more research is needed. Additionally, we acknowledge these are preliminary results and that a longer dataset is needed to account for annual variations in populations before more meaningful comparisons can be made between streams. However, given the relatively low-productivity of headwater mountain streams it is interesting to note that our estimates are equal to or larger than estimates for other regional streams at lower elevations with warmer temperatures which theoretically should be more productive (Sutcliffe and Carrick 1973, Buffagni and Comin 2000). The size-selectivity of electrofishing created sampling inefficiencies with young-of-the-year making it difficult to accurately determine density and biomass estimates. This is problematic because it has been well-documented that age-0 fishes contribute significantly to species production (Mathews 1971, Neves 1981) but will continue to be underrepresented in assemblage-level studies using electrofishing as the primary sampling method.

Additionally, we compared the fish assemblage found by Cope in two tributaries of the Kanawha/New River to the fish assemblages found in our three study sites. Using his descriptions of which species were highly abundant, common, and rare, we were able to compare his assemblages with ours (Table 5).

This study represents new natural history data in the relatively understudied region of southern West Virginia. The focus on game fishes like brook trout and smallmouth bass in the northern part of the state is well-documented. However, relatively little is known about the biology of fishes in southern West Virginia streams. These data can be used in ecosystem model validation, ecosystem productivity studies and food-web analyses. This study provides the first quantitative assessment and assemblage-level production estimates for stream fishes in a region currently undergoing rapid landscape and environmental change.

Acknowledgements

The authors wish to thank Frank Ratcliffe for providing site access at Camp Creek State Park. Additional thanks to Michael Strager for providing the Critical Forest Map and to Dr. Harold Bergman for comments and recommendations on the manuscript. Funding was provided by the Eppley Foundation for Scientific Research and a new faculty start-up grant from Virginia Commonwealth University. Supplemental funding was provided by the Society for Freshwater Science (Boesel-Sanderson Award) and the Mid-Atlantic chapter of the National Association of Environmental Professionals.

1 **Table 1. Cohort-based estimates of population density and biomass**

Species	Month	Slaunch Fork		Cabin Creek		Camp Creek	
		D _c	B _c	D _c	B _c	D _c	B _c
Blacknose Dace (<i>Rhinichthys atratulus</i>)	Jul	1378	15.6	--	--	5092	21.8
	Oct	3806	12.3	--	--	8277	27.9
	Mar	2398	25.3	--	--	1585	5.8
	May	4133	44.1	--	--	2569	9.8
Central Stoneroller (<i>Campostoma anomalum</i>)	Jul	102	2.4	--	--	--	--
	Oct	276	8.3	--	--	--	--
	Mar	367	14.6	--	--	--	--
	May	1398	86.4	--	--	--	--
Common White Sucker (<i>Catostomus commersonii</i>)	Jul	--	--	91	2.3	--	--
	Oct	--	--	205	5.9	--	--
	Mar	--	--	45	1.2	--	--
	May	--	--	136	8.3	--	--
Creek Chub (<i>Semotilus atromaculatus</i>)	Jul	1276	36.0	364	17.0	2169	213.1
	Oct	6510	1118.6	477	32.0	2400	229.4
	Mar	4041	673.6	932	19.1	585	38.2
	May	1969	289.0	318	12.7	1277	90.1
Fantail Darter (<i>Etheostoma flabellare</i>)	Jul	--	--	--	--	1262	5.3
	Oct	--	--	--	--	1492	3.8
	Mar	--	--	--	--	77	0.2
	May	--	--	--	--	508	1.9
Mottled Sculpin (<i>Cottus bairdii</i>)	Jul	2520	15.5	159	1.3	--	--
	Oct	1969	12.5	159	1.4	--	--
	Mar	1122	8.2	159	1.4	--	--
	May	4878	24.9	341	1.9	--	--
Rosyside Dace (<i>Clinostomus funduloides</i>)	Jul	980	11.6	--	--	--	--
	Oct	939	5.2	--	--	--	--
	Mar	296	2.7	--	--	--	--
	May	1041	11.3	--	--	--	--

2

3 **Table 2. Non-cohort estimates of population density and biomass**

Species	Month	Slaunch Fork		Cabin Creek		Camp Creek	
		D _o	B _o	D _o	B _o	D _o	B _o
Banded Darter (<i>Etheostoma zonale</i>)	Jul	173	0.38	--	--	--	--
	Oct	204	0.50	--	--	--	--
	Mar	20	0.04	--	--	--	--
	May	327	0.76	--	--	--	--
Common White Sucker (<i>Catostomus commersonii</i>)	Jul	214	4.86	--	--	0	0.00
	Oct	337	11.31	--	--	138	1.73
	Mar	102	2.63	--	--	46	0.49
	May	265	10.19	--	--	123	1.23
Fantail Darter (<i>Etheostoma flabellare</i>)	Jul	--	--	91	0.14	1399	0.24
	Oct	--	--	45	0.09	699	2.37
	Mar	--	--	23	0.08	350	0.13
	May	--	--	23	0.05	350	1.35
Johnny Darter (<i>Etheostoma nigrum</i>)	Jul	82	0.08	--	--	--	--
	Oct	214	0.26	--	--	--	--
	Mar	214	0.24	--	--	--	--
	May	582	0.65	--	--	--	--
Northern Hogsucker (<i>Hypentelium nigricans</i>)	Jul	112	2.85	--	--	--	--
	Oct	143	3.61	--	--	--	--
	Mar	61	1.38	--	--	--	--
	May	143	7.43	--	--	--	--

4

5

6 **Table 3. Summary of annual production by most abundant species at each site.**

Site	Species	Annual production
Slaunch Fork	Creek Chub	27.87
	Blacknose Dace	3.15
	Mottled Sculpin	1.65
	Central Stoneroller	3.94
	Rosyside Dace	0.91
Cabin Creek	Creek Chub	6.91
	Mottled Sculpin	0.62
	White Sucker	3.06
Camp Creek	Creek Chub	9.31
	Blacknose Dace	1.25
	Fantail Darter	0.46

7 Table 4. Studies on annual fish production in the eastern U.S. and Canada for comparison with West Virginia. Studies for comparison were selected based on location,
8 stream order, number of sites and species.

Location	Streams/Sites	Species	Production (kg·ha ⁻¹ ·y ⁻¹)			Source
			Range	Mean	Total	
<i>Slaunch Fork, WV</i>	<i>4th order, 1 site</i>	<i>All species</i>	--	--	37.52	<i>This study</i>
		<i>Add. sp. (Sim. Inc. Sum)</i>			(45.91)	
<i>Camp Creek, WV</i>	<i>4th order, 1 site</i>	<i>All species</i>	--	--	11.02	<i>This study</i>
		<i>Add. sp. (Sim. Inc. Sum)</i>			(12.45)	
<i>Cabin Creek, WV</i>	<i>3rd order, 1 site</i>	<i>All species</i>	--	--	10.59	<i>This study</i>
		<i>Add. sp. (Sim. Inc. Sum)</i>			(10.67)	
Calfpasture River, VA	3 sites, 2nd order	All species	28.4-39.6	33.2	--	Neves and Pardue 1983
		Guys Run	5.4-19.3	13.9	--	"
	"	Mottled Sculpin	5.7-10.8	8.2	--	"
	"	Blacknose Dace	2.7-3.8	3.2	--	"
	"	Fantail Darter	0.1-1.0	0.2	--	"
	"	Longnose Dace	0.4-1.5	0.9	--	"
	"	Rosyside Dace	0.2-1.2	0.7	--	"
Clear Creek, OH	7 sites, 1st and 2nd order	All species	--	--	--	Storck and Momot 1989
	Indian Creek	Creek Chub	--	--	136.0	"
	"	Redbelly Dace	--	--	27.7	"
Coweeta Creek, NC	3 sites, 3rd and 4th order	All species	13.6-35.9	21.0	--	Freeman et al. 1988
	Coweeta and Ball Creek	Sculpin	6.3-8.0	7.3	--	"
	"	Longnose Dace	2.6-4.5	3.5	--	"

	"	Rosyside Dace	0.9-1.3	1.1	--	"
	"	Greenside Darter	--	--	0.6	"
Speed River, ON	3 Sites, 6th order	All species	10.3-35.9	19.3	--	Mahon 1979
	3 Sites	Creek Chub	1.9-12.6	6.1	18.3	"
	3 Sites	Northern Hogsucker	0.5-9.9	4.1	12.3	"
	2 Sites	White Sucker	1.0-1.7	1.4	2.7	"
	1 Site	Blacknose Dace	--	--	2.5	"
	3 Sites	Fantail Darter	0.5-4.0	1.9	5.6	"
Steeles Run, KY	4 sites, 2nd and 3rd orders	All species	3.7-21.3	11.4	--	Small 1975
	2nd order	"	3.7-5.1	4.4	--	"
	3rd order	"	15.5-21.3	2.0	--	"

Table 5. Comparison of fish assemblages found at three study sites in southern West Virginia to assemblages found by E.D. Cope in 1867

Cope's Survey of 2 Tributaries of the Kanawha River 1867				Survey of 3 Sites in WV 2013-14		
Genus	Species	Formerly	Specimens	Genus	Species	Specimens
<i>Etheostoma</i>	<i>blennoides</i>		Rare	<i>Etheostoma</i>	<i>zonale</i>	Rare
<i>Lepomis</i>	<i>cyanellus</i>	<i>Lepomis mineopas</i>	Rare	<i>Nocomis</i>	<i>leptocephalus</i>	Rare
<i>Noturus</i>	<i>insignis</i>	<i>Noturus marginatus</i>	Rare	<i>Lepomis</i>	<i>macrochirus</i>	Rare
<i>Anguilla</i>	<i>l.</i>		Rare	<i>Etheostoma</i>	<i>flabellare</i>	Rare
<i>Rhinichthys</i>	<i>cataractae</i>	<i>Rhinichthys nasutus</i>	Common	<i>Moxostoma</i>	<i>erythrurum</i>	Rare
<i>Phenacobius</i>	<i>teretulus</i>		Common	<i>Etheostoma</i>	<i>blennoides</i>	Rare
<i>Notropis</i>	<i>photogenis</i>	<i>Photogenis leucops</i>	Common	<i>Etheostoma</i>	<i>nigrum</i>	Rare
<i>Notropis</i>	<i>scabriceps</i>	<i>Photogenis scabriceps</i>	Common	<i>Rhinichthys</i>	<i>cataractae</i>	Rare
<i>Notropis</i>	<i>rubellus</i>	<i>Alburnellus jaculus</i>	Common	<i>Nocomis</i>	<i>micropogon</i>	Rare
<i>Pimephales</i>	<i>notatus</i>	<i>Hyborhynchus notatus</i>	Common	<i>Ambloplites</i>	<i>rupestris</i>	Rare
<i>Hypentelium</i>	<i>nigricans</i>	<i>Catostomus nigricans</i>	Common	<i>Campostoma</i>	<i>anomalum</i>	Common
<i>Catostomus</i>	<i>commersonii</i>	<i>Catostomus communis</i>	Common	<i>Catostomus</i>	<i>commersonii</i>	Common
<i>Pylodictis</i>	<i>olivaris</i>	<i>Hopladelus olivaris</i>	Common	<i>Hypentelium</i>	<i>nigricans</i>	Common
<i>Ictalurus</i>	<i>furcatus</i>	<i>Ichthelurus cerulescens</i>	Common	<i>Clinostomus</i>	<i>funduloides</i>	Common
<i>Etheostoma</i>	<i>flabellare</i>	<i>Poecilichthys flabellatus</i>	Highly Abundant	<i>Notropis</i>	<i>rubellus</i>	Common
<i>Rhinichthys</i>	<i>atratus</i>	<i>Rhinichthys lunatus</i>	Highly Abundant	<i>Rhinichthys</i>	<i>atratus</i>	Highly Abundant
<i>Cyprinella</i>	<i>analostana</i>	<i>Hypsilepis analostanus</i>	Highly Abundant	<i>Semotilus</i>	<i>atromaculatus</i>	Highly Abundant
<i>Campostoma</i>	<i>anomalum</i>		Highly Abundant	<i>Cottus</i>	<i>bairdii</i>	Highly Abundant

Figure Captions

Figure 4. Map of the three study site locations and their parent river drainages.

Figure 5. Example illustration of the cohort recognition process, using the NORMSEP method of modal-progression analysis in the program FiSAT II (see text). Data are shown for creek chub at the Slaunch Fork site. Cohorts are denoted with the labels 'C1', 'C2', etc., moving from the youngest (i.e., smallest) to oldest (i.e., largest) groups detected within the length-frequency histograms.

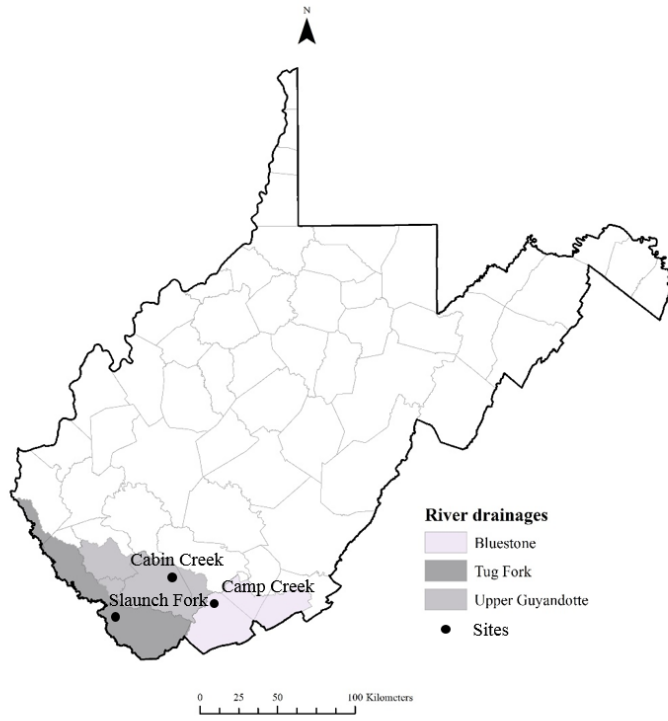


Figure 4. Map of Study Sites

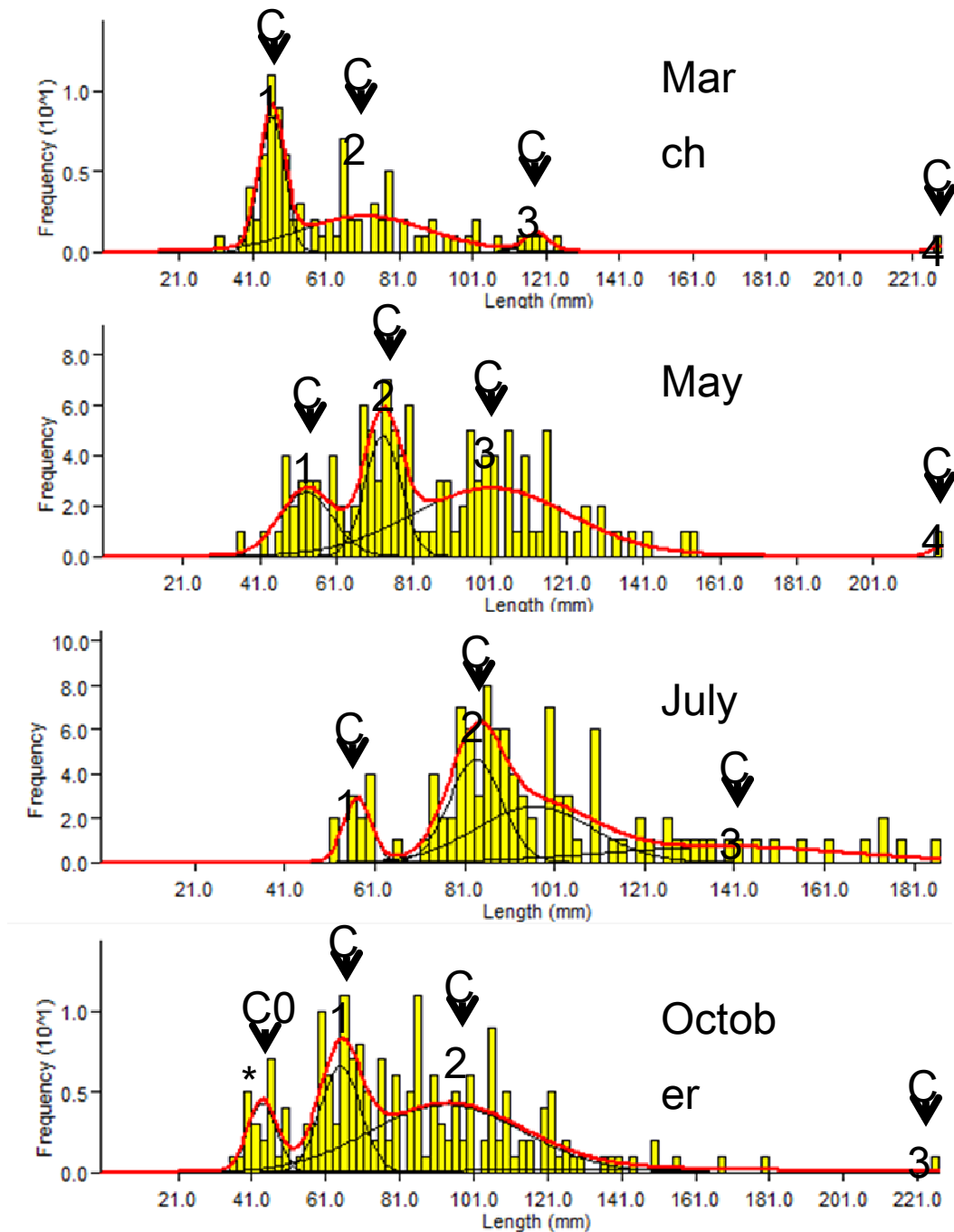


Figure 5. Length-frequency histogram data for Creek Chub at Slaunch Fork site.

Appendix 1. Complete fish survey results. Numbers shown are total counts as CPUE summed among three electrofishing passes. Species' absences or non-detects are indicated by '--' marks.

Species	Slauch Fork				Cabin Creek				Camp Creek			
	Mar	May	Jul	Oct	Mar	May	Jul	Oct	Mar	May	Jul	Oct
Petromyzontidae												
Least Brook Lamprey (<i>Lampetra aepyptera</i>)	--	--	--	1	--	--	--	--	--	--	--	--
Cyprinidae												
Blacknose Dace (<i>Rhinichthys atratulus</i>)	155	325	111	276	11	20	34	43	96	155	17	469
Central Stoneroller (<i>Campostoma anomalum</i>)	21	131	10	21	2	--	2	2	--	--	--	--
Creek Chub (<i>Semotilus atromaculatus</i>)	90	122	109	186	11	16	24	41	39	70	34	137
River Chub (<i>Nocomis micropogon</i>)	--	--	5	6	--	--	--	--	--	--	--	--
Rosyside Dace (<i>Clinostomus funduloides</i>)	24	93	85	91	--	--	--	--	--	--	--	--
Catostomidae												
Golden Redhorse (<i>Moxostoma erythrurum</i>)	--	1	--	--	--	--	--	--	--	--	--	--
Northern Hogsucker (<i>Hypentelium nigricans</i>)	6	14	11	14	--	--	--	1	--	--	--	--
White Sucker (<i>Catostomus commersonii</i>)	9	26	21	33	2	6	4	9	3	8	--	9
Cottidae												
Mottled Sculpin (<i>Cottus bairdii</i>)	87	247	139	149	7	7	7	15	--	--	--	--
Centrarchidae												
Bluegill (<i>Lepomis macrochirus</i>)	--	--	--	--	1	4	14	3	--	--	--	--
Rock Bass (<i>Ambloplites rupestris</i>)	1	--	--	--	3	--	4	3	--	--	--	--
Smallmouth Bass (<i>Micropterus dolomieu</i>)	--	--	--	--	--	--	--	2	--	--	--	--
Percidae												
Banded Darter (<i>Etheostoma zonale</i>)	2	23	10	20	--	--	--	--	--	--	--	--
Fantail Darter (<i>Etheostoma flabellare</i>)	1	--	--	8	1	1	4	2	5	33	10	39
Greenside Darter (<i>Etheostoma blennoides</i>)	--	2	--	--	--	--	--	--	--	--	--	--
Johnny Darter (<i>Etheostoma nigrum</i>)	11	26	8	17	1	--	--	--	--	--	--	--

CHAPTER 3. REFERENCE CONDITIONS AND INDICES OF BIOTIC INTEGRITY

Data collected from wadeable streams on fish and macroinvertebrates have been used to develop multiple Indices of Biotic Integrity (IBI). The Environmental Protection Agency (EPA) developed protocols for assessing the biological integrity of streams (Barbour 1999). James Karr's index for fish communities was the first index to hold up in court (Karr 1981). David Lenat developed an index for benthic macroinvertebrates for the state of North Carolina (Lenat 1994).

The movement towards the need for increased biomonitoring began in the 1970's, along with the environmental movement and passages of statutes such as the Federal Water Pollution Control Act of 1972. To effectively determine the level of impairment in streams and rivers, it is imperative to know something about the conditions in "pristine" or minimally-impacted streams so a quantitative comparison or bioassessment can be conducted. Without baseline data or reference conditions, these types of analyses are unable to be performed.

Studies like this one are important because they can help identify streams that have been stressed or impaired by anthropogenic activities. Fish and macroinvertebrates can be used to look at the long-term and short-term health of a stream. Streams that have been severely impacted are placed on EPA's list of impaired waters and states are required to put forth appropriate restoration efforts. IBI's, habitat assessments and macroinvertebrate assessments are useful in making these judgments, however some metrics used in the assessments can be ineffective and biased so there is room for improvement in developing these indices.

Fish as Biological Indicators

The use of fish as biological indicators is a subject that has been discussed by several biologists and ecologists (Ortmann 1909; Brinley 1942; Trautman 1957). Fish are good representatives of regional conditions because the dispersal of individuals through the stream network is influenced by local and regional characteristics (Freund and Petty 2007), whereas macroinvertebrates are more indicative of local conditions. Some of the advantages of using fish as indicators come from Karr (1981) and Hocutt (1981) and include the facts that they are: long-lived, widely-occurring in a variety of habitats, vast amounts of scientific literature on fish biology, ease of identification, and toxicity trends.

Some of the disadvantages are the amount of manpower and technology required to effectively sample fish populations, as well as the migratory behavior of fishes. However, metrics can still be built around different characteristics of fishes to serve as indicators. For example, a stream with more specialist feeders (piscivores or herbivores) would generally score higher than a stream with more generalist feeders (omnivores). This along with other metrics were part of the original index developed by Karr (1981). Others include the total number of species and the number of intolerant species. High diversity of fish fauna is usually correlated with a high degree of health. Pollution-intolerant species are very good indicators of water quality because these species are unable to tolerate even low-levels of pollution. Various darter species are used for this metric since they are largely benthic insectivores and very sensitive to pollution.

Selected Reference Condition Criteria for Study Sites

West Virginia does not currently have a fish IBI in place, although considerable effort has gone into developing one (Walters 2006). The West Virginia Department of Natural Resources only performs single-pass surveys at stocked trout sites in an effort to manage sport fish and indirectly assess stream health (WVDEP 2013). Therefore, in order to conduct an ad-hoc assessment, considerable research was performed to determine a list of accepted metrics that could be used for West Virginia streams.

Multiple approaches exist for determining a reference condition including regional comparisons, historical data and quantitative analysis (Davis and Simon 1995). To place our data in a regional context, we compared our fish production estimates to known minimally-disturbed streams and our assemblage compositions to current regional/non-regional data. Other regional production studies used for comparison included streams in Kentucky (Small 1975) and Virginia (Neves and Pardue 1983). Assemblages were also compared using these studies, as well as assemblages from non-regional and historic studies.

For historical context, our literature search revealed that Edward Drinker Cope had surveyed southern western Virginia in 1867, specifically Walker's Creek and Strouble's Creek – two tributaries of the New River, which is a tributary of the Kanawha River. These qualitative data served as our historical reference condition (Cope 1869). While our assemblages were similar to those found by Cope, abundances were generally lower in our sites. Additionally, more sensitive species such as fantail darter, satinfish shiner and central stonerollers were “highly abundant” in Cope's survey, where as in our survey they were considered “common” to “rare”. In fact, in our study more tolerant species like creek chub and blacknose dace were highly abundant. Given that abundances were generally lower and numbers of tolerant species were

higher in our survey could suggest conditions in these sites have already been altered since Cope. However a more in-depth study of historical fish assemblages in this region is needed before a more meaningful conclusion can be drawn.

Our quantitative analysis consisted of two specific metrics from Davis and Simon (1995) – a test of 100% native species (Hughes et al. 1998) and deviations from the Maximum Species Richness Line (Fausch et al. 1984). Species were determined to be native to the stream network after examining point-presence maps and researching if they historically occurred in these areas (Stauffer et al. 1995). A Maximum Species Richness Line (MSRL) is a common technique for assessing biotic integrity, it consists of a hand-drawn line, of which <5% of the points are above the line, through a plot of the relationship between species richness and watershed area. If a stream is found to be within a 25-33% deviation from this line this would indicated “excellent” biotic integrity. Additionally, we scored our streams, for each season, based on fish assemblages according to proposed metrics described in an EPA technical support document for the development of a fish IBI for West Virginia Streams (Walters 2006). While this document is not in use by the state of West Virginia, it still adds value by proposing metrics that are specifically related to streams in this region.

Similarly, a Non-Metric Multidimensional Scaling (NMDS) ordination was calculated using fish assemblages from current regional and non-regional streams, as well as historic data from Cope (1869; see Figure 6). The Slauch Fork site was the closest, in terms of assemblage composition, to representing regional sites and the historical assemblages found by Cope. The Cabin Creek site fell the second closest and the Camp Creek site had the greatest deviation from the group.

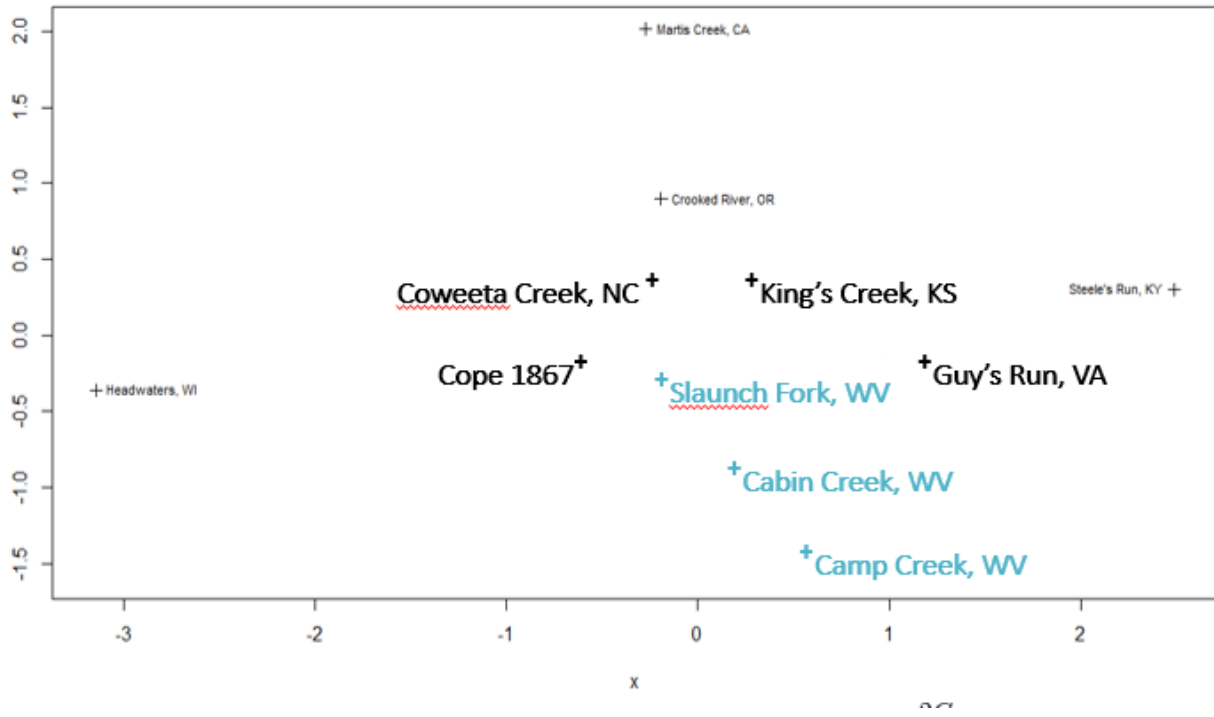


Figure 6. NMDS ordination of fish assemblages at study sites compared with regional and non-regional assemblages

According to Hughes (1998) a site that contains 100% native fish species could potentially be used as a reference condition. The fish assemblages at Slaunch Fork and Camp Creek were all determined to be native. The Cabin Creek site however contained rock bass (*Ambloplites rupestris*) and bluegill (*Lepomis macrochirus*), which are native to the state of West Virginia, but most likely introduced to this site by a stocked pond on a golf course upstream. Fausch et al. (1984) proposed a quantitative model termed the Maximum Species Richness Line (MSRL), which measures the relationship between species richness and watershed area. The maximum number of species that could be present at a site, with regards to its watershed area, would suggest excellent biotic integrity. The amount of deviation from this line would suggest some amount of impairment. A site within a 25-33% deviation from this line could potentially serve as a reference condition. The results of our sites versus the MSRL are

illustrated in Figure 7. Watershed areas were calculated using hydrology and elevation layers in ArcGIS 10.2. The Slaunch Fork site fell within a 33% deviation from the MSRL, indicating “excellent” biotic integrity. However, the Camp Creek and Cabin Creek sites fell outside of a 33% deviation which could indicate some level of impairment.

Fausch's Maximum Species Richness Line (MSRL)

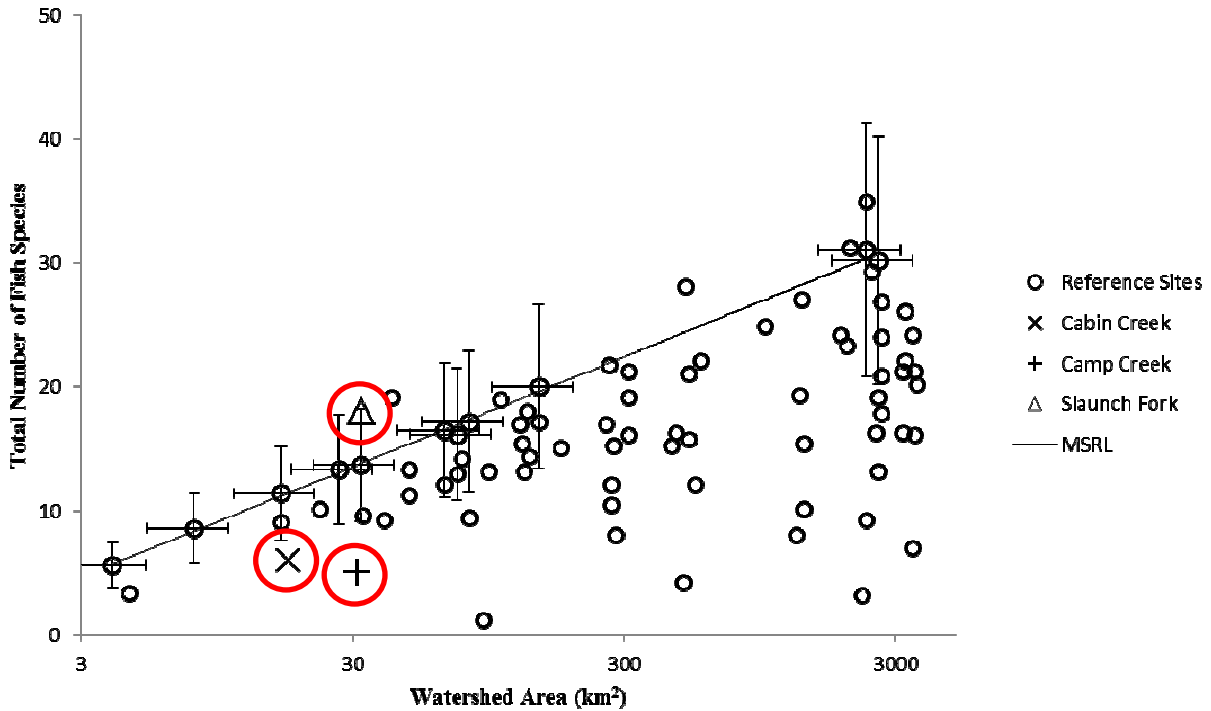


Figure 7. Fausch's Maximum Species Richness compared with our study sites

Finally, our sites were scored using metrics from a proposed EPA document calling for the development of fish IBI for West Virginia (Walters 2006) for all four sampling periods. The results are presented in Table 6. Across all four seasonal sampling events, the Slaunch Fork site had a mean score of 82.5 categorizing it as having “excellent” biotic integrity according to the EPA document. The mean score for the Camp Creek site was 37.5 categorizing it as having “poor” biotic integrity. Finally, Cabin Creek had a mean score of 75 categorizing it as having

“fair” biotic integrity. This was the only site that showed seasonal variation in IBI score. Cabin Creek’s score increased to 74 during the winter improving its classification to having “good” conditions. The Slaunch Fork and Camp Creek sites remained consistently “excellent” and “poor” respectively throughout each season.

A final tally of our IBI metrics for comparison is shown in Figure 8. This table is the result from our interpretation and synthesis of how these sites compare to all our selected metrics for this study. The Slaunch Fork site was found to be minimally-disturbed having excellent biotic integrity and should therefore be considered a reference condition according to these proposed metrics. The Cabin Creek site supported a more diverse fish assemblage than the Camp Creek site, but it was much less productive. This site is known to have some level of impairment due to impoundments and a golf course upstream which introduces non-native species to the stream. However we recommend that this site be categorized as “best-available” for this region given the lack of other candidate streams. The Camp Creek site had the least diverse fish assemblage causing it to score low on many IBI metrics, however the water quality data we collected did not suggest impairment nor is this site downstream of any surface mining or impoundments. It is not uncommon for headwater mountain streams to simply lack biodiversity with regards to fish assemblages and therefore low abundance or diversity of fishes in these streams does not necessarily indicate impairment (McCormick 2001). Thus, we categorize Camp Creek as a “best-available” reference condition. We acknowledge it is likely that all of these streams have some level of human impairment, which is contrary to the classic definition of a reference condition. However, the vast majority of streams throughout the world have some level of human impairment and yet some are still used as reference sites (Davis and Simon 1995).

We found it intriguing that when scored using various IBI metrics based on assemblage composition, the Camp Creek site scored very low and by traditional standards would have been classified as having poor biotic integrity. However, the Camp Creek site was almost twice as productive as the Cabin Creek site while supporting an assemblage with almost half of the number of species. Such a difference in production would indicate that Camp Creek is the overall healthier of the two sites, especially when considering the overall low-productivity and diversity of headwater mountain streams. These data could suggest that annual production is a better indicator of stream health in low-order high-elevation streams than simply assemblage composition alone.

Multi-metric indices and IBI's are useful tools in rapidly assessing stream health. The presence/absence, as well as abundance, of certain species or families of fishes are particularly good indicators of regional conditions in the stream network. However, in less abundant and diverse low-order mountain streams, examining the assemblage composition alone may not be a strong enough indicator of stream health. Our sites' scores varied from "excellent" to "poor" biotic integrity according to traditional metrics, but were comparable in terms of production to other studied stream sites. Perhaps an additional metric which includes some aspect of biomass or fish growth might be needed to gain a better understanding of the health of mountain streams.

Table 6. Summary of index metrics and scores

Metric	SUMMER			FALL			WINTER			SPRING		
	Slauch Fork	Camp Creek	Cabin Creek	Slauch Fork	Camp Creek	Cabin Creek	Slauch Fork	Camp Creek	Cabin Creek	Slauch Fork	Camp Creek	Cabin Creek
% Cottid Individuals	10	0	8	10	0	10	10	0	10	10	0	10
% Intolerant Clean Spawner Individuals	8	0	3	5	0	4	7	0	5	7	0	4
% Macro-ominvore Individuals (negative)	10	10	10	10	10	10	10	10	10	10	10	10
% Tolerant Individuals (negative)	5	1	3	5	1	1	5	1	6	6	1	2
% Non-native Individuals (negative)	10	10	10	10	10	10	10	10	10	10	10	10
Intolerant Species	10	4	8	10	4	8	10	4	8	10	4	8
Cyprinid Species	5	3	6	5	3	6	5	3	6	5	3	6
% Non-tolerant Piscivore/Invertivore Individuals	7	4	3	8	1	3	7	1	8	8	4	3
Intolerant Benthic Habitat Species	10	3	4	10	3	4	10	3	4	10	3	4
IBI Score	83	39	61	81	36	62	82	36	74	84	39	63
Rank	Excellent	Poor	Fair	Excellent	Poor	Fair	Excellent	Poor	Good	Excellent	Poor	Fair

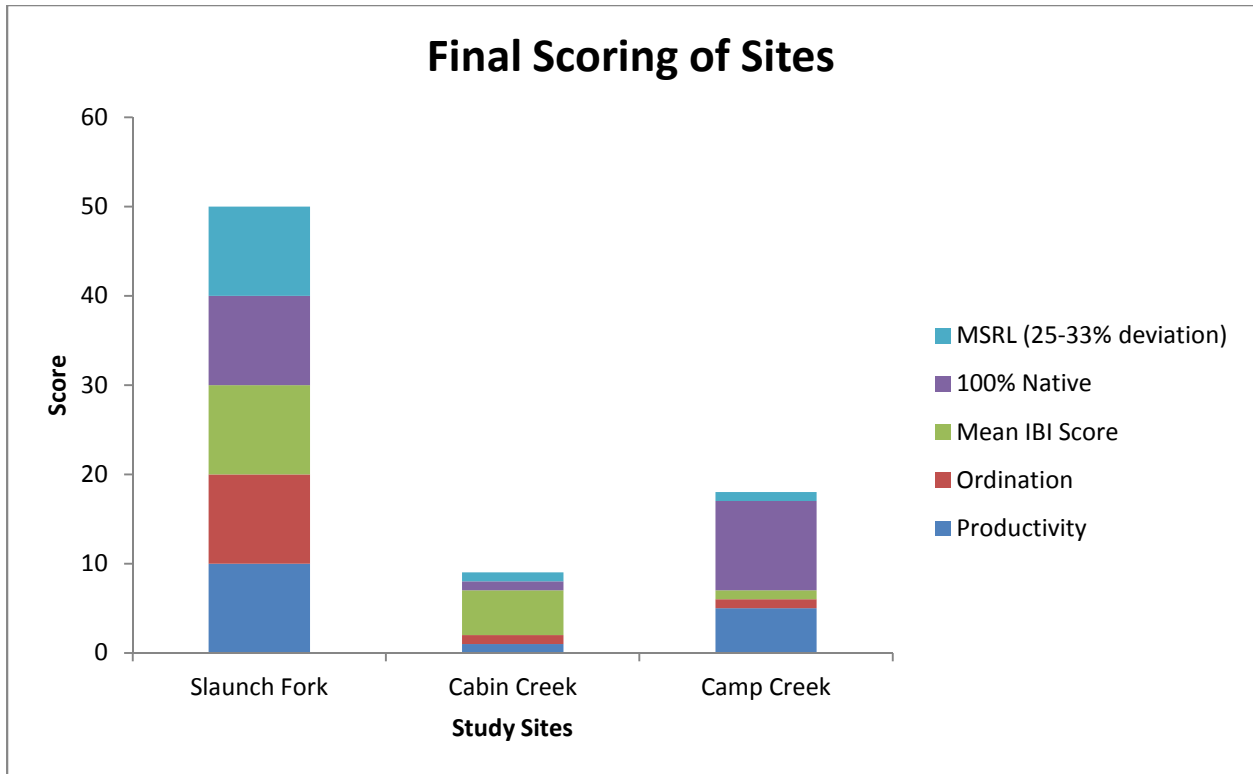


Figure 8. Scored study sites interpolated from selected metrics

CHAPTER 4. GIS ANALYSIS

Land Use

2011 National Land Cover Dataset (NLCD) data were used to assess the current land use status of West Virginia and the significant land cover statistics summarized below (Table 7). The state is primarily dominated by deciduous forest coverage with very minimal developed land overall. Most development is occurring along the Kanawha and Guyandotte Rivers in the western portion of the state, around the towns of Beckley and Bluefield in the southern portion, and around Morgantown in the northern portion. There is a small amount of evergreen forest in the eastern portion of the state in the higher elevations. There is a significant amount of hay and pasture land in the southeastern and northeastern portions of the state. There is little woody wetlands or emergent herbaceous wetlands in the state due to the mountainous terrain. Of note, most of the barren land occurs in southwestern portion of the state, an area known for high-levels of anthropogenic disturbance, such as surface mining activities.

Table 7. Statewide NLCD 2011 land cover classification

Land Cover Classification	Percentage	Acres
Forest Coverage	80.43	12,583,600
Developed (Open Space)	4.79	749,000
Developed Land	2.19	343,700
Barren Land	0.81	126,200
Herbaceous Vegetation	1.72	269,300
Hay/Pasture	7.8	1,220,100
Cultivated Crops	1.04	162,350
Woody/Emergent Wetlands	0.14	22,000

Using NLCD data from 2011, general land use in our study site counties was relatively minimal and the important statistics summarized in the table below (Table 8). McDowell, Wyoming and Mercer counties encompass total areas of 1384, 1298 and 1088 km², respectively. They are minimally-developed counties with minimal agricultural use and a high percentage of forest coverage. Our study sites are dominated by forests and herbaceous vegetation with Mercer County having a significant proportion of hay/pasture land.

Table 8. Study site counties NLCD land cover classification

Land Cover Classification	McDowell (Slauch Fork)	Wyoming (Cabin Creek)	Mercer (Camp Creek)
Forest Coverage (%)	87.3	70.2	85.7
Developed Land (%)	2.5	2.0	6.1
Barren Land (%)	1.3	1.1	0.3
Herbaceous Vegetation (%)	3.2	5.6	6.7
Hay/Pasture (%)	1.2	1.1	9.7
Cultivated Crops (%)	0.004	0.14	0.01

Mercer County has the highest percentage of developed land, herbaceous vegetation and hay/pasture land respectively. It also has the least amount of barren land while McDowell and Wyoming Counties have >1% barren land. This is significant because of the unique land-use situation in West Virginia, the NLCD classification of barren land initially appeared to be strongly correlated with the amount of land left barren due to surface mining activities. Upon further analysis, overlaying the Barren Land NLCD classification layer with satellite imagery of surface mining sites further illustrates this correlation (Figure 9). Of the 126,000 acres of barren

land in the state, about 9,000 acres or 9% of the total amount of barren land is contained within our three study counties.

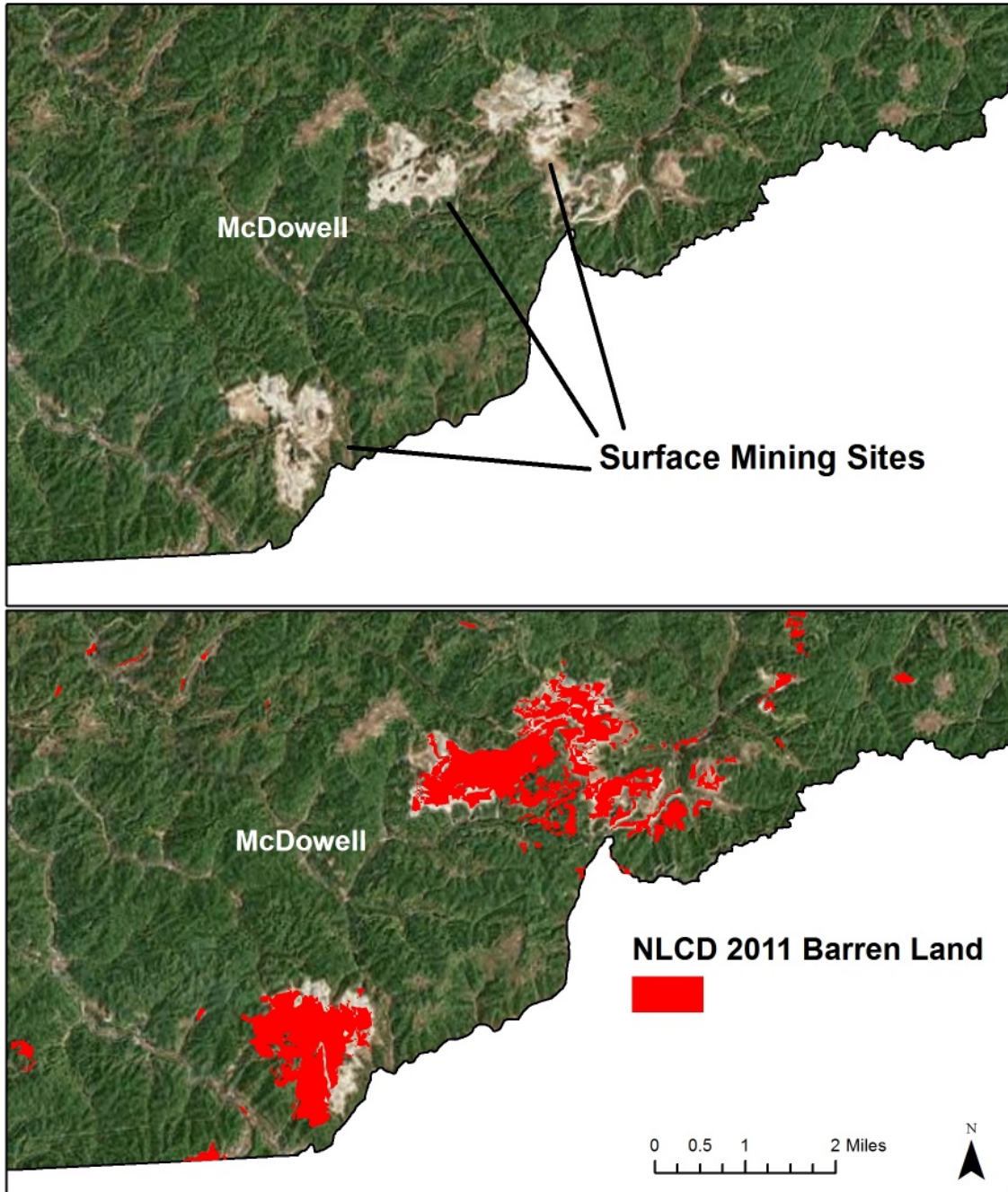


Figure 9. The comparison of satellite imagery showing surface mining sites and NLCD barren land classification for McDowell County, West Virginia

Current and Projected Extent of Mountaintop Mining Operations

Mountaintop mining, and other surface mining operations, are the major anthropogenic disturbance in this region. The high-levels of surface mining are occurring in the Cumberland Plateau and Eastern Allegheny Plateau regions of West Virginia, where our three sites are located. The concern here is the rate that mountaintop mining and surface mining operations have spread since the 1990's. In 1998, the projected current area of surface mining operations was constrained to the southern central region of the state. According to data from 2007, that area has doubled in size with expansion into the southeastern portion of the state (Figure 10).

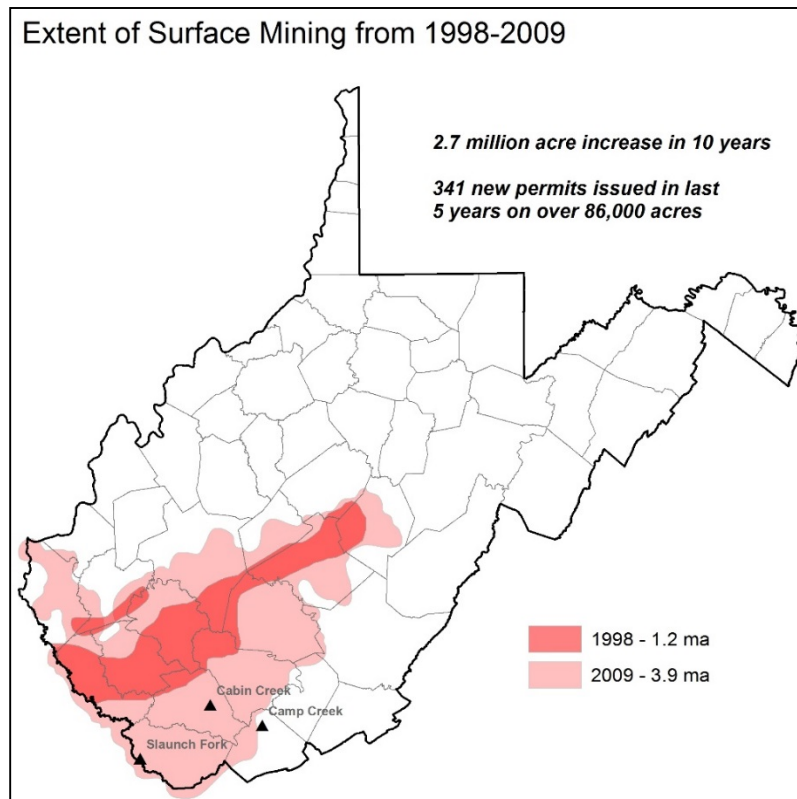


Figure 10. The expansion of surface mining operations in West Virginia from 1998 to 2009

Mapping operations continue throughout the state and thousands of acres of potential minable coal beds have been identified (Figure 11). According to the West Virginia Geologic and Economic Survey (WVGES), mining operations are expected to expand to the east and the north in the state (WVGES 2014). Of particular interest is the fact that ongoing mapping operations are occurring in Mercer, McDowell and Wyoming counties – the three counties where our sites are located. This implies continued expansion into our watersheds of interest and potentially increasing the risk to these aquatic and terrestrial ecosystems. While expansion of mining operations have slowed in the last year or two, current expansion rates are between 17,000 to 25,000 acres per year. This rapid increase significantly threatens the ecological integrity of the region.

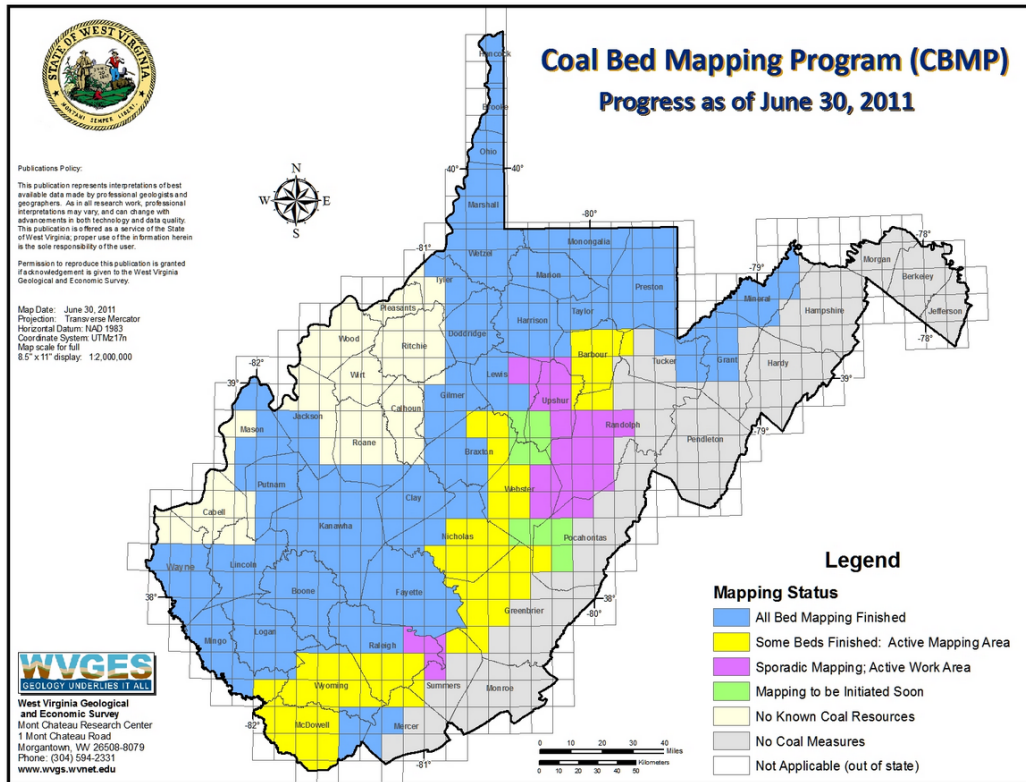


Figure 11. Progress of coal bed mapping programs in West Virginia

Predicting Occurrence of Similar Fish Assemblages

Climate Analysis

The first objective of the GIS analysis was to predict where we would expect to find similar fish assemblages throughout the state based upon two climate variables, i) mean annual air temperature and ii) mean annual rainfall. These are two important variables in determining fish assemblage composition (Godinho et al. 1998). Data on mean annual rainfall and air temperature were obtained from WorldClim.org which provides global climate spatial data that can be used in GIS analyses. Stream layers were obtained from the U.S. Geological Surveys' National Hydrography Dataset (NHD) (<http://nhd.usgs.gov/data.html>).

First, climate spatial data were intersected with the NHD statewide stream layer to associate climate attributes with streams. This produced two separate layer files – one for streams now with attributes on mean annual air temperature and another for mean annual rainfall. Examining our sites, according to the climate data they have a mean annual air temperature range of 50-53°F and receive an average of 38-47" in annual rainfall. In order to predict where we would expect to find similar assemblages, those same attributes need to be searched throughout the rest of the stream network in the state. By selecting the attributes of the streams that match the attributes of our sites a map of sites with similar climatic variables can be produced (Figure 12).

The result of this analysis shows that the streams in the rest of the state that are climatically similar to our sites are primarily located in southern central portion of the state, where the vast majority of surface mining operations are being conducted. These operations could be putting an increasing number of surface waters in peril. A potential next step would be a

more comprehensive modeling effort to quantify the amount of similar stream systems that are threatened by expanding surface mining activities.

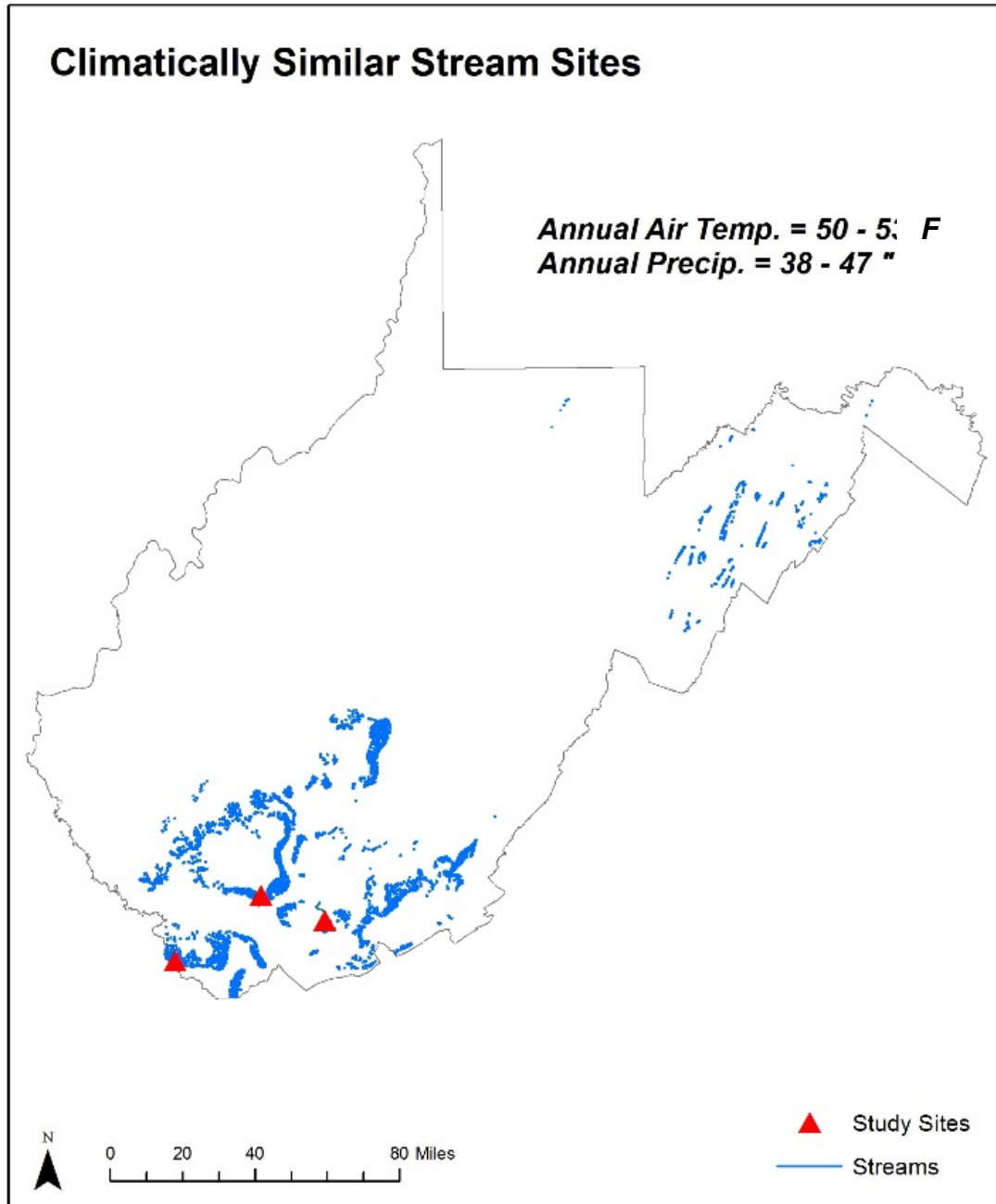


Figure 12. Streams with similar climate attributes to our study sites

Physical Stream Characteristics Analysis

The second objective of the GIS analysis was to examine three physical characteristics of our streams – stream order, gradient and elevation – and determine which other streams throughout the state had similar characteristics. Data sources included two raster layers for slope and stream order, a digital elevation model and National Hydrography Dataset stream shapefile layers from the U.S. Geological Survey. Among our three study sites, elevation ranged from 500 – 750 meters above sea level, gradient ranged from 3 – 6 %, and stream order ranged from 3rd to 4th.

First, elevation values between 500 and 750 meters were extracted from the statewide digital elevation model. These values were then associated with NHD flowlines using the 3D analyst tool in ArcMap. The streams with matching elevation ranges were then extracted from the statewide NHD dataset. The raster layers containing data on slope and stream order were converted to polygon shapefile layers. As before, the range of slope values and stream orders were extracted from these statewide datasets. These files were then merged into one layer. These data were then intersected with the extracted stream layer containing elevation values. The result (Figure 13) produced a map showing sites with similar physical habitat characteristics to our study sites which might be a good indicator or predictor of similar fish assemblages.

This analysis is the first-step in a modelling effort to predict the occurrence of fish assemblages similar to our study sites throughout the rest of the state. From a scientific perspective, it is intriguing to examine what climatic and physical characteristics have influenced the distribution of this assemblage. From a management standpoint, knowing that this assemblage is widely-spread throughout the state and occurs at similar elevations to surface

mining operations, it begins to place in context the amount of stream networks and fishes that are endangered.

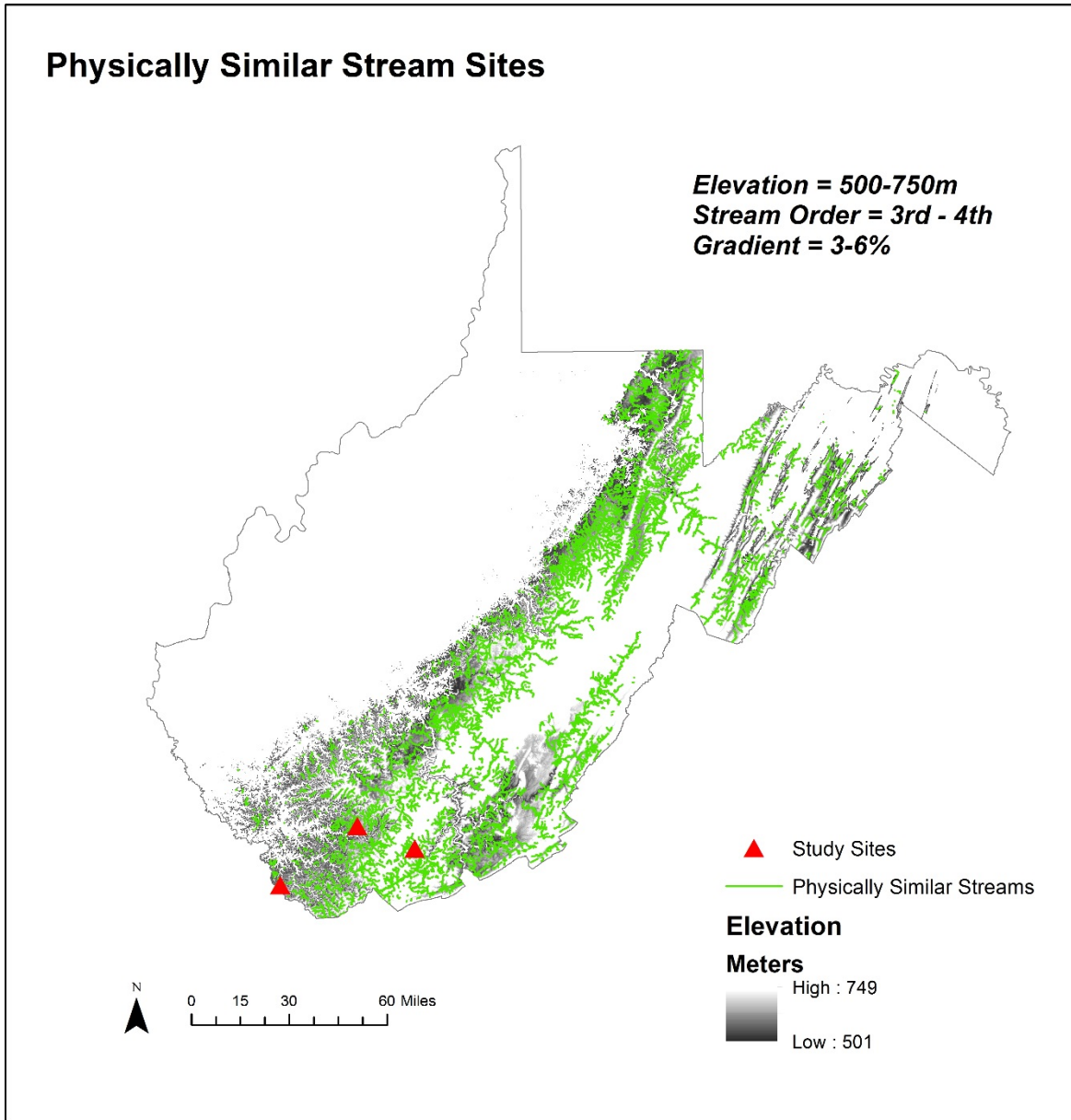


Figure 13. Streams with similar physical attributes to our study sites

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