Native Brook Trout Restoration on Anthony Creek
Great Smoky Mountains National Park
2016-2018

Great Smoky Mountains National Park
Resource Management & Science
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INTRODUCTION

Brook Trout (*Salvelinus fontinalis*) across the southern portion of their range have been shown to be highly genetically differentiated across populations, even within the same major watersheds (Kazyak et al. 2018; Weathers et al. 2019). Brook Trout are the only native salmonid to the Southeast United States. Unfortunately, anthropogenic activities, such as logging, and the introduction of non-native trout, e.g., Rainbow Trout (*Oncorhynchus mykiss*), have eliminated nearly 75% of their documented range, including roughly 50% of the original Brook Trout streams in the southern Appalachian Mountains. From the 1930s-1970s, the presence of Brook Trout in Great Smoky Mountains National Park (GRSM) declined from 157.8 km (98.1 mi) of streams to 63.6 km (39.5 mi) while Rainbow Trout expanded by 94.2 km (58.5 mi) (Larson and Moore 1985).

While timber logging of watersheds and non-native fish stockings no longer occur in GRSM, Brook Trout range loss continues today, especially at high elevations. Water quality surveys indicate increasing stream acidity in high elevation streams (>915 m or 3,000 ft), many to levels beyond the tolerance of Brook Trout populations (Fakhraei et al. 2016; Zhou et al. 2015). As such, restoration of streams at mid to low elevations (i.e. <1,070 m or 3,500 ft), such as Anthony Creek, is necessary to stabilize populations in the Park and expand Brook Trout range to its historical status, where feasible, in accordance with the GRSM Fisheries Management Plan. Successful completion of this portion of Anthony Creek to allopatric populations of Brook Trout will see the first time since the 1950s that these fish are present in the Abrams Creek Watershed.

OBJECTIVES

1. Remove non-native Rainbow Trout from 2.8 km (1.75 mi) of Anthony Creek with cooperation from the Tennessee Wildlife Resource Agency and Trout Unlimited.
2. Collect native Brook Trout from proximate streams within GRSM and release them into Anthony Creek in 2017 and subsequent years as needed.
3. Monitor Brook Trout populations in Anthony Creek during subsequent years to determine success of the project.

STUDY AREA

Anthony Creek is a headwater stream of Abrams Creek, located immediately upstream of the Cades Cove Picnic Area (*Figure 1*). The restoration site encompasses a drainage area of 10.07 km^2 (3.89 mi^2), all of which is within the boundary of GRSM. Anthony Creek is of special interest for Brook Trout restoration because of its relatively low elevation. The project area ranges from an elevation near 634 m (2,080 ft) to approximately 853 m (2,800 ft) with a mean stream width of 5.3 m (17.4 ft).

An old mill dam (*Figure 2*), approximately 2 m (6.6 ft) in height, marks the bottom of the restoration site, which is similar to the LeConte Creek restoration site of 1999. While non-game fish species, like Blacknose Dace (*Rhinichthys atratulus*), Longnose Dace (*Rhinichthys cataractae*), and Creek Chub (*Semotilis atromaculatus*) are found below this barrier, the upstream fish community was exclusively Rainbow Trout prior to the restoration.

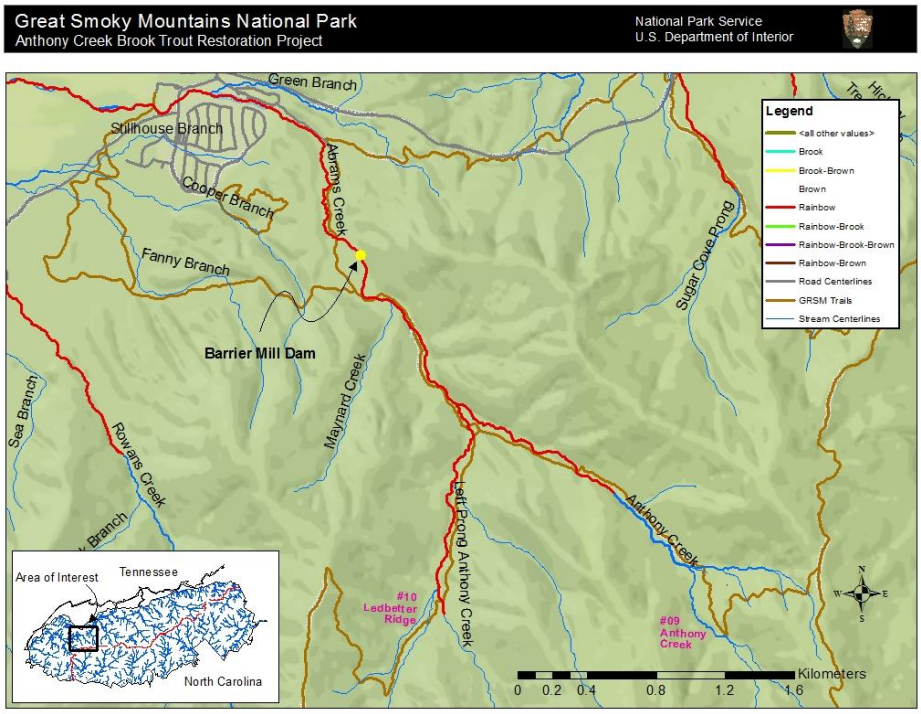

Figure 1: Anthony Creek restoration site with fish barrier and pre-treatment trout distributions.

Figure 2: Measuring the height of the mill dam fish barrier for the Anthony Creek Restoration Project.

METHODS

ENVIRONMENTAL PLANNING

National Park Service (NPS) Management Policies state that management of exotic species, up to and including eradication will be undertaken whenever such species threaten Park resources or public health and when control is prudent and feasible (National Park Service 1988). GRSM management staff determined that the eradication of non-native fish species and the reestablishment of native Brook Trout populations are feasible in select stream segments and that such action is justified under its legislative mandates and management policies.

SITE PREPARATION

Prior to treatment, the project area was sampled to determine Rainbow Trout distribution and organized into 34 sites, each being 100 m (328 ft) stream lengths (*Figure 3*). Prior to fish removal, Rainbow Trout distribution was found to end on the left fork of Anthony Creek near the top of site LF_ANC_4, at a large cascade. On the main stem, distribution ended below site ANC_23. These 26 sites were then identified as the target restoration zone. Once sites were established, marker tags were placed on streamside trees to identify specific monitoring and restoration sites.

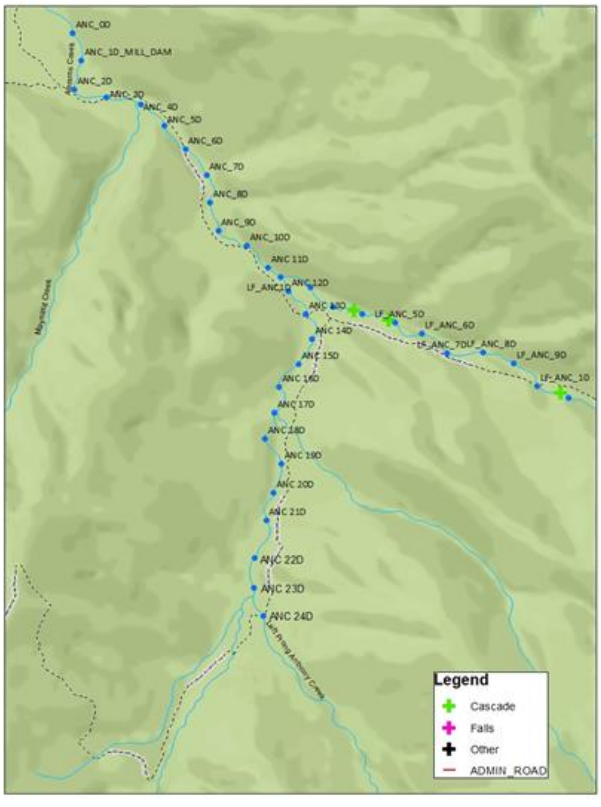

Figure 3: Anthony Creek project area with site designations.

NON-NATIVE FISH REMOVAL IMPLEMENTATION

Each removal consisted of setting block nets at the downstream and upstream limits of each 100m site and conducting three consecutive passes with electro-fishing units. Lower block nets were then pulled and placed at the top of the next site to continue progression upstream. Each team consisted of at least 2 GRSM staff to operate electro-fishing units, 2 back-up netters, and 1-2 bucket carriers to hold captured fish. Each trout encountered was counted and categorized as either juvenile or adult. All Rainbow Trout were humanely euthanized on-site and, due to a large number of recent bear/human encounters in the area, were carried out of the treatment area in sealed plastic bags via backpacks and disposed of at a pre-determined carrion dump site. Estimations of remaining populations were calculated following each removal effort to determine the necessity of repeat treatments.

POPULATION ESTIMATES

Using the software MicroFish™ 3.0, population estimates were made after removal efforts to determine a probable number of fish remaining in the project area. MicroFish™ 3.0 is used to estimate populations sampled via the 3-Pass Depletion method. GRSM staff treated each removal effort as a single pass to model the trout population of the entire treatment zone as one segment. This estimation methodology is valid because it meets the same three assumptions required of the 3-Pass Depletion method:

1. *Individuals sampled are members of a closed population; without immigration or emigration between sampling efforts.*
   This first assumption is met by the project area being upstream of a fish barrier, in this case a pre-existing mill dam. The upper reaches of the project site are above trout distribution limits, thus creating a closed population.

2. *Equal effort is expended on each sampling attempt.*
   Equal effort per removal attempt was ensured by using the same number of backpack electro-fishing units, experienced crew members, and back-up netters on each removal attempt.

3. *All members of the population have an equal chance of capture during the sampling effort.*
   The equal catchability assumption was met by using the same voltage output on electro-fishing units on every attempt and putting the same effort into capture of all fish, regardless of fish size and location.

RESULTS

PRE-TREATMENT TROUT ASSESSMENTS

Prior to the first removals in 2016, fish populations were sampled using the 3-Pass Depletion method to set a reference point for Brook Trout population metrics in the future. Site ANC_0, outside of the restoration area, was sampled as a control and sites ANC_2 and LF_ANC_2 were sampled to assess the project area. Although the control site and ANC_2 had similar trout abundances (98 and 100 individuals, respectively), ANC_2 had generally smaller fish with a mean mass of 23.6 g. The left fork of Anthony Creek had a similar mean mass to ANC_2 but many fewer individuals, particularly the 0-20 g weight class, which indicates a lack of young-of-year fish (*Figure 4*).

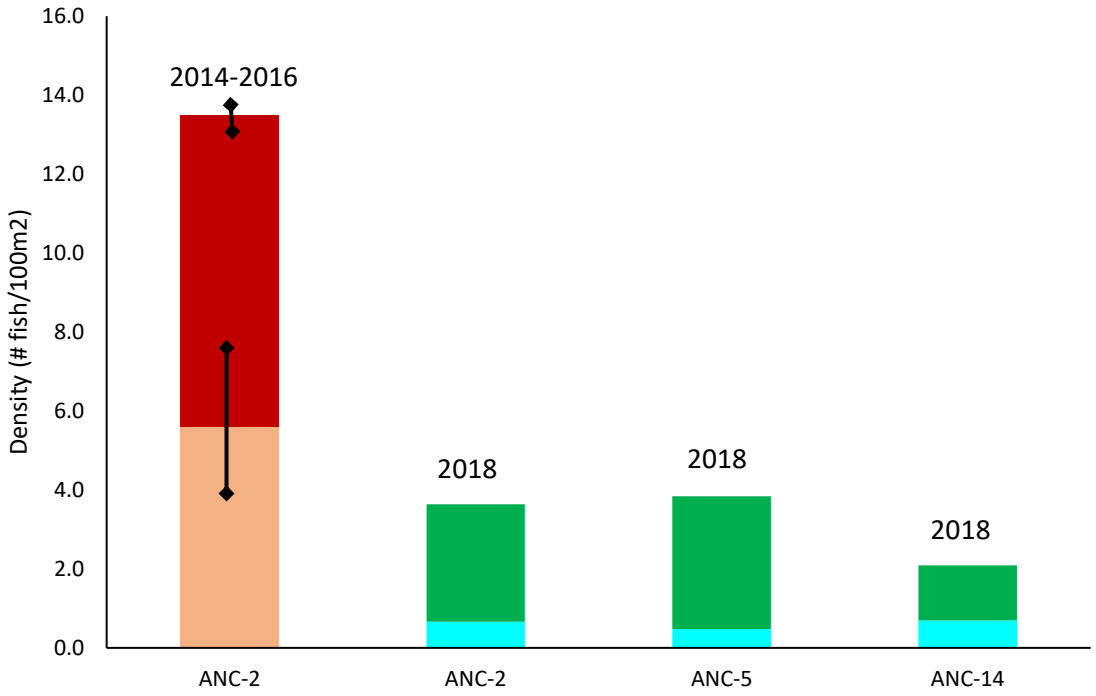

Figure 4: Pre- and post-treatment density of rainbow (pink/red) and Brook Trout (cyan/green) sampled at 3 monitoring sites in Anthony Creek. Note that pre-treatment data reflects mean density for the period and includes standard error (SE) bars.

REMOVALS

Successful electro-fishing removal projects depend upon significant effort between breeding seasons to eliminate potential breeding stock (Kulp and Moore 2000). Four complete removal efforts were made throughout 2016, plus a targeted fifth effort in October to take advantage of severe drought conditions, which improved capture efficiency by reducing stream volume.

Treatment began on June 7, 2016, with a total of 1,432 Rainbow Trout being removed from Anthony Creek during the initial effort. 1,243 individuals (371 juveniles and 872 adult fish) were taken from Anthony Creek proper, while the left fork yielded 189 individuals (11 juveniles and 178 adults) (Table 1). The second round of removals, beginning June 27, 2016, resulted in a total of 528 individuals overall, with 489 trout (283 juveniles and 206 adults) from Anthony Creek and 39 trout (16 juveniles and 23 adults) from the left fork. August 1, 2016 marked the start of the third effort, which produced 229 individuals (183 juveniles and 46 adults) from the main stem and 14 individuals (6 juveniles and 8 adults) from the left fork, for a total of 243 fish. A fourth complete pass was made in October, with 71 individuals coming from Anthony Creek (66 juvenile fish and 5 adults) and only 1 juvenile fish from the left fork, for a total of 72 specimens. Finally, to exploit local drought conditions, on November 1, 2016, the lower 6 sites of Anthony Creek were shocked for a fifth time with 8 juvenile fish and one adult fish being captured (*Table 1*).

Ultimately, 2,284 Rainbow Trout were removed from the restoration site over five removal efforts during the summer/fall of 2016. The total was comprised of 1,339 mature fish and 945 juveniles. Population estimates calculated with MicroFish™ 3.0 software estimate that 99.3% of the trout population within the project area had been removed and that 17 trout (16.10 actual) remained (6 trout/km or 10 trout/mi) (*Figure 5*).

Table 1: Rainbow Trout, by size class, removed from project area during 5 electro-fishing efforts in 2016.

	1ST REMOVAL	2ND REMOVAL	3RD REMOVAL	4TH REMOVAL	5TH REMOVAL	TOTAL
TOTAL	1,432	528	243	72	9	2,284
ADULTS	1,050	229	54	5	1	1,339
JUVENILES	382	299	189	67	8	945

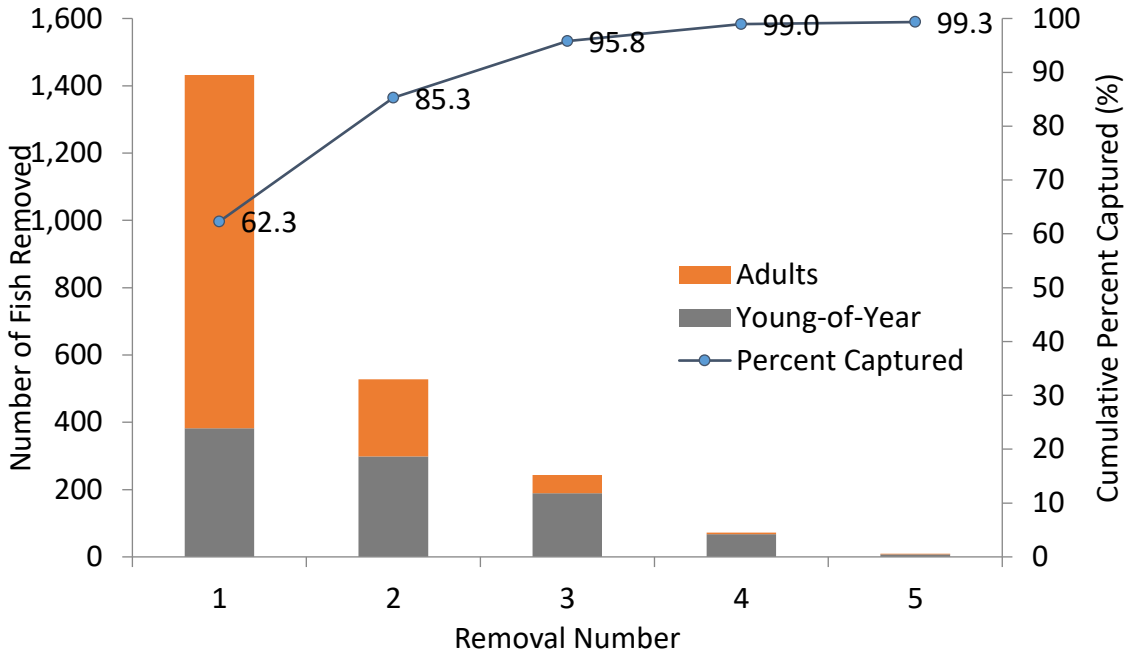

Figure 5: Number of trout removed and associated capture efficiency during the 5 removal efforts of 2016.

2017 REMOVALS

On July 10, 2017, the entirety of the restoration project was electro-fished again to assess the success of the previous year's removal efforts and to capture any remaining Rainbow Trout. Seventeen (17) adult fish were removed from 10 of the original 26 stream sites. Unfortunately, 24 young-of-year fish were found concentrated in a section of about 170 m (558 ft) with high habitat complexity. This setback meant that at least a pair of adult Rainbow Trout had reproduced and additional removal efforts would be required.

A total of four subsequent removals were conducted, in July, September, and October (*Table 2*). Immediately after the complete effort in July 2017, a second attempt, focusing on the lower 700m was undertaken. Efforts in September and October revisited the lower 700 m where the young-of-year fish had been recorded. This targeted approach was justified by MicroFish™ 3.0 population estimates for each section, which predicted 0 fish left in all but the focus area where reproduction had occurred.

Table 2: Rainbow Trout, by size class, removed in 2017.

	1ST REMOVAL	2ND REMOVAL	3RD REMOVAL	4TH REMOVAL	TOTAL
TOTAL	41	4	6	1	52
ADULTS	17	2	4	0	23
JUVENILES	24	2	2	1	29

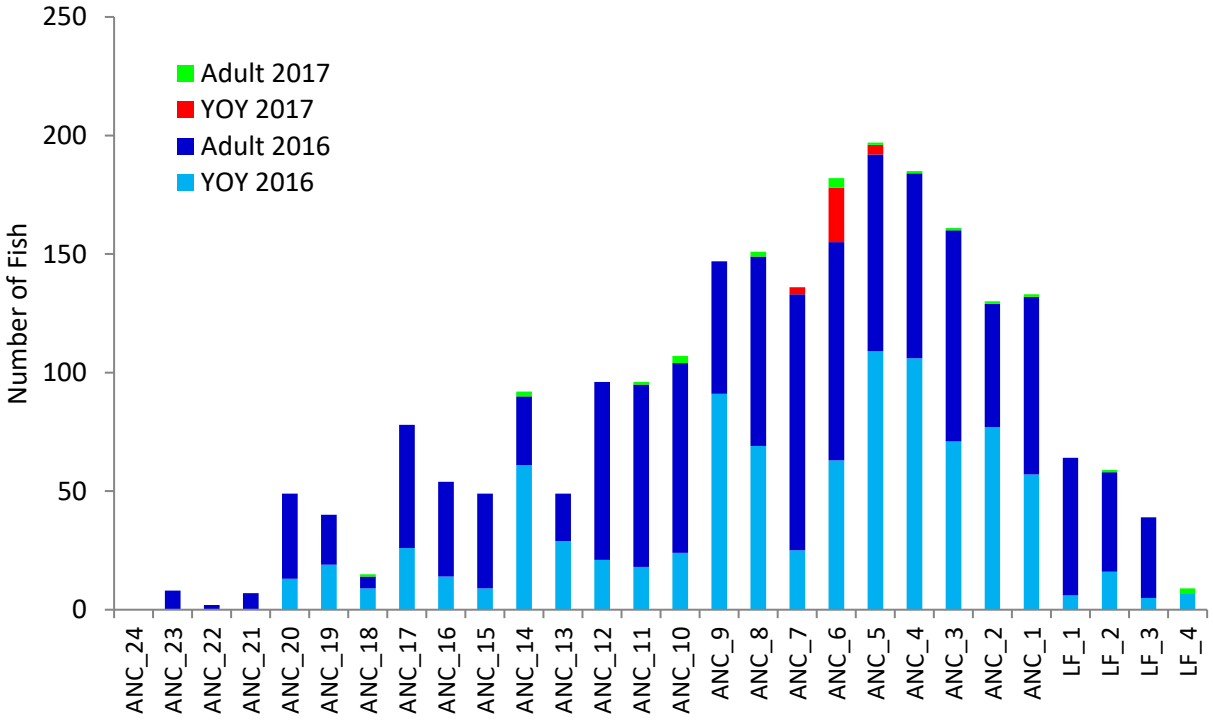

Figure 6. Number of Rainbow Trout removed by site and size class in 2016 and 2017 using backpack electrofishing gear during the Anthony Creek Brook Trout restoration project. Note the barrier mill dam was located at the bottom of site ANC_1 and the left fork Anthony Creek sites are labeled 'LF'. No fish were captured upstream of site ANC_23.

DISCUSSION

POST-REMOVAL FISH SAMPLING

Fish populations will be monitored via the 3-Pass Depletion method for the following 3 years, or until trout meet pre-project densities. The LF_ANC_2 and ANC_2 will be monitoring sites while ANC_0 will be used as a reference site during these years. In 2018, seven (7) locations were surveyed for census data; sites ANC_1, 3, 4, 5, 6, 7, and ANC_14. Total Brook Trout collected numbered 99 individuals; 24 YOY and 75 adult. It should be noted that 2018 was an extremely wet year and that frequent downpours and high water events made more extensive sampling of the restoration zone difficult.

REINTRODUCTION AND MONITORING OF NATIVE BROOK TROUT

The 2017 source population selected for this reintroduction came from Bunches Creek, a tributary of Raven Fork on the North Carolina-side of GRSM, which was chosen due to its high Brook Trout densities and evidence showing high levels of genetic diversity within the population. A total of 269 fish were removed from Bunches Creek, transported, and equally distributed between sites ANC_12D, ANC-17D, and LF_ANC_3D.

In June, 2018, GRSM Fishery Division and Trails Crew staff used Park-owned mules to help transport 237 Brook Trout from the upper Deep Creek watershed to HWY 441, where they were offloaded into a larger transport tank before translocation to Anthony Creek. There were 135 fish collected from Deep Creek and 102 fish collected from Sahlee Creek. These fish were distributed in the lower 1000m of the main stem of Anthony Creek. No fish experienced mortality during the transfer.

ACKNOWLEDGEMENTS

This Brook Trout restoration project was accomplished using volunteer time, financial assistance, and cooperative assistance of numerous agencies, groups, and individuals. We would like to thank the partnership assistance of the Tennessee Wildlife Resource Agency (TWRA), Trout Unlimited (TU), and the University of Tennessee. We are very thankful for the project funding provided by the Eastern Brook Trout Joint Venture (EBTJV), Duke Energy, U.S. EPA, the Tennessee Brook Trout License Plate Fund and Friends of the Smokies. These funds supported the seasonal employees and interns necessary to restore native Brook Trout to 1.6 miles of Anthony Creek.

In 2016, Trout Unlimited members donated 234-hours (29.25 man-days) to the project; TWRA assisted in 132-hours (16.5 man-days); while other volunteers represented by the Smoky Mountain Hiking Club, The Tremont Institute, American Conservation Experience (ACE) High School interns, Tennessee Technology University, The University of Tennessee, and Friends of The Smokies volunteered 318-hours (39.75 man-days) of their time. Partner project hours for 2017 are as follows: Trout Unlimited, 191-hours (23.8 man-days); Americorps interns, 32-hours (4 man-days); Friends of the Smokies, 11-hours (1.37 man-days); Tennessee Wildlife Resources Agency, 59-hours (7.38 man-days); University of Tennessee, 12-hours (1.5 mand-days); and non-affiliated volunteers, 22-hours, (2.75 man-days). These folks were essential 'boots on the ground' help to GRSM staff and invested plenty of 'sweat equity' during this project.

Special thanks also go to all the seasonal fisheries technicians and interns at Great Smoky Mountains National Park's Fishery Division who assisted with project preparation, setup, and implementation. Their professional attitudes, constructive

comments, and affirmative work ethics of everyone involved helped make the project a success.

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Native Brook Trout Restoration on Little Cataloochee Creek - Great Smoky Mountains National Park

2019 Project Report

June 2019

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INTRODUCTION

Historically, Brook Trout (*Salvelinus fontinalis*) were ubiquitously distributed across eastern North America from Georgia to Ontario and are the only salmonid native to the southeastern United States (U.S.). Once abundant in the southeastern U.S., the range of the Brook Trout has declined 75% in Great Smoky Mountains National Park (GRSM) since 1900, primarily due to extensive logging prior to creation of the park in 1934, followed by the introduction of non-native Rainbow Trout (*Oncorhynchus mykiss*), and more recently, acidic deposition. Today, southern Appalachian Brook Trout are often restricted to marginal headwater streams above 1,067m elevation (3,500 ft.), characterized by steep gradients and low stream pH. Continued acid deposition and nitrogen saturated soils have led to decreased stream pH, poor Brook Trout condition and reproduction, and loss of Brook Trout populations in seven headwater streams. These observations increase the urgency of restoring Brook Trout to lower elevation (<1,067 m.) streams that are not as susceptible to the impacts of atmospheric acidic deposition.

Approximately 25% of the remaining Brook Trout habitat designated for conservation on public lands occurs in GRSM; therefore, it is critically important that the Park expand the range where feasible. If successful, re-established fish in these streams could be used as a brood stock for future restoration projects in streams that have recovered sufficiently from acid deposition impacts so as to provide suitable habitat for Brook Trout. Completion of the Little Cataloochee Creek project will increase the quantity of streams that have been restored for native Brook Trout (53.3 km) in GRSM by approximately 12%.

OBJECTIVES

1. Remove non-native Rainbow Trout from 6.4 km (4.0 mi) of Little Cataloochee Creek with help from NC Wildlife Resources Commission, Trout Unlimited volunteers and NPS staff.
2. Collect as many native Brook Trout from Little Cataloochee Creek and its tributaries to retain while chemical restoration is in progress and re-release once piscicide treatment is complete.
3. Monitor Brook Trout populations in Little Cataloochee Creek and its tributaries during subsequent years to determine success of the project.
4. Assist Tennessee Tech University personnel with pre- and post-treatment aquatic insect surveys to determine potential impacts to trout food sources.

STUDY AREA

Little Cataloochee Creek is a fourth-order tributary to Cataloochee Creek located roughly 4.8 km (3.0 mi) southwest and upstream of Waterville Lake in Haywood County, North Carolina. Little Cataloochee Creek drains an area of 21.9 km^2 (8.46 mi^2) the entirety of which is located within Great Smoky Mountains National Park. The watershed is 98.9% forested with a maximum elevation of 5,550 ft. The restoration project location is distinguished by a natural waterfall, approximately 3 m in height,

downstream of the confluence of Coggins Branch (*Figure 1*). This area contains 12.7 km^2 (4.90 mi^2) of drainage which is 99.3% forested with a maximum elevation of 5,500 ft (*Table 1*).

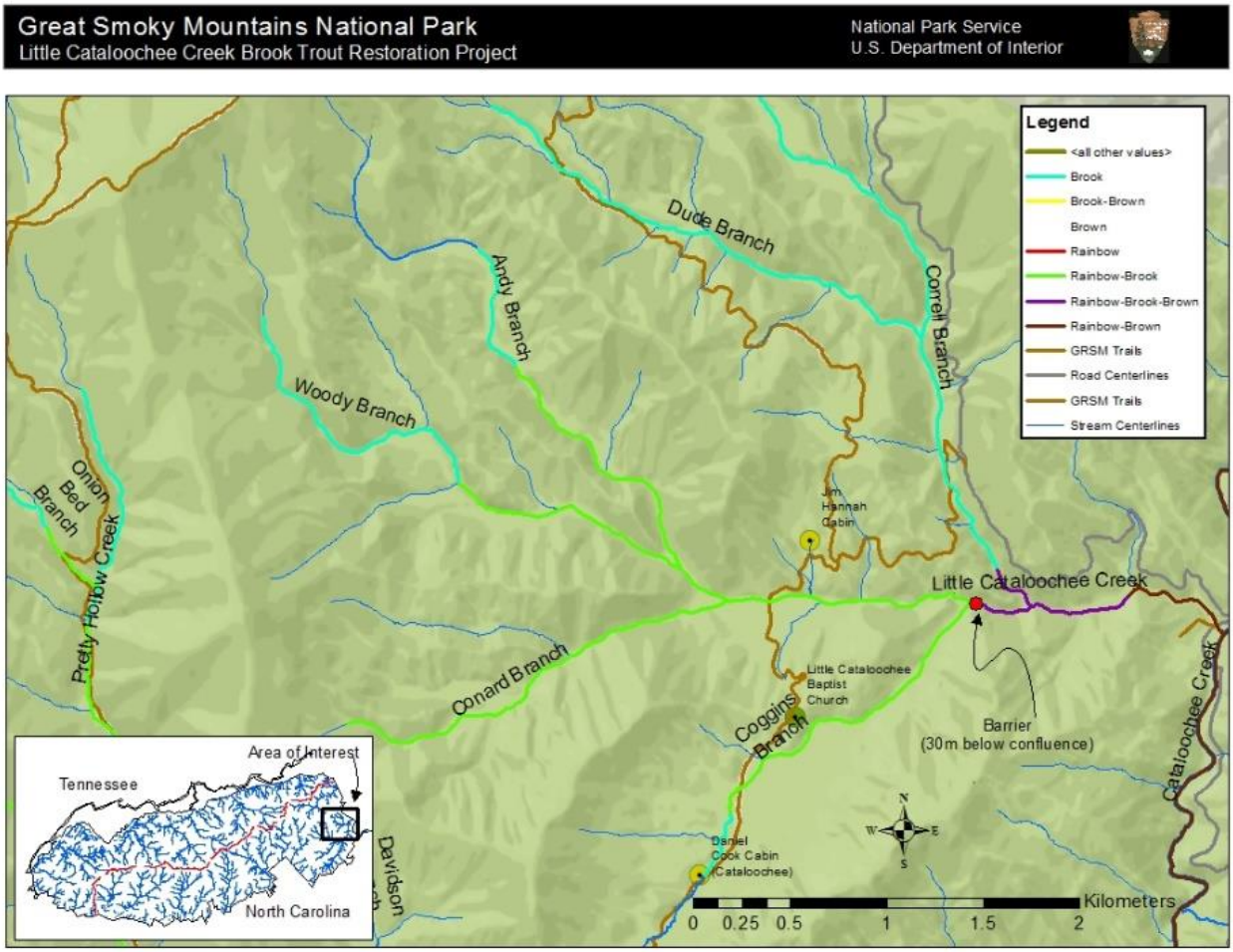


Figure 1: Little Cataloochee Creek project area with historic trout distributions.

Table 1: Basin Characteristics of Little Cataloochee Creek project area and sub-watersheds.

	Little Cataloochee Project Totals	Tributaries			
		Andy Branch	**Woody Branch**	**Conard Branch**	**Coggins Branch**
Drainage Area (km$^{2)}$	12.69	2.49	3.44	2.38	2.9
Maximum basin elevation (ft)	5,550	5,550	5,440	5,120	4,370
Minimum basin elevation (ft)	2,710	3,070	3,070	2,990	2,730
Percent (%) Forested	99.251	98.651	99.644	100	99.752
Mean Annual Precipitation (in)	60	62.4	63.8	60.1	55.5

METHODS

ANTIMYCIN BACKGROUND

Fishery staff determined Little Cataloochee Creek was too large to effectively restore using backpack electrofishing gear (project length of 6.4 km, roughly 2.5 times the stream length restored by electroshockers in Anthony Creek GRSM), therefore, the piscicide Antimycin-A was chosen as the tool for effective fish removal. Antimycin ($C_{28}H_{40}N_2O_9$) is an antibiotic produced in cultures of Streptomyces bacteria that is toxic to fish and sold under the trade name of Fintrol (*Figure 2*). It is an EPA approved piscicide that kills fish by inhibiting cellular respiration. The toxicity of antimycin is diminished by high alkalinity, low temperatures (<5°C), sunlight, and the metabolic activity of aquatic organisms. Antimycin has a half-life of only a few hours in fast moving non-acidic waters and neutralizes as aeration and tumbling break up its large molecules. Overall, Antimycin is less harmful to the macroinvertebrate community, crayfish, and salamanders, than the recommended lethal concentration of Rotenone. Lennon et al. (1971) stated that Antimycin is the ideal fish toxicant because of its selective effects: effectiveness at low concentrations, short exposure times, a wide range of water qualities, is not repulsive to fish (they don't sense its presence), effective on multiple size classes, and it leaves no residue. Bruce Rosenlund, a U.S. Fish and Wildlife Service biologist with many years of experience using Antimycin in the removal of non-native trout in Rocky Mountain National Park, noted that other advantages include lessened effect on eggs in the gravel substrate and colder water temperatures do not reduce the toxicity to fish (personal communication).

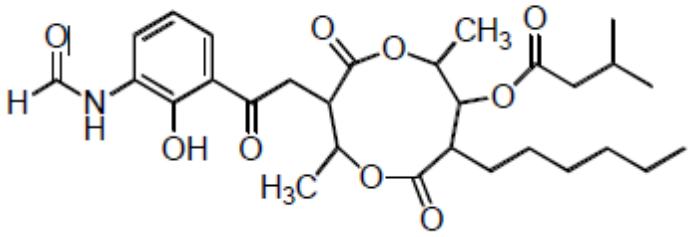


Figure 2: Diagram of the antimycin molecule ($C_{28}H_{40}N_2O_9$) used in Little Cataloochee Creek restoration activities, GRSM 2017.

DETOXIFICATION OF ANTIMYCIN

To restrict chemical effects to the desired zone only, a detoxification technique was also necessary. Potassium permanganate ($KMnO_4$) is a strong oxidizing agent that has been used for various purposes in agriculture, industry, medicine, and water treatment (Rose and Rose 1966). Walker (1967) pointed out that potassium permanganate could be used to detoxify the fish toxicant antimycin, however, Marking and Bills (1975) showed that potassium permanganate is toxic to trout at low concentrations (< 4 mg/l). On site toxicity tests at Sams Creek, GRSM, indicate that concentrations of 3 mg/l or less of potassium

permanganate resulted in zero mortality and that 4 mg/l resulted in only 20 percent mortality. These tests also showed that 2 mg/l of potassium permanganate are sufficient to detoxify antimycin in Sams Creek (Moore et al. 1998, unpublished data).

ENVIRONMENTAL PLANNING

As a unit of the National Park system, legislation mandates that Park resources are to be managed in such manner and by such means as will leave them unimpaired for the enjoyment of future generations (NPS Organic Act 1916). NPS Management Policies state that management of exotic (non-native) species, up to and including eradication, will be undertaken whenever such species threaten Park resources or public health and when control is prudent and feasible (NPS Management Policies 1988).

GRSM management staff determined that the eradication of non-native fish species and the reestablishment of native Brook Trout populations are feasible in select stream segments and that such action is required under its legislative mandates and management policies. Based upon the National Environmental Policies Act (NEPA), an Environmental Assessment (EA) was initiated in 1998 to investigate the use of antimycin to restore native Brook Trout in six GRSM streams. The Environmental Assessment for Using a Piscicide for Brook Trout Restoration in Great Smoky Mountains National Park was approved for use in Sams Creek on February 10, 2000. Follow-up monitoring demonstrated no effect to the aquatic community of Sams Creek; therefore, the remaining five streams could also be reclaimed for native brook trout using antimycin. Antimycin application to Little Cataloochee Creek was also conducted in compliance with North Carolina Department of Agriculture & Consumer Services, license/certificate number 031-9658.

LITTLE CATALOOCHEE RESTORATION

Prior to treatment, stream discharge (ft^3/sec), stream gradients (%), site elevations (m), and hydrological travel times (hr) were determined at multiple locations within the treatment area. Stream discharges were gathered each day to calculate the amount of antimycin needed to maintain toxicity of 8 ppb for 8 hours during treatment. Additionally, dye retention studies were used to determine water travel times for every 100 m (328 ft) of measured stream length to estimate the time required for the chemical to travel between treatment stations and coordinate the release of chemical at each station (*Table 2*). These data assisted with determining logistically feasible and effective daily treatment segments, as well as timing and quantities for detoxification station operation.

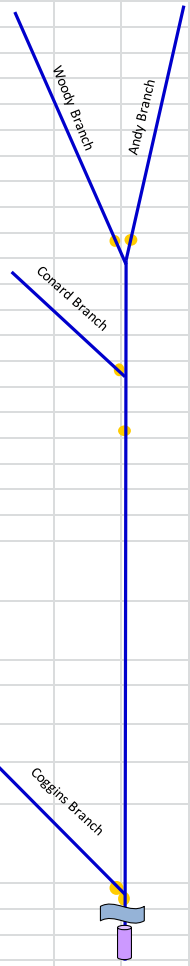
The effective range of antimycin in Little Cataloochee Creek was determined to be over a 50 ft. of vertical drop in elevation. With this parameter, aerial LIDAR mapping determined appropriate locations of treater stations. As a result, linear stream distances between treater stations ranged from 90-300 m (295-984 ft), with intervals tending to increase with progression downstream (*Figure 3*).

For logistic purposes, Brook and Rainbow Trout were used as indicator specimens in live-cages above each treater station to be observed during treatment. Cages were checked hourly during treatment and

the following morning to track chemical effectiveness. Flagging was attached to each cage to record notes such as: date, time, number of fish added, number of dead fish removed, and other observations.

Little Cataloochee Creek Restoration Project Map

Site #	Elev.	Flow (Q)	Time Per Site (min)	Cum. Time (min)	Notes
WDY_10					Treatment Start
WDY_9			8		
WDY_8			11		
WDY_7			9		
WDY_6			12		
WDY_5			11		
WDY_4			12		
WDY_3			12		
WDY_2			15		
WDY_1		1.39	15	105	
LCT_18					Site 18 is only 30m long
LCT_17			2		
LCT_16			7		
LCT_15			8		
LCT_14		3.75	7		
LCT_13			8		
LCT_12		3.11	8		
LCT_11			7		River right on large birch tree
LCT_10			4		River right on little buckeye
LCT_9			6		River Right on Poplar
LCT_8			6		River left, on 'Y' strunk birch tree
LCT_7			7		tree. On opposite stream bank is large rotting stump. A potential braid here during higher flows
LCT_6			6		birch - Historic stream channel appears to be off river right side. May be a braid during higher flows
LCT_5			7		River left, large buckeye
LCT_4			8		River left, on small tulip. Large, slanted, bedrock cascade
LCT_3			6		River left, on maple tree. Immediately at upstream start of braid
LCT_2			8		River right, on large birch tree with root sprouts. Large flat rock at base of tree
LCT_1			9		River right, on birch tree - an existing tag was already at this site. It was marked as LCT_2D. This tag was left in place and the LCT_1D tag was placed directly above it
LCT_0		6.42	8	122	Very treacherous around falls



Site #	Elev.	Flow (Q)	Time Per Site	Cum. Time
AND_8				
AND_7			7	
AND_6			9	
AND_5			11	
AND_4			9	
AND_3			14	
AND_2			10	
AND_1		1.06	18	78

Site #	Elev.	Flow (Q)	Time Per Site	Cum. Time
CON_8			9	
CON_7			11	
CON_6			11	
CON_5			14	
CON_4			14	
CON_3			13	
CON_2			13	
CON_1		0.97	11	96

Site #	Elev.	Flow (Q)	ime Per Sit	Cum. Time	Notes
COG_8			12		Start Treatment
COG_7			17		
COG_6			21		
COG_5			23		
COG_4			23		
COG_3			23		
COG_2			21		
COG_1		1.68	20	160	

Legend

- Flow Monitoring Site
- Detox Site
- Barrier
- Stream Segment *with* RBT

Detox

- 15 gal of $KMnO_4$/water applied per hour
  - Need 946 ml/min, OR
  - *Need 237 ml/15sec*

example:

Detox Station cfs = 3.0 cfs

- $KMnO_4$ for 4ppm for 1 hr = 1224g/hr
- Mix 2,448 g in each 30-gal drum
- Apply at 15gal/hr

*** Sprayer Uses 10% of TOTAL DAILY Antimycin ***

*** Non-Oxyl-9 Applied at 13% of ml Antimycin ***

Figure 3: Little Cataloochee Creek dye retention times and discharge data.

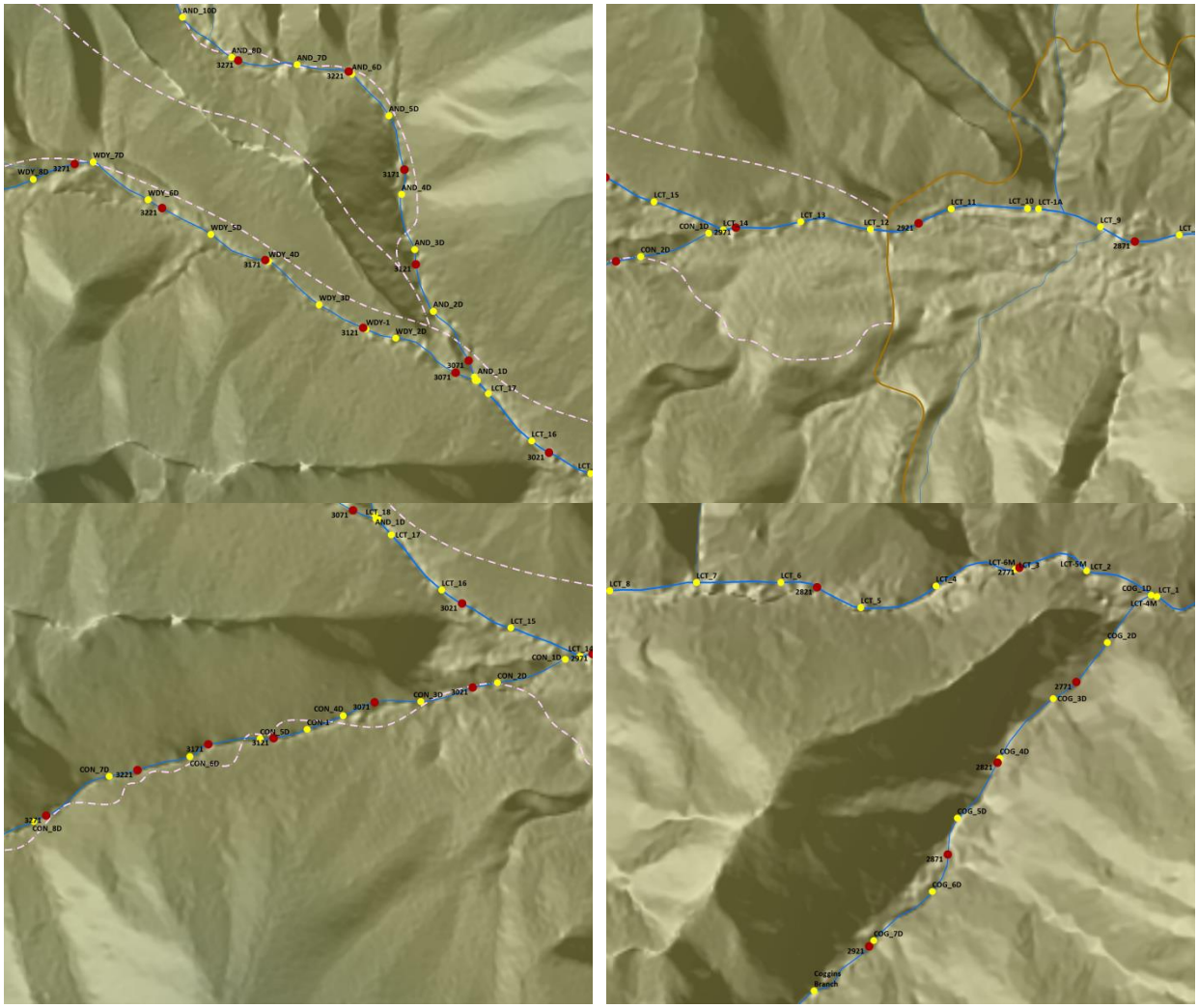


Figure 4: Distribution of antimycin treater stations in Little Cataloochee Creek project, September 2017. From clockwise in the upper left, are treater locations for: Woody and Andy Branch, Little Cataloochee Creek, Coggins Branch, and Conard Branch.

RESULTS

ANTIMYCIN TREATMENT

Antimycin treatment began on the upstream areas of Woody Branch and Andy Branch on September 8, 2017. Treatment proceeded downstream to the main stem of Little Cataloochee Creek and Conard Branch on September 9-10, 2017, treating 675-1,275 m stream segments per day (*Table 2*). Treatment was suspended on September 10, 2017 due to safety concerns related to Hurricane Irma and resumed/completed on Little Cataloochee Creek and Coggins Branch on September 19, 2017.

A total of 3,057 mL of Antimycin was administered throughout the treatment (7 units). Many trout appeared to show signs of Antimycin impact after 1 hour of exposure (loss of color or 'ghosting'). The majority of sites observed total expiration after 8 hours of exposure with remaining sites presenting 100% mortality the morning after treatment (i.e. 16-24 hours post-treatment).

Table 2:-- Summary of Antimycin and Potassium permanganate volumes used during the Little Cataloochee Creek restoration project.

Date	Number of Stations	Total Antimycin (ml)	Water Temp.°C	Maximum Discharge (ft^3/sec)	Stream Length Treated (m)	Conc.$KMnO_4$ (ppm)/hours	Total $KMnO_4$ Applied (g)*
9/8/17	8	1,012	11-13	2.0	1,275	0	0
9/9/17	9	1,075	10-14	3.0	1,205	0	0
9/10/17	3	490	12	3.0	805	0	0
Treatment suspended due to Hurricane Irma							
9/19/17	4	480	13-15	4.0	675	4ppm/8hrs	14,688

*** No $KMnO_4$ used during first 3 days of treatment because treated portion was in upper end of project site, providing sufficient time for natural breakdown of Antimycin before leaving the project area**

DETOXIFICATION OF ANTIMYCIN

Based upon the results of previous antimycin restoration projects, detoxification was delayed until treatment stations were within 100 vertical feet of the detox station, equating to just over 0.4 km (0.25 mi) of the project site. On September 19, 2017, the detox station was in operation as the lower 200 m of the Little Cataloochee Creek project area and 450 m of Coggins Branch were treated. Based on the initial stream discharge of 4.0 ft^3/sec, 1,632 g of potassium permanganate was needed per hour for 8 hours to maintain 4 ppm. As such, a total of 14,688 g of potassium permanganate was applied to complete the restoration attempt.

DISCUSSION

REINTRODUCTION OF NATIVE BROOK TROUT

Prior to treatment, Little Cataloochee Creek had a sympatric trout population of Rainbow and Brook Trout. To maintain genetic integrity within the project site, steps were taken to retain these Brook Trout during treatment for reintroduction after treatment was complete. Brook Trout in each tributary to Little Cataloochee Creek were collected by electro-fishing and relocated into pools above the most upstream treatment station on each respective branch. Additionally, roughly 400 Brook Trout from the main stem were placed in a portable, 1,000 gal holding tank on September 7, 2017 and returned to the upper portion of Little Cataloochee Creek on September 10, 2017.

PRE- AND POST-TREATMENT FISH MONITORING

Original distribution surveys were conducted in the Little Cataloochee Creek watershed in 1990. Prior to the 2017 restoration effort, Rainbow Trout distributions were verified by GRSM staff in both 2016 and 2017 to ensure treatment would encompass complete range of Rainbow Trout within the project area.

Pre-treatment 3-pass depletion surveys were conducted on Little Cataloochee Creek and Woody Branch in 2012, 2015, and 2017. Total trout density at site LCT-10 (Little Cataloochee Creek 1 km upstream of the treatment barrier) in 2017 was roughly 20 fish/100m^2 (14.6 and 5.4 fish/100m^2 for Brook and

Rainbow Trout) (Figure 5). Brook Trout densities in Woody Branch at WDY-1 (Woody Branch site 1) for Brook and Rainbow Trout were 16.1 and 0.5 fish/100m^2 during the 2017 sampling period (Figure 6).

Post-treatment population surveys began in 2018 and will continue in Little Cataloochee Creek and Woody Branch annually for 3 years or until Brook Trout populations reach pre-treatment levels of all trout. No Rainbow Trout were found in either Little Cataloochee Creek or Woody Branch during post-treatment surveys. Total Brook Trout densities in Little Cataloochee sites LCT-7, 10 and 16 ranged from <1-12.1 fish/100m^2 (Figure 5). Brook Trout may be relocated from the upper reaches of Little Cataloochee headwater streams to the treatment portion in 2019 in order to accelerate recovery.

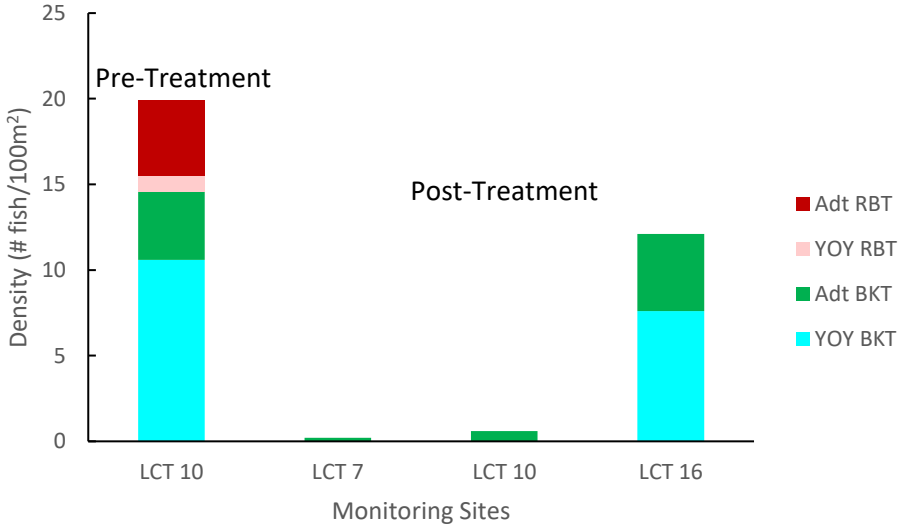


Figure 5.— Pre- and post-treatment densities (# fish/100m^2) at three monitoring sites located in Little Cataloochee Creek. Note site numbers represent each 100m site above the barrier falls.

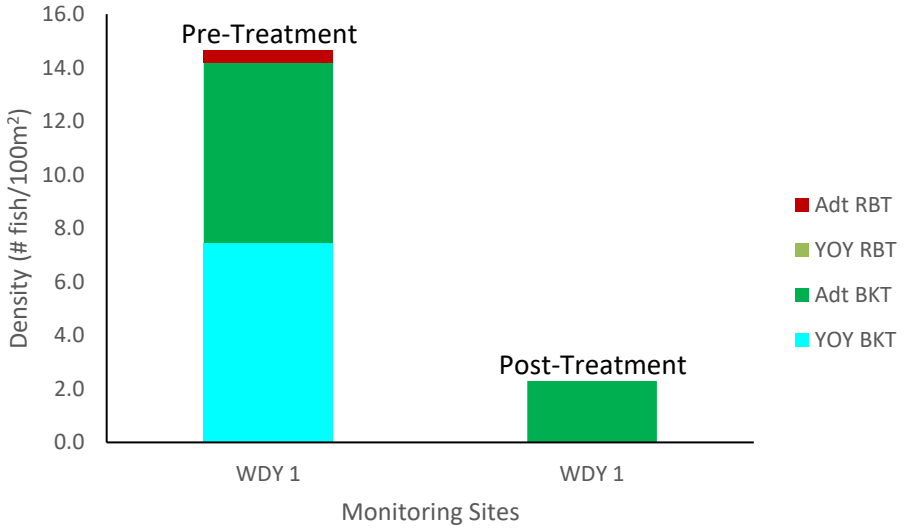


Figure 6.— Pre- and post-treatment densities (# fish/100m^2) at monitoring site located in Woody Branch, an upper tributary to Little Cataloochee Creek. Note Woody Branch site 'WDY-1' is located approximately 300m upstream of the confluence with Little Cataloochee Creek.

AQUATIC INSECT SURVEYS

In cooperation with GRSM, Tennessee Tech University (TTU) is conducting a long term pre- and post-treatment survey of Little Cataloochee Creek to better evaluate the effect of antimycin and potassium permanganate on the benthic macroinvertebrate community.

Nine (9) sites were sampled in the Little Cataloochee Creek watershed (Figure 7) for long-term monitoring after Brook Trout restoration that was conducted in September 2017. Macroinvertebrate assemblages were assessed in October 2015 (2y-Pre), October 2016 (1y-Pre), August 2017 (1m-Pre), September 2017 (1w-Pre, 1d-Post, and 1w-Post), October 2017 (1m-Post), and October 2018 (1y-Post). Three replicate samples were collected from each site. Nutrient and periphyton samples were collected in all samples from August-October 2017 to determine additional short-term effects of antimycin treatment on aquatic biota. The potassium permanganate detoxification site below the confluence of Little Cataloochee Creek and Correll Branch was not sampled in 2015 due to low light conditions and safety concerns. However, this site was sampled during all other sampling events.

All sampling for the project was completed in 2018, with 1y-Post treatment samples collected in October 2018. All macroinvertebrate samples have been processed, organisms have been separated from debris, and individuals have been sorted and identified to order. One and two-year pre-treatment samples have been identified to the lowest taxonomic status possible (i.e., genus). All nutrient and periphyton samples have been processed and these data being analyzed. The remaining macro-invertebrate samples are being identified to the lowest possible taxonomic status for final analysis.

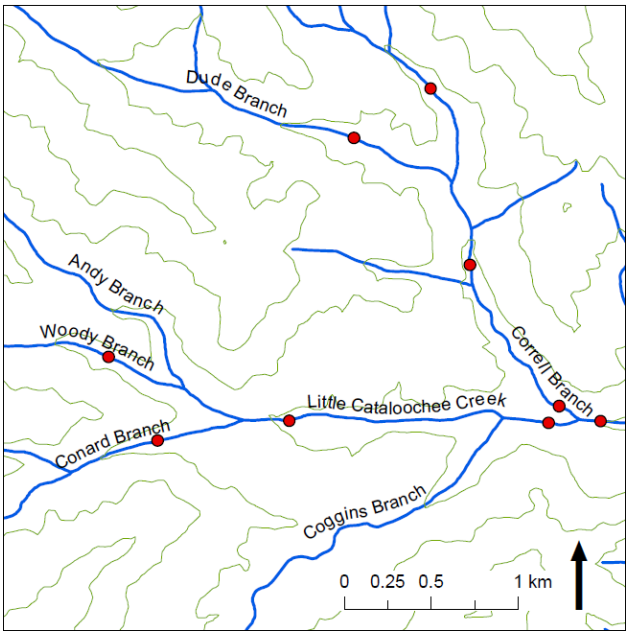


Figure 7.—Macroinvertebrate sampling locations in Little Cataloochee Creek watershed

Non-metric multidimensional scaling (NMDS) depicts post-treatment separation between control and treatment and detoxification sites 1 week and 1 month after treatment (Figure 8) indicating a short term shift in macroinvertebrate assemblages in response to antimycin and potassium permanganate detoxification. We conducted an Analysis of Similarity (ANOSIM) among control, treatment, and detoxification samples over time for major macroinvertebrate taxonomic orders [i.e., Coleoptera (beetles), Diptera (flies), Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)] using Primer Statistical Software (2016). These analyses showed a significant difference between control and treatment sites in pooled data across all times ($p = 0.001$), a significant difference between control and detoxification sites ($p = 0.002$), and a moderately significant difference between treatment and detoxification sites ($p = 0.098$).

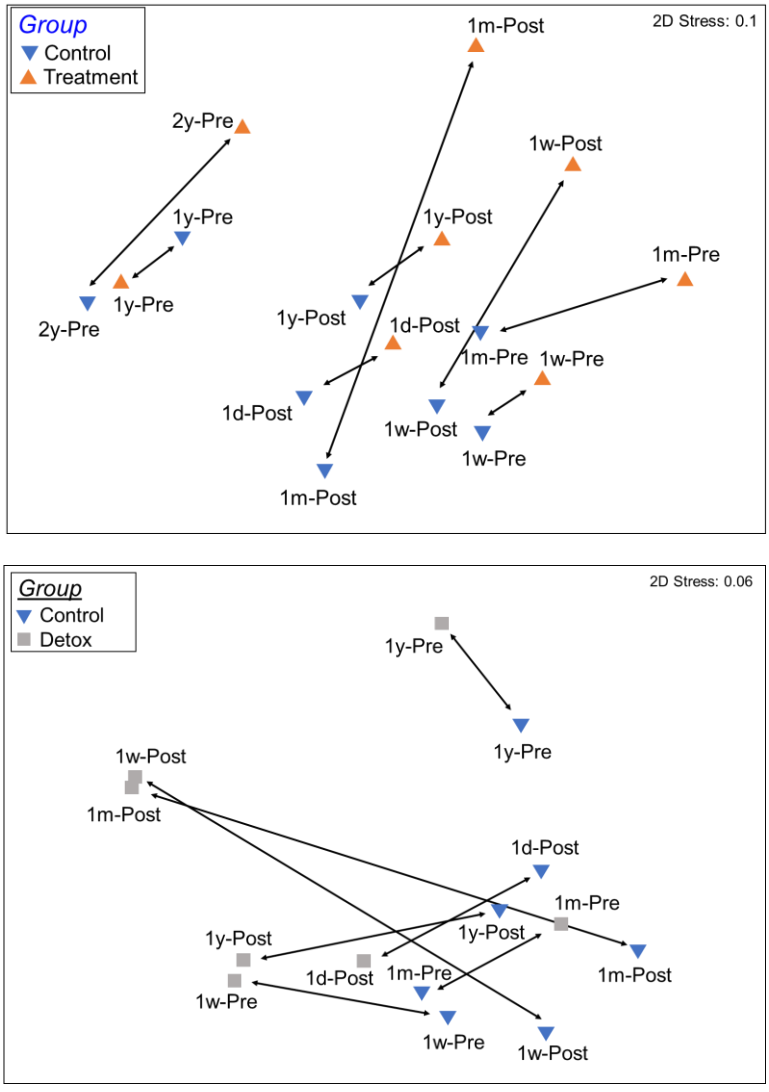


Figure 8. Non-metric multidimensional scaling of macroinvertebrate samples, identified to taxonomic order, collected in the Little Cataloochee Creek (Treatment and Detox) and Correll Branch (Control) subwatersheds before and after September 2017 antimycin application. Arrows indicate samples collected during the same period.

Mixed models were analyzed using R version 3.5.2 to determine effects of antimycin application and detoxification on the macroinvertebrate orders over time (Figure 9). Based on global NMDS results (i.e., Control vs. Treatment vs. Detox), sampling times were pooled as Long-term Pre-Treatment (2y-Pre and 1y-Pre), Immediate Pre-Treatment (1m-Pre and 1w-Pre), Immediate Post-Treatment (1d-Post and 1w-Post), and Long-term Post-Treatment (1m-Post and 1y-Post) to determine effects among the three groups during each time period. Ephemeropterans were significantly different from pre-treatment controls in immediate post-treatment samples for both treatment and detoxification sites ($p = 0.0219$ and $p = <0.0001$, respectively). Ephemeropterans remained significantly different in long-term detoxification sites ($p = <0.0001$). Plecopterans also were significantly different from pre-treatment controls in immediate and long-term post-treatment detoxification sites ($p = 0.0153$ and $p = <0.0001$, respectively). Collectively, these analyses suggest a significant, short-term reduction of Ephemeropterans after antimycin exposure with recovery within 1 year (Figure 8). Similar reductions in Ephemeropterans and Plecopterans occurred after exposure to potassium permanganate, but were still lower than pre-treatment conditions after one year (Figure 9).

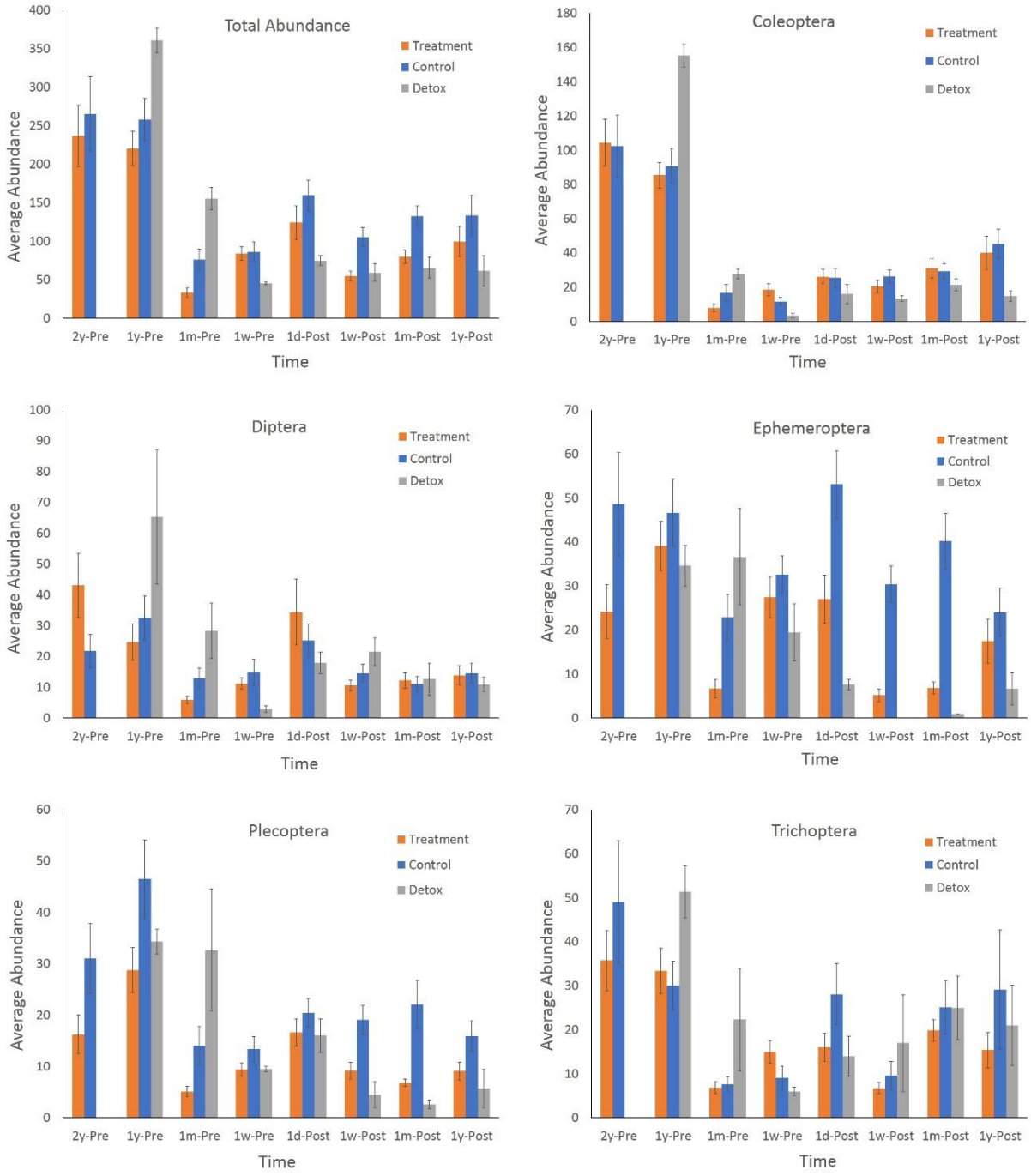


Figure 9.— Comparisons of major taxonomic orders of macroinvertebrate samples collected in Little Cataloochee Creek (Treatment and Detox) and Correll Branch (Control) subwatersheds before and after September 2017 antimycin application.

ACKNOWLEDGEMENTS

Brook Trout restoration projects could never be accomplished without the volunteer time, financial assistance, and cooperative assistance of numerous agencies, groups, and individuals. On behalf of over 11 million annual visitors and the staff of Great Smoky Mountains National Park, we would like to thank

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