2013-2014 EMBRACE-A-STREAM
PROJECT EVALUATION & REPORT (3/3/2015 Update)

General Information

Chapter or Council Name & State: **Little River Chapter (#644), Maryville, TN**

Name of Project, Stream, or Watershed:
(2013) Identification of Metapopulations of Brook Trout in the Little River Watershed & (2014) Insuring Genetic Integrity and Long Term Survival of Brook Trout in Source and Restored Streams

Chapter or Council Contact Information:

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Financial Information

Date of Award: March 2013; May 2014

EAS Grant Amount: $4,800 (2013); $5,000 (2014)

Total Project Cost: $75,577 (2013); $66,036 (2014)

Total Volunteer Hours: 140 (2013); 292 (2014)

Did the Embrace-A-Stream grant leverage or help match any additional funding?

Yes___X____ No_____ If so, please clarify in the table below.

Source of Leveraged or Matched Funds	2013 Amount	2014 Amount
NPS	$22,959	$24,149
USGS Labor, Analysis and Supplies	$26,104	$26,887
TN Council TU (license plate sales)	$10,000	$10,000
Project Total	**$ 59,063**	**$61,036**

Outcomes and Evaluation

All TU chapters and councils should evaluate the impact of their projects. Evaluation involves identifying and measuring specific indicators to demonstrate the impacts of your work. To truly evaluate a project you must measure conditions before you begin work and after the project is completed. Examples of indicators are water flow or fish populations. Other volunteers may want to evaluate their projects by measuring the number of new members gained, the amount of publicity generated, or the increase in participant conservation knowledge. The indicators for your project will depend on what you are trying to accomplish. Please feel free to contact Rob Roberts (703.284.9424 or rroberts@tu.org) with questions on project evaluation.

Please list the specific objectives of the project. What did you want to accomplish?

The objective of the study in the upper Little River watershed (Little River proper & Goshen Prong sub-watersheds) by Great Smoky Mountains National Park (GRSM) was to determine if connected headwater populations are functioning as metapopulations. This would be accomplished by collecting Brook Trout tissue samples from a minimum of six locations in the upper segments of the Little River watershed and three locations in the Goshen Prong portion of the watershed. The primary objectives are:

1. To evaluate the relative genetic health of previously un-typed Brook Trout populations by determining the effective population size (N_e); and
2. To determine if these populations are functioning as a metapopulation because of the connectivity between streams.

Please list the indicators used to evaluate these objectives.

***Indicator 1 (2013)*:** Have effective population size estimates been calculated for headwater Brook Trout populations with tissue collected from a minimum of 30 individuals across a significant portion of the distribution in each stream segment?

Before: Some effective population estimates have been calculated for GRSM populations; however, most of these samples were of a small sample size (<25 individuals) sampled within a short segment of stream.

After: GRSM fisheries crews collected at least 30 tissue samples across a 300m reach of 30 different stream segments in the upper Little River (2013) and Goshen Prong (2014) watersheds (Figure 1 & 2). Samples were delivered to the USGS Science Center lab in Leetown, WV where some of the DNA has been extracted, but many more populations remain to be analyzed. Effective population size analyses are being conducted by PhD candidate Casey Weathers at Penn State University in support of a PhD Dissertation. Analyses have not been completed to date; however, samples are being processed and researchers anticipate final results by 31 April 2015.

***Indicator 2 (2013)*:** Are GRSM Brook Trout populations in the upper EP Little River and Fish Camp Prong watersheds functioning as metapopulations?

Before: Although some Brook Trout tissue collections have been made for genetics analyses, data has not been collected that would reliably determine if Brook Trout populations in select watersheds were functioning as a metapopulation.

After: Data was collected in the upper EP Little River (2013) and Goshen Prong (2014) watersheds that will reliably determine if Brook Trout populations are functioning as a metapopulation. Metapopulation structure analyses are being conducted by PhD candidate Casey Weathers at Penn State University in support of a PhD Dissertation. Analyses have not been completed to date; however, samples are being processed and researchers anticipate final results by 31 August 2015.

Indicator 1 (2014): Complete genetic analysis on Brook Trout tissue samples from Toms Creek, Indian Camp Creek, Cosby Creek and Greenbrier Creek, and compare the results to previous samples from those streams to determine if the effective population size (N_e) of the source streams has been altered. ***Note:*** *we changed our study streams to those mentioned above as these streams had more numerous prior collections (3-4) and afforded a more statistically robust analysis of effective size changes over time.*

Before: Previous genetic analyses had been conducted on Cosby Creek (2000, 2004, 2009), Greenbrier Creek (2000, 2004, 2009), Indian Camp Creek (2000, 2009) and Toms Creek (2009), providing comparative effective population size data. Although these collections had been made, no attempts have been made to determine the trajectory of effective population size in these streams. Further collections were made in 2014 in order to compare effective population size over time. These data were compared to the number of brook trout removed from each study stream for transplant into recently restored stream segments (Table 1).

After: Effective population size (N_e), is defined the actual number of adults that are passing on their genes to the next generation. Effective population size was determined for the four previously mentioned source streams used as founder stocks for the Lynn Camp Prong restoration. Geneticists at the USGS Leetown Science Center, WV, determined that after multiple stock removals from those streams, repeated removals had *not* significantly altered the effective population size of the founding Brook Trout populations (Table 2), nor the allelic richness of any populations (Figure 3). These results will be very useful for guiding future restoration projects and guiding management decisions for not only GRSM, but other agencies working to restore Southern Appalachian Brook Trout.

Indicator 2 (2014): Complete genetic analysis of Brook Trout tissue samples from Lynn Camp Prong prior to the supplemental stocking of Brook Trout to determine if the existing effective population size (Ne) is similar to that of the source streams.

Before: Numerous transplants of Brook Trout have occurred since 2000 from the source streams listed above as well as others across the park. Effective population size values were calculated for source streams; however, these analyses have not been conducted for the founder population (Lynn Camp) since the transplants have been in place. Our hope is that the effective population size of the Lynn Camp population is greater than those of the source streams.

After: Transplanted individuals need at least one year opportunity (post introduction) to spawn after transplant into the restored stream segments. Fish were removed from Cosby and Toms Creek in 2014 and transplanted into Lynn Camp Prong (Table 1). Collections will be made on Lynn Camp Prong in 2015 in order to address this indicator. Results will determine if the effective population size of Lynn Camp Prong (founder population) is significantly greater than the effective sizes of the source populations.

If you did not achieve your target objectives, please briefly explain why.

2013 Objectives: Originally, samples were to be collected from both the upper Little River and upper Goshen Prong watersheds; however, excessive summer rains in 2013 (wettest year on record for region) precluded the samples from being collected in the Goshen Prong watershed. The campsites for Little River and Fish Camp Prong, from which staff will base operations to collect tissue, are 5.7 and 6.6 miles from the trailhead. Logistically, acquiring samples from these remote areas is a challenge in a typical weather year. Goshen Prong samples were successfully collected in 2014 and sent to the USGS lab for analyses. Sample processing has been progressing very well, although final genetic analyses have not been completed. Metapopulation structure analyses are challenging and require a series of comparative analyses in order to prepare final results. Accurate final results will take time and diligent guidance from USGS and PSU geneticists to ensure data are properly interpreted and checked for data quality. Final results are anticipated by 31 August 2015.

2014 Objectives: Target data collection objectives were met; however, we are awaiting final results of the effective population analyses. Final results are anticipated by 31 April 2015. Lynn Camp Prong collections will be made in 2015, so that the founder populations have an opportunity to spawn and pass their genes onto the Lynn Camp population.

Other information

Did you collaborate with TU field staff during the implementation of this project? If so, please describe briefly.

- ***2013:*** LRCTU members were scheduled to participate in sample collections on both watersheds; however, record rainfall precluded one watershed to be completed (Fish Camp Prong) in 2013. In addition, one watershed was rescheduled so many times that volunteers were unable to assist when the sampling effort was finally completed during one of the few open weather windows. LRCTU members participated in sample collections in the Goshen Prong watershed in 2014. Although two were scheduled, only one was able to stay for the four days required to collect all samples totaling 48 hours of volunteer service.
- ***2014:*** Numerous TU members and other park volunteers assisted with collection of Brook Trout fin clips to address the 2014 EAS study goals. Members helped collect clips via electrofishing, transport fish to the work up area, clip fins and record data for each collection. Total volunteers hours for this project in 2014 were 288 hours.

Do you have any plans to continue or expand the project? If yes, please describe.

Depending upon the metapopulation results, we may look at other watersheds to see if there is metapopulation structure in them, as well. In addition, if we determine that repeated transplants of Brook Trout from the source streams have caused declines in the effective population sizes, we will repeat the analyses in future years to monitor population recovery.

Please describe any publicity the project received, including dates, source, and type of coverage. *(Please attach any press clippings.)*

- The study design and results were presented at the 24th Annual Wilderness Wildlife Week and will be presented at the 25th Annual Wilderness Wildlife Week, numerous Trout Unlimited meetings in TN and NC, as well as, local presentations to NPS and other local park visitors.
- The study design was presented at the Little River Chapter of Trout Unlimited (#644) meeting in January 2015.

Other comments:

- **Mail** one copy to Rob Roberts, Trout Unlimited, 1300 N. 17th St, Suite 500, Arlington, VA 22209; or,
- **Fax** to Rob Roberts at 206-222-2381; or
- **Email** the Microsoft Word, PDF, or scan to eas@tu.org.

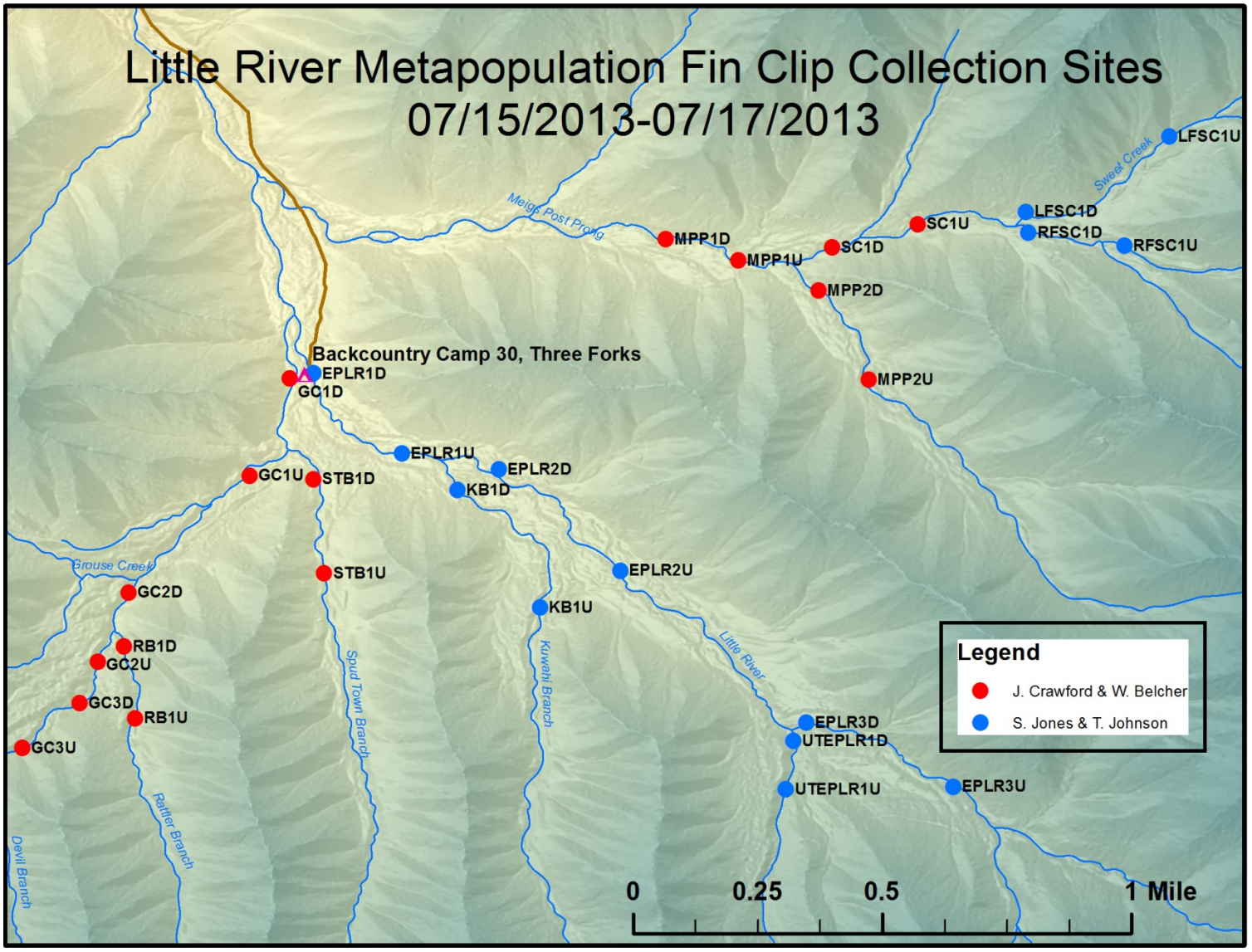

Figure 1.— Map of upper East Prong Little River watershed illustrating where Brook Trout fin clips were collected in order to estimate effective population size (Ne) and determine if metapopulation structure exists in the watershed.

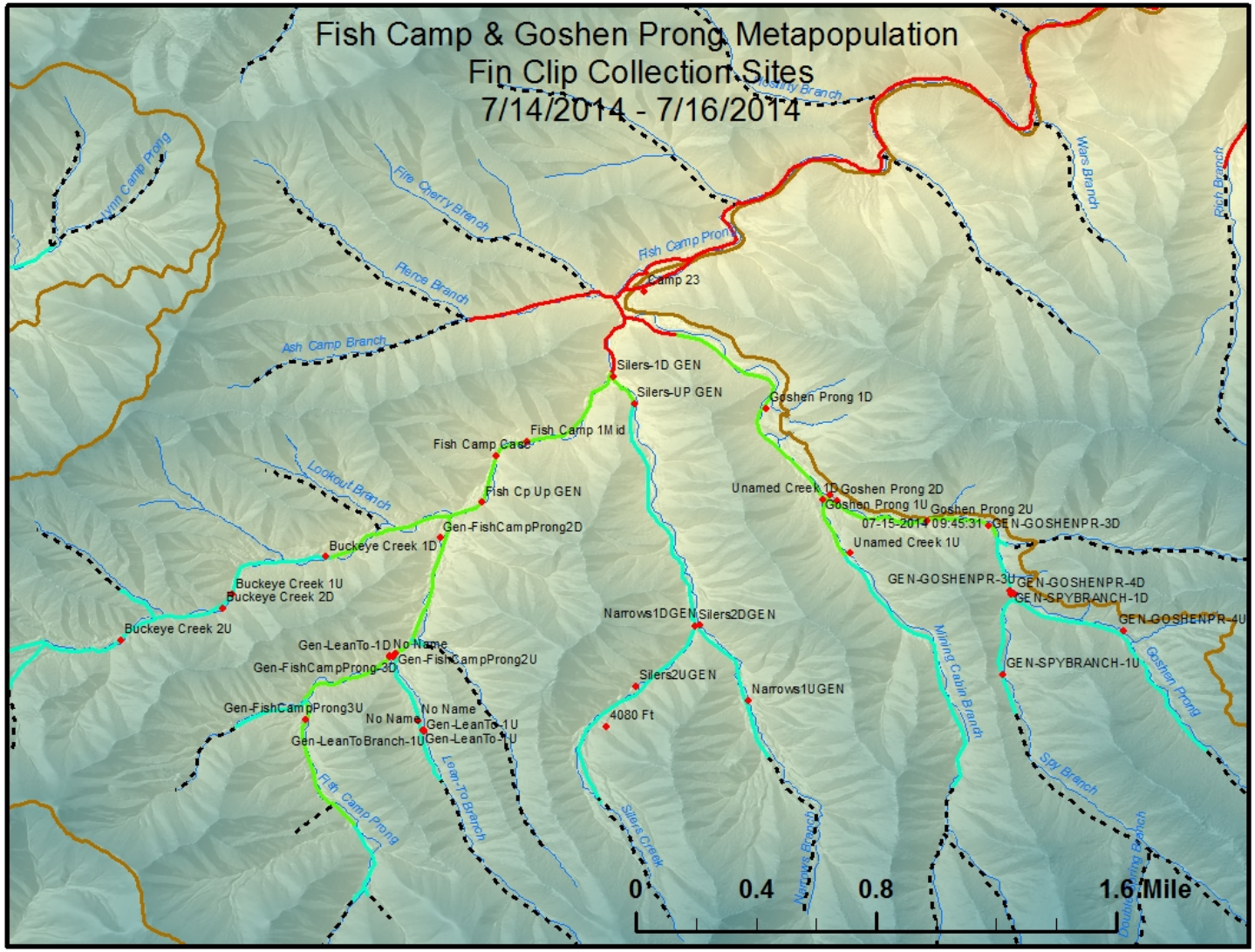

Figure 2.— Map of upper Goshen Prong watershed illustrating the upper and lower limits of where Brook Trout fin clips were collected in order to estimate effective population size (Ne) and determine if metapopulation structure exists in the watershed.

Figure 3. Figure depicting the allelic patterns of four Southern Appalachian Brook Trout populations over the course of 14 years following repeated removals of all size classes for transplant to recently restored stream segments. All fish were genotyped at 13 microsatellite DNA loci. N_A = the average number of alleles per locus; A_e = the effective number of alleles per locus; No. Private Alleles = the number of private alleles per locus; and H_E = the expected unbiased heterozygosity.

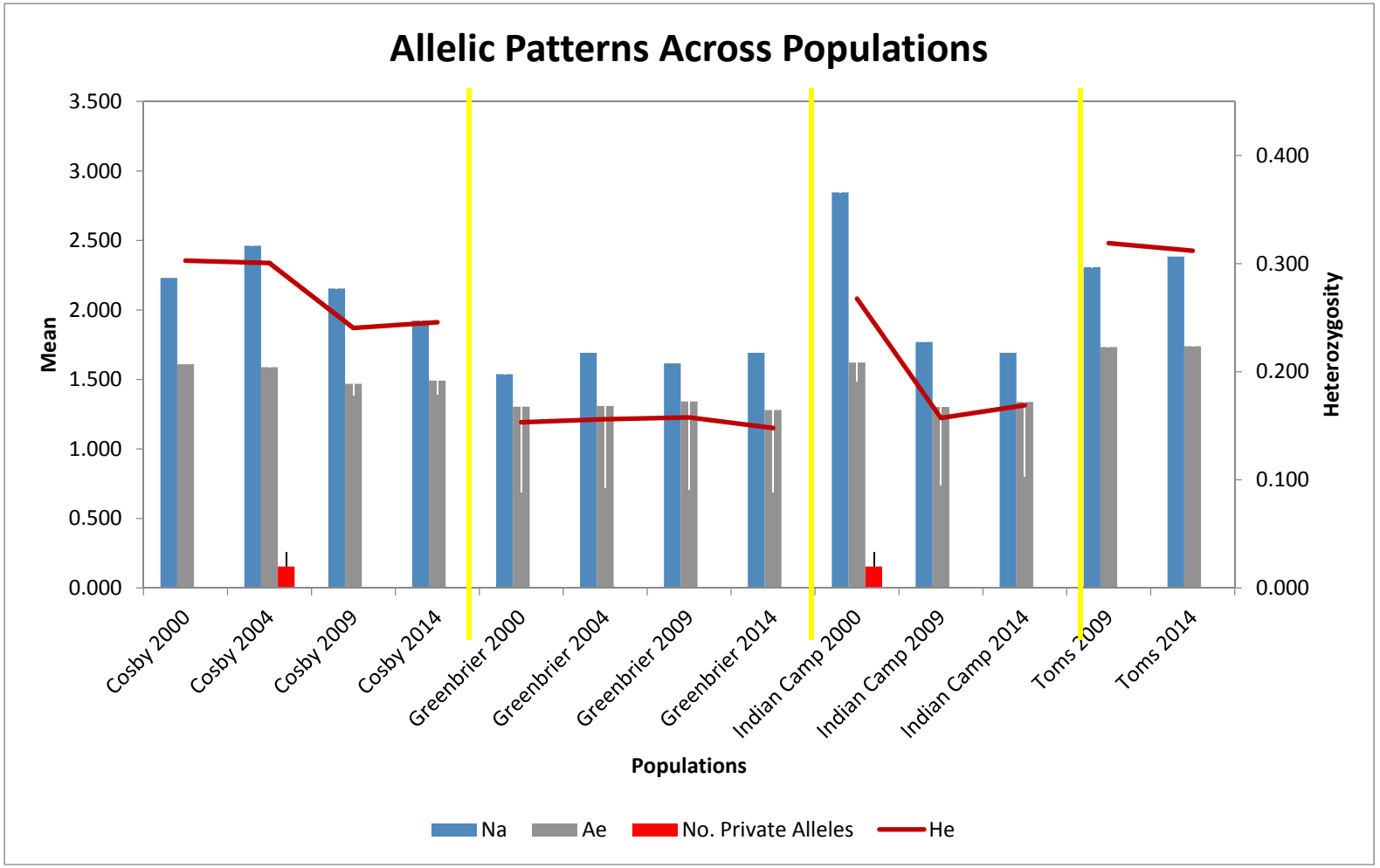

TABLE 1.—Numbers of Southern Appalachian Brook Trout collected and transplanted to various restoration streams between 1996 and 2014 within Great Smoky Mountains National Park.

Source Stream	*Date*	*Number of SABT Removed*	*Receiving Stream*
Cosby Creek	Sep-99	111	Leconte Creek
Cosby Creek	Jun-04	65	Sams Creek
Cosby Creek	Jun-06	50	Sams Creek
Cosby Creek	Jul-09	132	Lynn Camp Prong
Cosby Creek	May-14	161	Lynn Camp Prong
Indian Camp Creek	Oct-96	50	Mannis Branch
Indian Camp Creek	Sep-99	106	Leconte Creek
Indian Camp Creek	Jun-06	50	Sams Creek
Indian Camp Creek	Sep-12	112	Mannis Branch
Little Greenbrier Creek	Oct-96	55	Mannis Branch
Little Greenbrier Creek	Sep-99	103	Leconte Creek
Little Greenbrier Creek	Jun-04	65	Sams Creek
Little Greenbrier Creek	Jun-06	50	Sams Creek
Toms Creek	Jul-09	132	Lynn Camp Prong
Toms Creek	Jul-09	191	Lynn Camp Prong
Toms Creek	Sep-11	399	Lynn Camp Prong
Toms Creek	May-14	412	Lynn Camp Prong

TABLE 2.—Diversity statistics for 13 wild Brook Trout collections (N=429) sampled among four streams utilized for supplemental and restorative stocking. All fish were genotyped at 13 microsatellite DNA loci. N_A = the average number of alleles per locus; A_e = the effective number of alleles per locus; A_P = the average number of unique alleles per locus; H_E = the expected unbiased heterozygosity; F = the inbreeding coefficient; and N_e = the effective population size. Mean values across all loci and populations are given with standard error and 95% confidence interval (N_e) values provided in shaded columns.

Collection	N_A	N_A SE	A_e	A_e SE	A_P	A_P SE	H_E	H_E SE	F	F SE	N_e	$L\ N_e$	$U\ N_e$
Cosby 2000	2.2	0.3	1.6	0.2	0.0	0.0	0.30	0.07	-0.010	0.046	13.6	3.3	78.9
Cosby 2004	2.5	0.3	1.6	0.1	0.2	0.1	0.30	0.06	0.048	0.036	21.2	5.4	153.3
Cosby 2009	2.2	0.2	1.5	0.2	0.0	0.0	0.24	0.06	-0.091	0.022	45.0	9.1	170.0
Cosby 2014	1.9	0.2	1.5	0.2	0.0	0.0	0.25	0.07	-0.067	0.054	17.9	1.4	154.0
Greenbrier 2000	1.5	0.2	1.3	0.1	0.0	0.0	0.15	0.06	-0.080	0.073	59.4	1.5	200.0
Greenbrier 2004	1.7	0.3	1.3	0.1	0.0	0.0	0.16	0.06	0.107	0.036	37.9	6.3	160.0
Greenbrier 2009	1.6	0.2	1.3	0.2	0.0	0.0	0.16	0.07	0.062	0.067	18.3	1.8	165.0
Greenbrier 2014	1.7	0.2	1.3	0.1	0.0	0.0	0.15	0.06	-0.097	0.036	27.2	2.8	146.0
Indian Camp 2000	2.8	0.5	1.6	0.2	0.2	0.1	0.27	0.08	-0.068	0.027	52.1	14.2	180.0
Indian Camp 2009	1.8	0.3	1.3	0.1	0.0	0.0	0.16	0.06	0.055	0.043	19.0	3.3	145.0
Indian Camp 2014	1.7	0.3	1.3	0.1	0.0	0.0	0.17	0.07	-0.184	0.098	20.3	3.2	127.0
Toms 2009	2.3	0.3	1.7	0.2	0.0	0.0	0.32	0.08	-0.144	0.049	31.6	6.1	138.0
Toms 2014	2.4	0.4	1.7	0.2	0.0	0.0	0.31	0.08	-0.006	0.029	38.9	6.7	146.0