

**Inventory and Monitoring of Brook Trout in the Santeetlah Creek
Watershed, Nantahala National Forest, 2016 - 2017**



Report prepared by:

**Craig Roghair¹, Colin Krause¹, Thomas Franklin², Jason Farmer³, and C. Andrew Dolloff¹
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¹USDA Forest Service
Southern Research Station
Center for Aquatic Technology Transfer
1710 Research Center Dr.
Blacksburg, VA 24060
540-230-8126
<http://www.srs.fs.usda.gov/catt/>

²USDA Forest Service
Rocky Mountain Research Station
National Genomics Center for Wildlife & Fish Conservation
800 E. Beckwith Ave.
Missoula, MT 59801
406-542-4171

³USDA Forest Service
Nantahala National Forest
1070 Massey Branch Rd.
Robbinsville, NC 28771
828-479-6431

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Introduction

Brook Trout (*Salvelinus fontinalis*) in the Southern Appalachians face myriad challenges; warming streams and acid deposition (McDonnell et al. 2015), competition with introduced Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*) (Clark and Rose 1997; Hitt et al. 2017), and intraspecific genetic insult (Hayes et al. 1996; Humston et al. 2012; Wesner et al. 2011) are just a few of the many stressors (Hudy et al. 2008) threatening the long term viability of remaining southern strain Brook Trout populations. The North Carolina Wildlife Resources Commission (NCWRC) is working throughout the mountains of western North Carolina to identify, protect, enhance, and restore southern strain Brook Trout populations (NCWRC 2013). Many of the streams with the healthiest remaining populations and highest potential for population restoration fall within the borders of the National Forests in North Carolina (NFNC).

The NFNC and NCWRC are working together to find and monitor changes in existing trout populations, and to assess genetic differentiation among Brook Trout populations in the mountains of North Carolina. They also seek to maintain or increase Brook Trout distribution through stream restoration and reintroduction projects where conditions allow. In 2012, the NFNC and NCWRC buffered two episodically acidic streams in the Santeetlah Creek watershed with limestone sand to maintain existing Brook Trout populations. Water quality monitoring in these streams indicated that episodic drops in pH associated with precipitation events were becoming more pronounced over time, and NFNC was concerned about impacts on the existing Brook Trout populations. Post-treatment monitoring included water quality monitoring and annual multiple-pass depletion electrofishing to estimate Brook Trout population size.

In fall 2016, NFNC and NCWRC partnered with the U. S. Forest Service Southern Research Station Center for Aquatic Technology Transfer (CATT) to sample fish in several western North Carolina streams. Our goals were to: 1) complete the annual multiple-pass electrofishing monitoring at 6 sites within the 2012 lime restoration project area, 2) assess trout distribution within several streams in the Santeetlah Creek watershed using with single-pass electrofishing, and 3) document significant fish passage barriers encountered during trout distribution surveys.

Fish distribution sampling in headwater mountain streams often entails accessing small, shallow, rhododendron covered streams on foot over rough terrain in remote areas; conditions that can lead to inefficient sampling with traditional backpack electrofishing. Additionally, the number of field teams available to complete sampling has decreased as natural resource agencies address significant capacity challenges. Given these challenges it is prudent to explore new approaches that could improve sampling

efficiency. Environmental DNA (eDNA) sampling has recently emerged as an alternative to traditional sampling approaches such as backback electrofishing (Evans et al. 2017). The current project provided the opportunity to compare single-pass electrofishing with eDNA for describing trout distributions in headwater mountain streams. In spring 2017, we used an eDNA approach to re-sample several of our fall 2016 single-pass electrofishing sites. Here, in addition to our original three objectives we compare results of single-pass electrofishing versus eDNA, as well as cost-benefit analysis.

Methods

Multiple-pass depletion

Limestone sand was added to Sand Creek and Wolf Laurel Branch on the downstream side of their respective road 81F crossings beginning in 2012. Monitoring sites were subsequently established by NCWRC and NFNC upstream and downstream of the 81F road crossings, at a site downstream of the confluence of Sand Creek and Wolf Laurel Branch (Figure 1, Table 1), and at an untreated reference site on Whigg Branch. The sites were sampled annually from 2012 - 2015, with the intent of completing 4 years of post-lime monitoring.

The NCWRC provided GPS coordinates for locating the downstream end of each monitoring site; several sites were also marked with blue paint. We set a blocknet at the downstream end of each site, measured a distance of 100 m upstream, and set a second blocknet at the upstream end of the site. We used 1 – 2 backpack electrofishers outfitted with 2 hoop probes (1 netted) per site (Table 1). We paired each backpack electrofisher with a dipnetter and completed a total of 3 - 4 passes, dependent on successful depletions, in an upstream direction. We paused 5 – 10 minutes between passes to process fish and to allow water to clear. For each individual fish we recorded species, total length and weight, and inspected for gill lice. Multiple-pass depletions were completed in September, 2016.

Distribution

Single-pass electrofishing

The NFNC and NCWRC identified several stream reaches within the Santeetlah Creek watershed for which Brook Trout distribution information was unknown or out of date. Within each stream reach we generated sample points every 500 m using the high resolution (1:24,000) national hydrography dataset and the ArcMap Construct Points editing tool. Sample points for tributaries entering our sample reaches were established in the same manner. All sample points were loaded onto Garmin Oregon 400t GPS units for field navigation (Figure 2, Table 2).

Stream access and safety considerations determined whether we traversed between sample points in an upstream or a downstream direction. Regardless, when we reached a sample point we completed a single 100 m pass in an upstream direction using 1 backpack electrofisher (Aqua Shock Solutions Model AS2) outfitted with 2 hoop probes (1 netted) and a single dip netter; we did not set block nets. In tributaries we completed a pass through the first 100 m upstream of the confluence and if fish were collected we proceeded upstream to the next sample point. We identified all collected fish to species, and for trout we recorded total length and collected a small fin clip for genetic analysis. All fin clips were stored in vials of 95% ethanol until they were turned over to NCWRC personnel. Single-pass electrofishing was completed in September, 2016.

eDNA

We established eDNA sample points at 11 locations in the Little Santeetlah watershed, 8 within the mainstem of Little Santeetlah Creek and 1 each from tributaries Adamscamp Creek, Indian Spring Branch, and the unnamed tributary flowing through Poplar Cove (Table 3). Sample points largely overlapped with those used in the 2016 single pass electrofishing samples, with the exception of an additional eDNA sample collected near the confluence of Little Santeetlah Creek and Santeetlah Creek (Figure 2). All samples were collected from downstream to upstream in order to avoid any possible contamination via personnel or sample equipment entering DNA into the stream.

We obtained kits containing sampling materials and field equipment for eDNA collection from the National Genomics Center for Wildlife and Fish Conservation (NGCWFC), U.S. Forest Service Rocky Mountain Research Station in Missoula, MT. At each sample point we followed the methods in Carim et al (2016a) to collect an eDNA sample. In short, we used a portable peristaltic pump to pass 5 L of water through a glass microfiber filter which capture the DNA. The filters were then stored inside a sample bag with silica beads, and labeled with a site identifier and GPS coordinates. Samples were stored out of direct sunlight in a cool, dry location until they could be stabilized in a freezer at the CATT office and then shipped to NGCWFC for analysis within 2 weeks of collection. The eDNA samples were collected in March, 2017.

Upon receipt of samples at the NGCWFC, sampling data were catalogued and samples were stored at -20 °C until analyzed. NGCWFC performed DNA extractions on half of the sample filter using the Qiagen DNEasy® Blood and Tissue Kit with a modified protocol described in Carim et al. (2016b). The other half of the sample filter was retained and stored at -20 °C. All 11 samples were analyzed for Brook, Brown, and Rainbow Trout DNA. All eDNA markers used for sample analysis were developed at the NGCWFC (Carim et al. 2016c; Wilcox et al. 2015; Wilcox et al. 2013). Each sample was analyzed in

triplicate on a StepOne Plus qPCR instrument (Life Technologies). A sample was considered positive for the presence of the target species if at least one of the three PCR reactions amplified DNA of that species.

All reactions included an internal positive control to ensure that the reaction was effective and sensitive to the presence of the target species' DNA. The internal positive control was also used to check for sample inhibition (i.e., chemical compounds on the filter reduced amplification of the target DNA; Figure S2). There were no inhibited samples in this dataset and all laboratory experiments were conducted with negative controls to insure there was no contamination during DNA extraction or qPCR setup.

Barriers

As we traversed between single-pass electrofishing points we documented the location of natural and manmade barriers that could impede upstream fish passage. Barriers could include features such as waterfalls, cascades, road crossings, dams and other features that may impede upstream fish passage. At each potential barrier, we recorded a geo-referenced photo and waypoint using a Garmin Oregon 450t GPS.

Results

Multiple-pass depletion

We completed multiple-pass depletions at all 6 annual monitoring sites within the liming project area in the Santeetlah Creek watershed in 3 days from September 7 – 11, 2016. Sampling was challenging due to extremely low water conditions, and we had to complete a 4th pass on the most downstream Sand Creek site due to an incomplete depletion. We collected young-of-year (YOY) and adult Brook, Brown, and Rainbow Trout from the furthest downstream site on Sand Creek and only Brook Trout YOY and adults from all other sites (Tables 4 and 5). We did not detect gill lice at any of the monitoring sites. We delivered the multiple-pass depletion data to the NCWRC (Jake Rash) and NFNC (Jason Farmer) in a Microsoft Access database in February 2017.

Distribution

Single-pass electrofishing

We visited a total of 46 sample points distributed among 20 streams in the Santeetlah Creek watershed in 6 days between September 9 and 15, 2016. At 10 sample points the water was too shallow to allow for effective electrofishing. At the remaining 36 points, we collected no fish at 13 points, Brook Trout at 10 points, Rainbow Trout at 9 points, and Brown Trout at 6 points (Figure 2, Table 6). All trout occurrences were allopatric with the exception of Indian Creek point 0.0, where Brown and Rainbow

Trout were collected together. The longest continuously occupied reach for Brook Trout was 1.5 km (i.e. four consecutive sample points) on Whigg Branch. Transition from Rainbow Trout to Brook Trout distribution could be quite abrupt, as was noted near the upstream end of sample point 4.0 on Little Santeetlah Creek. The transition between species near sample point 4.0 was marked by a bedrock cascade approximately 20 m in length; we collected only Rainbow Trout downstream and only Brook Trout upstream of the cascade.

eDNA

We collected eDNA samples from all 11 eDNA sample points in Little Santeetlah Creek and its tributaries in 4 days between March 6 and 9, 2017. Trout were detected at 8 of the 11 sample points (Table 7). No trout were detected in the unnamed tributary flowing through Poplar Cove, Indian Spring Branch, nor the most upstream sample point in the mainstem of Little Santeetlah Creek. Rainbow Trout DNA was detected in the 6 downstream most sample points in the mainstem and at the Adencamp Branch sample point. Brook Trout DNA was detected at 2 sample points upstream of the Rainbow Trout detections in the mainstem. There were no Brown Trout DNA detections or sympatric detections.

Barriers

We identified 11 potential barriers on 6 streams (Table 8). Barriers included both road-stream crossing culverts and steep boulder and bedrock cascades. The identification of barriers was not comprehensive as we were unable to traverse the entire length of most streams due to safety concerns and access issues.

Discussion

Brook trout continue to be present and reproducing in the limed streams. Our samples likely were the last collected for the 4-year post-treatment monitoring program, and the NFNC and NCWRC will soon produce a final monitoring report. Regardless of the monitoring project results, it is important to note that lime treatment provides only temporary relief from acidification in areas where acid deposition continues today (Downey et al. 1994) and that episodic acidity remains a problem even in streams where acid deposition is reduced (Lawrence et al. 2016). In addition, both Rainbow Trout and Brown Trout are present and reproducing downstream of the lime sites on Wolf Laurel and Sand Creek. The potential encroachment of Brown and Rainbow Trout into the limed area coupled with the potential for continued episodic acidity warrants periodic sampling at the multiple-pass depletion sites in the future.

Results from the distribution surveys highlight the current plight of Brook Trout in the southern Appalachians. Habitat loss, acid deposition, and encroachment by introduced trout species have

restricted Brook Trout in most streams to isolated headwater reaches. Under present conditions Brook Trout populations can at best maintain their distribution within streams and at worst may be lost altogether. In the case of Little Santeetlah Creek, the distribution of adult Brook Trout appears to have changed little over the past 30 years. An intensive basinwide survey in 1988 by the Forest Service Southern Research Station (unpublished data) employing both snorkeling and electrofishing did not detect any adult Brook Trout downstream of river kilometer (rkm) 5.5 (rkm 0.0 = confluence with Santeetlah Creek), nearly identical to the present results. The survey did detect very low density of young-of-year Brook Trout between rkm 2.0 and rkm 5.5, which we did not find with the present survey.

Even where Brook Trout persist today, questions remain as to their genetic lineage, and testing is underway on the fin clips we collected to examine for genetic differentiation and to assess impacts of hatchery integration on the populations we sampled (Jake Rash, pers. comm.). Where water quality and habitat conditions allow, reintroduction of appropriate strains of Brook Trout may enhance the potential for long-term persistence (Kanno et al. 2016).

The eDNA approach we employed in 2017 holds promise for increasing sampling efficiency. The sampling equipment was relatively light weight and fit inside a small backpack making it easy to transport to remote sample sites in rough terrain, and sampling in shallow water and rhododendron thickets was less problematic than with backpack electrofishing. Furthermore, teaming with the NGCWFC provided easy access to sampling equipment at no cost, a standard field sampling protocol that required minimal training time, and a high level of quality control for sample analysis, sample archiving, and reporting.

Collecting eDNA samples is undeniably faster than collecting backpack electrofishing samples. Our experience on the current project suggests that even under challenging sample collection conditions (remote sampling locations in rough terrain, accessed on foot), the number of eDNA samples collected per day is 3 - 4 times the number that can be collected with backpack electrofishing, and more experienced eDNA practitioners working in less remote settings report collecting 24 – 60 eDNA samples per day (Mike Schwartz NGCWFC, pers. comm.). Clearly, eDNA should be carefully considered where there is a need to collect a large number of samples in a short period of time. However, each eDNA sample collected requires lab analysis at an additional cost not associated with backpack electrofishing which calls into question the relative cost-effectiveness of the two approaches (Evans et al. 2017).

In May, 2017 the lab analysis costs at NGCWFC were \$75 for a single species, with an additional \$25 for each additional species, bringing our total lab analysis costs for 3 trout species to \$125 per sample. Under the sampling conditions we experienced on the current project the additional lab costs

were offset by a decrease in salary, hotel, and per diem associated with fewer days of field work (Figure A1). Because the level of technical expertise needed to collect an eDNA sample is low and samples can be collected by one person, the cost per sample can be taken even lower by incorporating a volunteer into the sampling team or by using one person to collect samples. Under these scenarios eDNA becomes less expensive than backpack electrofishing, which will always require at least two trained technicians (Figure A2).

While eDNA has become a viable option under many sampling scenarios, there are still times when backpack electrofishing is advantageous. Currently, backpack electrofishing is the only option for collecting data that require fish handling (e.g. length and weight) or when individual tissue samples are required. Backpack electrofishing is also required to produce population estimates, though recent studies have demonstrated that eDNA can be used to describe relative abundance or biomass in rivers (Doi et al. 2017) and lakes (Lacoursiere-Roussel et al. 2016). The total cost associated with backpack electrofishing can be less than eDNA where sampling teams are not required to enter travel status, however these cost savings must be weighed against the additional time required to complete backpack electrofishing sampling (Figure A3), and may be negated where volunteers can be used to supplement eDNA sampling teams or where the target is a single species (Figure A4). For agencies facing personnel capacity challenges, the ability to free technicians for additional projects may make eDNA an attractive option even under scenarios where total cost for eDNA sampling is higher than backpack electrofishing.

We found eDNA to be a viable and cost-effective tool for examining trout distribution in remote mountain streams. The science of eDNA is rapidly advancing and as the suite of species available for analysis increases, cost per sample decreases, and population estimation approaches are refined its application to aquatic management and conservation efforts will continue to expand.

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Figures

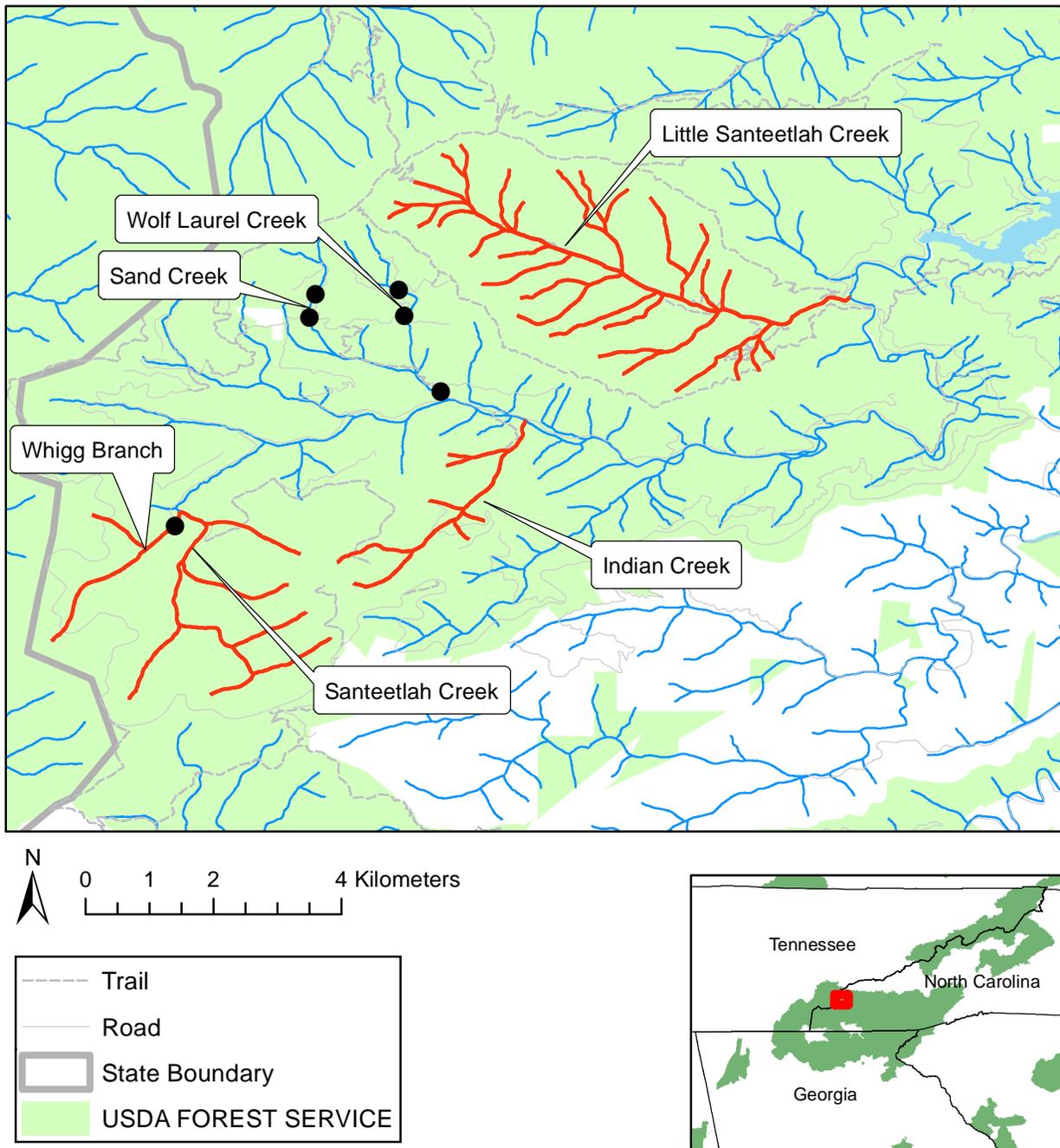


Figure 1. Project area in Santeetlah Creek watershed upstream of Santeetlah Lake. Black dots are multiple-pass depletion electrofishing sites. Streams highlighted red were targeted for distribution surveys.

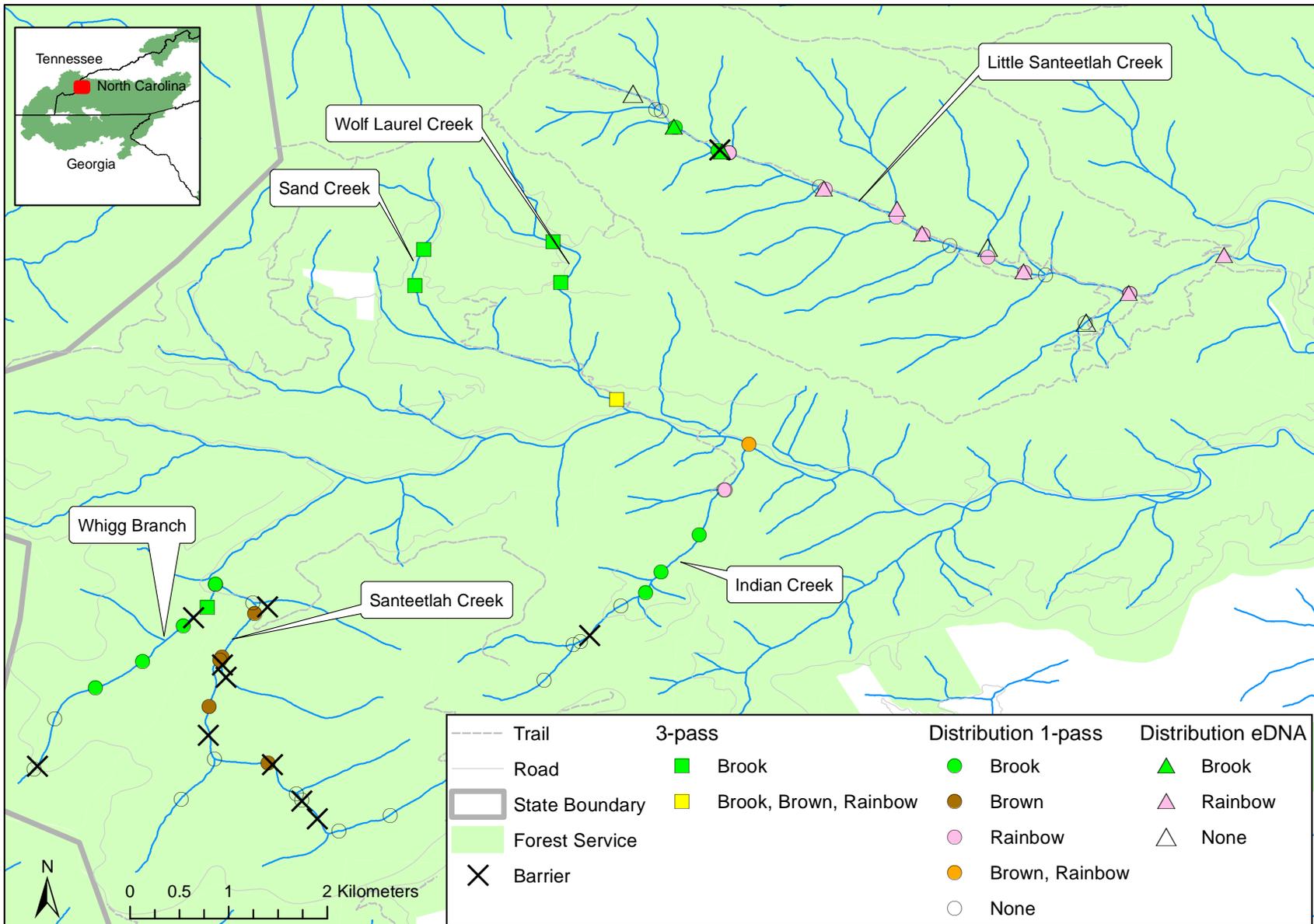


Figure 2. Trout species collected during multiple-pass depletions and distribution surveys in the Santeetlah Creek drainage, 2016 – 2017.

Tables

Table 1. Location of multiple-pass depletion monitoring sites and number of backpack electrofishers used to sample each site. Coordinates mark the downstream starting point for electrofishing. Depletion sampling was completed over 3 days from 9/7 – 9/11, 2016.

Site	Coordinates	Electrofishers	Description
Lower Sand Creek	N35.3491 W83.9774	2	Approximately 80 m upstream of road 81 crossing, 150 m upstream of Santeetlah Creek confluence
Middle Sand Creek	N35.3595 W83.9958	1	Approximately 300 m downstream of road 81F crossing
Upper Sand Creek	N35.3628 W83.9950	1	Approximately 50 m upstream of road 81F crossing
Lower Wolf Laurel Branch	N35.3598 W83.9825	2	Approximately 500 m downstream of road 81F crossing
Upper Wolf Laurel Branch	N35.3635 W83.9832	2	Approximately 50 m upstream of road 81F crossing
Whigg Branch	N35.3302 W84.0147	2	Approximately 150 m upstream of road 81C crossing

Table 2. Single-pass electrofishing sample points for trout distribution surveys. Coordinates mark the downstream starting point for each 100 m sample reach. Single-pass distribution surveys were completed over 6 days from 9/9 – 9/15, 2016.

Sample Point ID	Stream	Coordinates
AC0	Aden Camp Branch (tributary to Little Santeetlah)	N35.36582 W83.95190
GB0.0	Grassy Branch (tributary to Little Santeetlah)	N35.36321 W83.94703
GH0	Groundhog Branch (tributary to Little Santeetlah)	N35.36851 W83.95891
IC0	Indian Creek	N35.34508 W83.96535
IC0.5	Indian Creek	N35.34094 W83.96752
IC1.0	Indian Creek	N35.33680 W83.96987
IC1.5	Indian Creek	N35.33339 W83.97335
IC1.75	Indian Creek	N35.33153 W83.97498
IC2.0	Indian Creek	N35.33032 W83.97703
IC2.5	Indian Creek	N35.32708 W83.98070
IC3.0	Indian Creek	N35.32353 W83.98403
UT1IC0	Indian Creek, Unnamed Tributary 1	N35.34088 W83.96762
UT2IC0	Indian Creek, Unnamed Tributary 2	N35.32676 W83.98136
IS0	Indian springs (tributary to Little Santeetlah)	N35.36212 W83.94354
LST0.0	Little Santeetlah Creek	N35.35879 W83.93061
STARTLST1.0	Little Santeetlah Creek	N35.36030 W83.93818
LST2.0	Little Santeetlah Creek	N35.36418 W83.94947
LST3.0	Little Santeetlah Creek	N35.36836 W83.95839
LST4.0-START	Little Santeetlah Creek	N35.37163 W83.96700
LST4.0-END	Little Santeetlah Creek	N35.37185 W83.96807
LST4.5	Little Santeetlah Creek	N35.37396 W83.97207

Sample Point ID	Stream	Coordinates
UT1LST0.0	Little Santeetlah Creek, Unnamed Tributary 1	N35.35879 W83.93061
UT1LST0.5-START	Little Santeetlah Creek, Unnamed Tributary 1	N35.35599 W83.93430
UT2LST0	Little Santeetlah Creek, Unnamed Tributary 2	N35.36050 W83.93830
UT3LST0	Little Santeetlah Creek, Unnamed Tributary 3	N35.37165 W83.96716
UT4LST0-START	Little Santeetlah Creek, Unnamed Tributary 4	N35.37599 W83.97351
UT5LSTSTART	Little Santeetlah Creek, Unnamed Tributary 5	N35.37556 W83.97474
SC0	Santeetlah Creek	N35.33228 W84.01398
SC0.5	Santeetlah Creek	N35.32963 W84.01044
SC1.0	Santeetlah Creek	N35.32564 W84.01337
SC1.5	Santeetlah Creek	N35.32116 W84.01458
SC2.5	Santeetlah Creek	N35.31599 W84.00921
SC3.0	Santeetlah Creek	N35.31261 W84.00612
SC3.5	Santeetlah Creek	N35.30978 W84.00274
SC4.0	Santeetlah Creek	N35.31126 W83.99806
UT1SC0	Santeetlah Creek, Unnamed Tributary 1	N35.33059 W84.01056
UT2SC0	Santeetlah Creek, Unnamed Tributary 2	N35.32535 W84.01358
UT3SC0	Santeetlah Creek, Unnamed Tributary 3	N35.31637 W84.01407
UT3SC0.5	Santeetlah Creek, Unnamed Tributary 3	N35.31272 W84.01714
UT4SC0	Santeetlah Creek, Unnamed Tributary 4	N35.31322 W84.00662
WB0	Whigg Branch	N35.33228 W84.01398
WB0.5	Whigg Branch	N35.32851 W84.01691
WB1.0	Whigg Branch	N35.32525 W84.02061
WB1.5	Whigg Branch	N35.32283 W84.02493
WB2.0	Whigg Branch	N35.31998 W84.02867
WB2.5	Whigg Branch	N35.31546 W84.03052
UTWB0	Whigg Branch, Unnamed Tributary 1	N35.32712 W84.01858

Table 3. Coordinates for eDNA samples to determine trout distribution in the Little Santeetlah watershed. River kilometer (rkm) 0.0 is at the confluence with Santeetlah Creek. Samples were collected over 4 days from 3/6 – 3/9, 2017.

Stream	Coordinates	Date Collected	Description
Little Santeetlah Creek	N35.3623 W83.92203	3/7/2017	rkm 0.25
Poplar Cove	N35.35599 W83.9343	3/6/2017	At trail crossing
Little Santeetlah Creek	N35.35846 W83.93089	3/7/2017	rkm 1.25
Little Santeetlah Creek	N35.36082 W83.94031	3/8/2017	rkm 2.25
Indian Spring Branch	N35.36275 W83.94324	3/8/2017	At trail crossing
Little Santeetlah Creek	N35.36409 W83.94968	3/8/2017	rkm 3.25
Adamscamp Creek	N35.36629 W83.95255	3/8/2017	At trail crossing
Little Santeetlah Creek	N35.36821 W83.95871	3/8/2017	rkm 4.25
Little Santeetlah Creek	N35.37182 W83.96773	3/9/2017	rkm 5.25
Little Santeetlah Creek	N35.37403 W83.97218	3/9/2017	rkm 5.75
Little Santeetlah Creek	N35.37672 W83.97599	3/9/2017	rkm 6.35

Table 4. Fish collected from multiple-pass depletion monitoring sites.

Site	Species	Gill Lice (yes/no)	Length (mm) min	Length (mm) max	Weight (g) min	Weight (g) max
Lower Sand Creek	Brook Trout	No	74	140	3.1	22.8
	Brown Trout	No	58	169	2	46.6
	Rainbow Trout	No	54	215	1.4	86.5
Middle Sand Creek	Brook Trout	No	55	175	1.5	46.1
Upper Sand Creek	Brook Trout	No	58	155	1.9	32.2
Lower Wolf Laurel Creek	Brook Trout	No	58	180	1.2	45.4
Upper Wolf Laurel Creek	Brook Trout	No	54	156	1.2	31.5
Whigg Branch	Brook Trout	No	69	192	4.2	65.5

Table 5. Total fish collected per pass during multiple-pass depletions. We completed a 4th pass in Lower Sand Creek due to unsuccessful depletion after 3 passes.

Inventory_ID	Species	Pass 1	Pass 2	Pass 3	Pass 4	Total
Lower Sand Creek	Brook Trout	8	0	1	2	11
	Brown Trout	1	0	0	2	3
	Rainbow Trout	13	1	1	0	15
Middle Sand Creek	Brook Trout	33	9	3	--	45
Upper Sand Creek	Brook Trout	35	12	3	--	50
Lower Wolf Laurel Creek	Brook Trout	43	11	6	--	60
Upper Wolf Laurel Creek	Brook Trout	32	13	3	--	48
Whigg Branch	Brook Trout	21	9	4	--	34

Table 6. Fish species collected at single-pass distribution sample sites, September 2016. See Table 2 for stream name and coordinates associated with each sample point. 'No sample' indicates that the stream was too small to effectively sample with backpack electrofishing.

Sample Point ID	Brook Trout <i>S. fontinalis</i>	Brown Trout <i>S. trutta</i>	Rainbow Trout <i>O. mykiss</i>	Blacknose Dace <i>R. atratulus</i>	Longnose Dace <i>R. cataractae</i>	No fish	No sample
AC0			X				
GB0.0							X
GH0							X
IC0		X	X		X		
IC0.5			X				
IC1.0	X						
IC1.5	X						
IC1.75	X						
IC2.0						X	
IC2.5						X	
IC3.0						X	
UT1IC0							X
UT2IC0							X
IS0			X				
LST0.0			X	X	X		
STARTLST1.0			X		X		
LST2.0			X		X		
LST3.0			X				
LST4.0-START			X				
LST4.0-END	X						
LST4.5	X						
UT1LST0.0						X	
UT1LST0.5-START						X	
UT2LST0							X
UT3LST0						X	
UT4LST0-START						X	
UT5LSTSTART						X	

Sample Point ID	Brook Trout <i>S. fontinalis</i>	Brown Trout <i>S. trutta</i>	Rainbow Trout <i>O. mykiss</i>	Blacknose Dace <i>R. atratulus</i>	Longnose Dace <i>R. cataractae</i>	No fish	No sample
SC0	X						
SC0.5		X					
SC1.0		X					
SC1.5		X					
SC2.5		X					
SC3.0						X	
SC3.5						X	
SC4.0							X
UT1SC0							X
UT2SC0		X					
UT3SC0						X	
UT3SC0.5						X	
UT4SC0							X
WB0	X						
WB0.5	X						
WB1.0	X						
WB1.5	X						
WB2.0						X	
WB2.5							X
UTWB0							X

Table 7. Trout species detected using eDNA approach for trout distribution in the Little Santeetlah watershed.

Stream	Description	Brook Trout <i>S. fontinalis</i>	Brown Trout <i>S. trutta</i>	Rainbow Trout <i>O. mykiss</i>	No Trout
Little Santeetlah Creek	rkm 0.25			X	
Poplar Cove	At trail crossing				X
Little Santeetlah Creek	rkm 1.25			X	
Little Santeetlah Creek	rkm 2.25			X	
Indian Spring Branch	At trail crossing				X
Little Santeetlah Creek	rkm 3.25			X	
Adamscamp Creek	At trail crossing			X	
Little Santeetlah Creek	rkm 4.25			X	
Little Santeetlah Creek	rkm 5.25	X			
Little Santeetlah Creek	rkm 5.75	X			
Little Santeetlah Creek	rkm 6.35				X

Table 8. Barriers encountered during fish inventories on several streams in the Santeetlah Creek watershed, fall 2016. This list should not be considered comprehensive as the entire length of each stream was not traversed. Waypoint is the closest GPS waypoint downstream of the barrier and is provided for reference only. Coordinates are the actual barrier location.

Stream	Waypoint	Barrier Coordinates	Type	Comment
Indian Creek	IC2.0	N35.32767 W83.97987	Cascade	Long boulder cascade with several vertical drops of 1 m or greater
Whigg Branch	WB0	N35.32925 W84.01598	Cascade	Estimated 6 ft. near vertical cascade that extend across 90% of the channel. Appears to be a steep cobble/boulder cascade section on right side that may have flow during high water events.
	WB2.0	N35.31574 W84.03023	Road Crossing	Culvert under Rd 143
Santeetlah Creek	SC1.5	N35.31853 W84.01465	Cascade	Boulder cascade that could block trout
	SC2.5	N35.31588 W84.00878	Cascade	Bedrock cascade estimated 2 m long
	SC3.0	N35.31257 W84.00611	Cascade	Bedrock cascade with vertical drop at end. May not be barrier but several of these in this area.
	SC3.0	N35.31093 W84.00470	Cascade	Another example of the bedrock cascades that are abundant here. Not a full barrier.
Unnamed Tributary 1, Santeetlah Creek	UT1SCO	N35.33029 W84.00924	Road Crossing	Corrugated culvert with estimated 0.5 m outlet drop, no natural substrate and shallow water inside
Unnamed Tributary 2, Santeetlah Creek	UT2SCO	N35.32496 W84.01334	Cascade	Boulder cascade about 4 m long and steep, caught all fish downstream
Unnamed Tributary 1, Santeetlah Creek	UT2SCO	N35.32380 W84.01302	Cascade	Bedrock about 2.5 m high nearly vertical with sheet flow
Little Santeetlah Creek	LST4.0	N35.37190 W83.96801	Cascade	Bedrock chute; estimated 20m in length; probably not a complete barrier, but only Rainbow Trout collected immediately downstream and only Brook Trout collected immediately upstream of this chute

Appendix A: Electrofishing and eDNA comparison

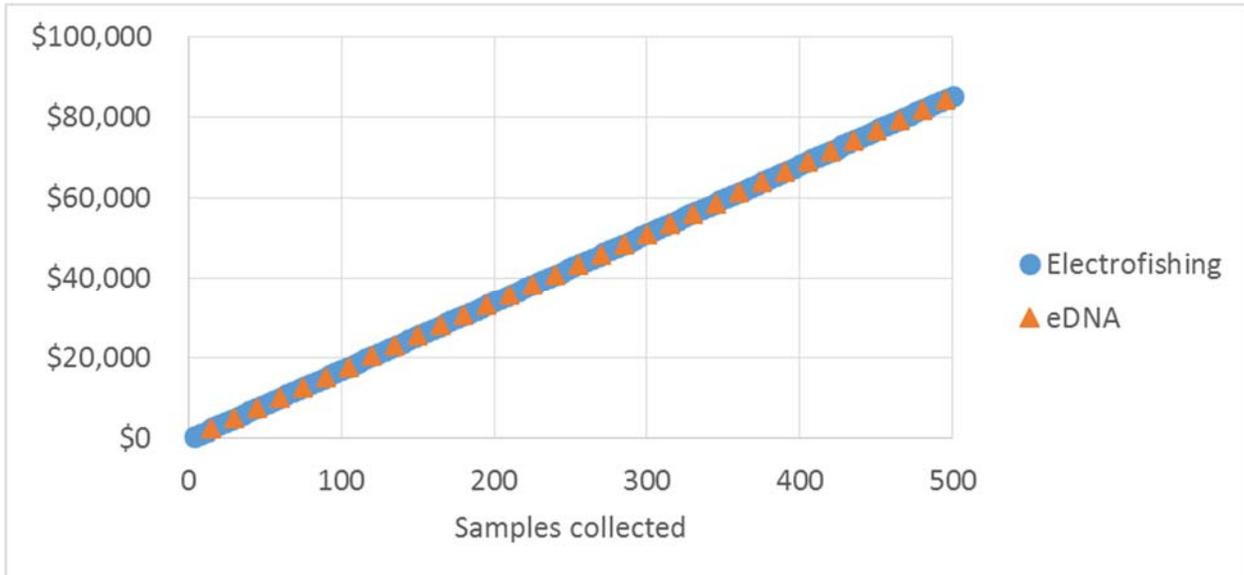


Figure A1. Cost for collecting up to 500 samples using scenario from Table A1. Here, the costs per sample are identical (\$171 per sample), but it takes 125 field days to collect 500 samples with electrofishing, as compared to 34 field days with eDNA. This scenario is similar to our experience sampling for the current project.

Table A1. Variables used to produce Figure A1.

	Single-pass electrofishing	eDNA
Crew size	2	2
Total Salary per day	\$400	\$400
Total Travel per day (hotel + per diem)	\$284	\$284
Average samples collected per day	4	15
Lab costs per sample (3 species)	\$0	\$125

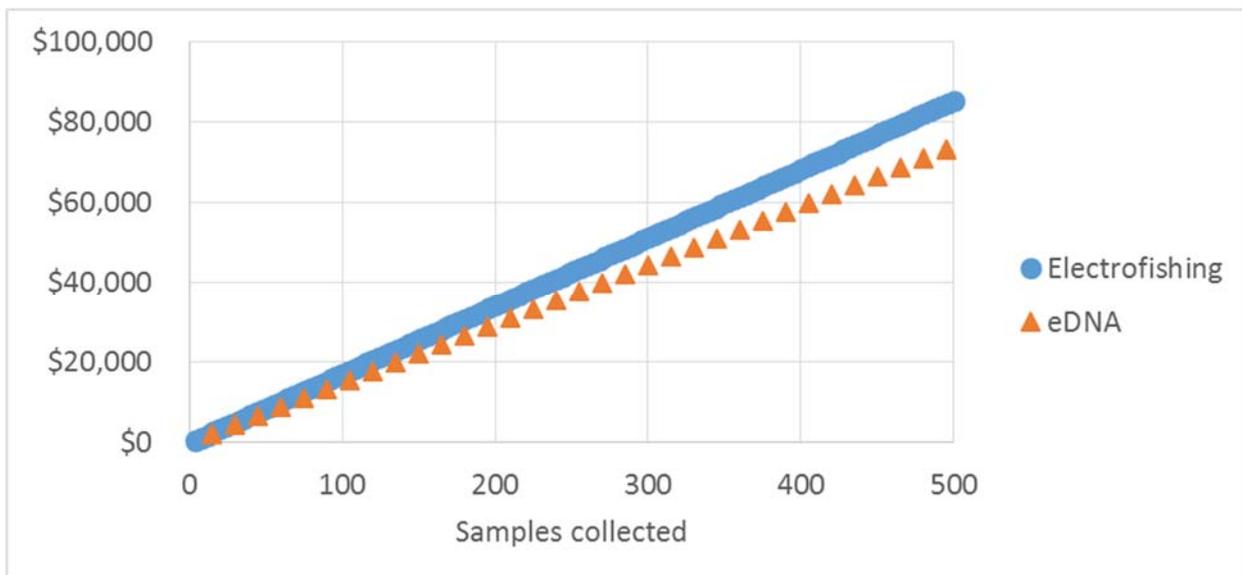


Figure A2. Costs for collecting up to 500 samples using scenario from Table A2. Here, the costs per sample for electrofishing is \$171 and it takes 125 days to collect 500 samples, whereas the cost per sample for eDNA is \$148 and it takes 34 field days to collect 500 samples. This scenario represents the cost savings gained by recruiting a volunteer to the field sampling team or using 1 person to collect samples, both viable options for eDNA, but not for electrofishing.

Table A2. Variables used to produce Figure A2.

	Single-pass electrofishing	eDNA
Crew size	2	1
Salary per day	\$400	\$200
Travel per day (hotel + per diem)	\$284	\$142
Average samples collected per day	4	15
Lab costs per sample (3 species)	\$0	\$125

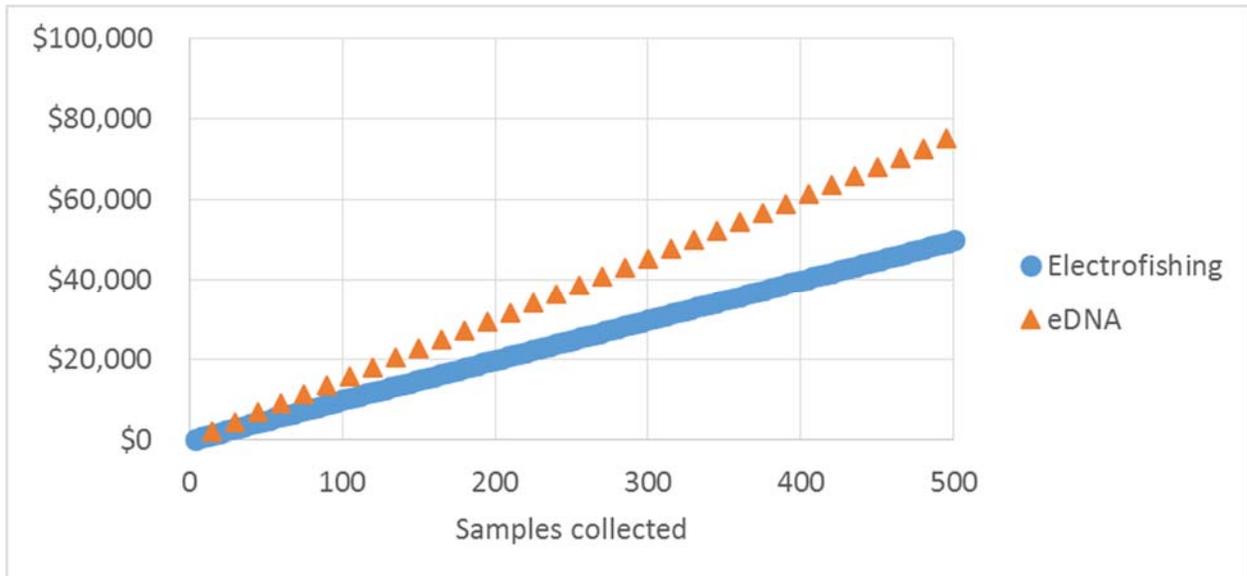


Figure A3. Costs for collecting up to 500 samples using the scenario from Table A3. Here, the costs per sample for electrofishing is \$100 and it takes 125 days to collect 500 samples, whereas the cost per sample for eDNA is \$152 and it takes 34 field days to collect 500 samples. In this scenario, the field team is not in travel status and fixed costs associated with lab analysis of eDNA samples cannot be overcome by salary savings alone, making electrofishing the more cost-effective option. However, the time in the field remains substantially less for eDNA when compared with electrofishing.

Table A3. Variables used to produce Figure A3.

	Single-pass electrofishing	eDNA
Crew size	2	2
Salary per day	\$400	\$400
Travel per day (hotel + per diem)	\$0	\$0
Average samples collected per day	4	15
Lab costs per sample (3 species)	\$0	\$125

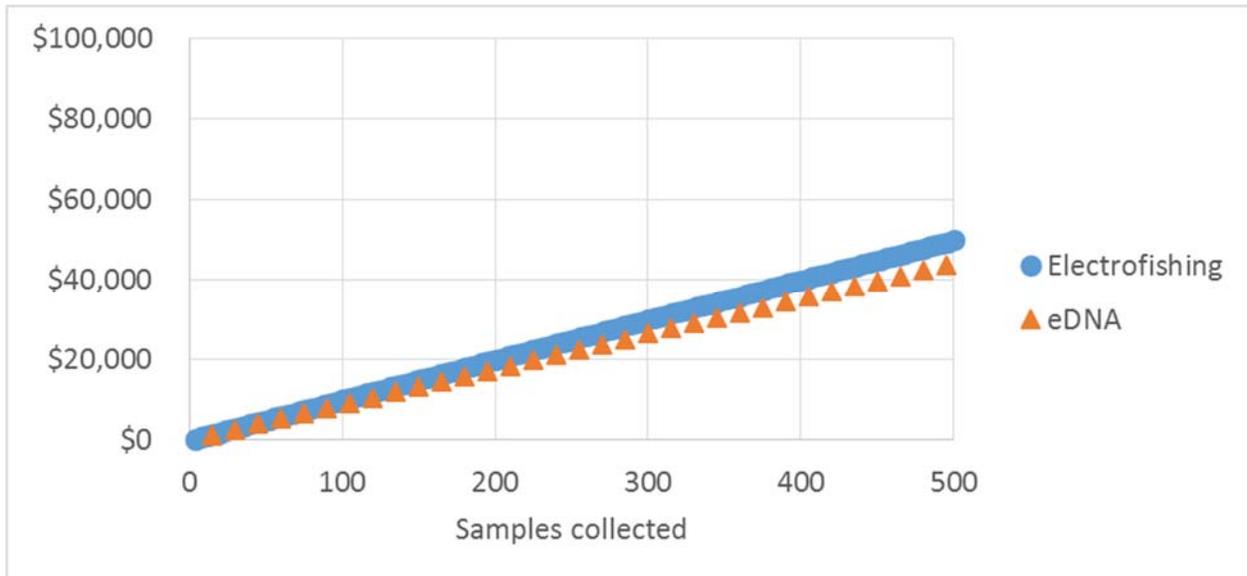


Figure A4. Costs for collecting up to 500 samples using scenario from Table A4. Here, the costs per sample for electrofishing is \$100 and it takes 125 days to collect 500 samples, whereas the cost per sample for eDNA is \$88 and it takes 34 field days to collect 500 samples. In this scenario, the field sampling team is not in travel status and we analyze each eDNA sample for 1 species (as opposed to 3 in the previous examples). In addition, we gain additional cost savings by recruiting a volunteer to the field sampling team or using 1 person to collect eDNA samples, both viable options for eDNA, but not for electrofishing.

Table A4. Variables used to produce Figure A4.

	Single-pass electrofishing	eDNA
Crew size	2	1
Salary per day	\$400	\$200
Travel per day (hotel + per diem)	\$0	\$0
Average samples collected per day	4	15
Lab costs per sample (1 species)	\$0	\$75