Genetic Analysis of Brook Trout from Isolated Populations in the Western Branch of the Susquehanna Watershed

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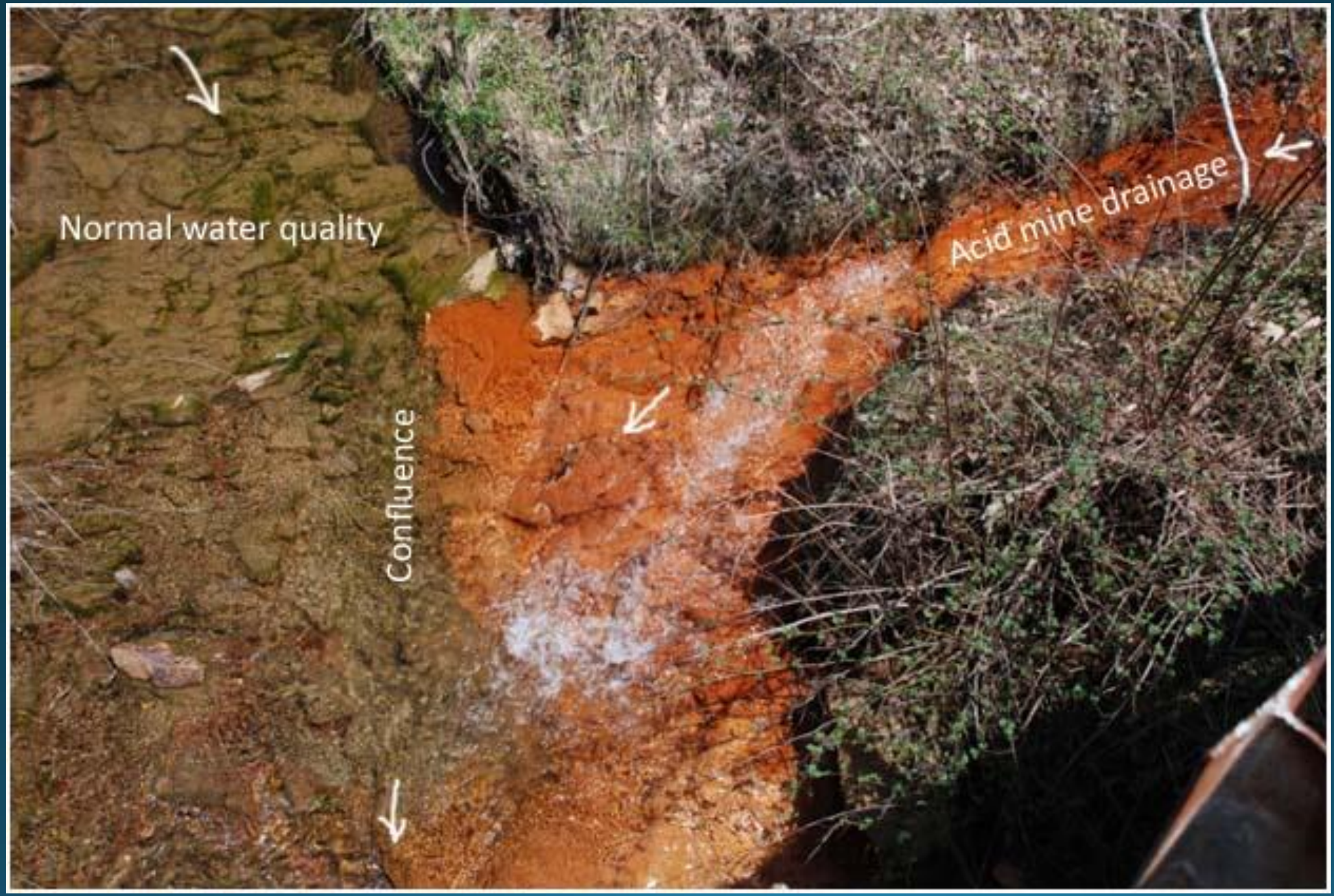
Background

- Trout Unlimited
  - Eastern Abandoned Mine Program (EAMP)
- Pennsylvania Fish and Boat Commission (PFBC)

Introduction

- The brook trout is the only native trout species to Pennsylvania
- Abandoned mine drainage (AMD) has been discharged into streams throughout the West Branch of Susquehanna for at least a 100 years.
- This acidic discharge has prohibited fish movement within these tributaries and has resulted in the isolation of brook trout populations creating a genetic bottleneck
- Microsatellite analysis of these populations will determine if they are truly isolated


Normal water quality
Confluence
Acid mine drainage

Sampling Methods

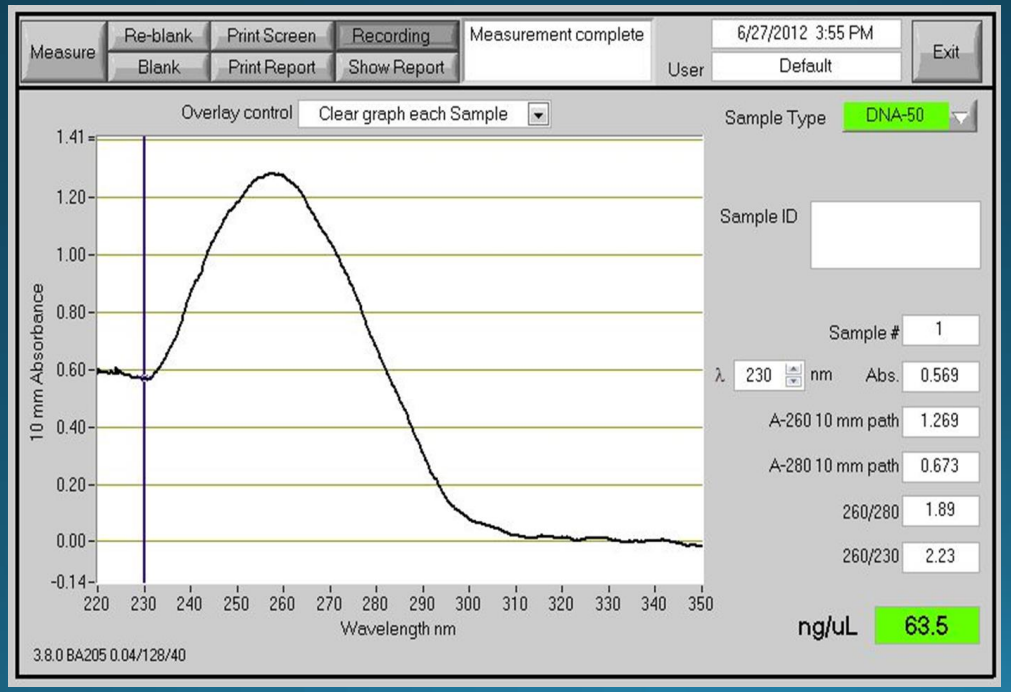
- Surveys are completed in low-flow summer conditions to reduce sampling bias and allow the capture of young-of-the-year fish.
- Sample sites are approximately 100 meters in length and contain habitat representative of the stream.
- The end point of each site contains a natural barrier to prevent upstream movement.
- Sampling is conducted with a Smith-Root, Model LR-24 backpack electrofisher.
- Caudal fin clips of *Salvelinus fontinalis* greater than 100 millimeters are taken for genetic analysis. The fin clips are preserved in ethanol

Laboratory Methods

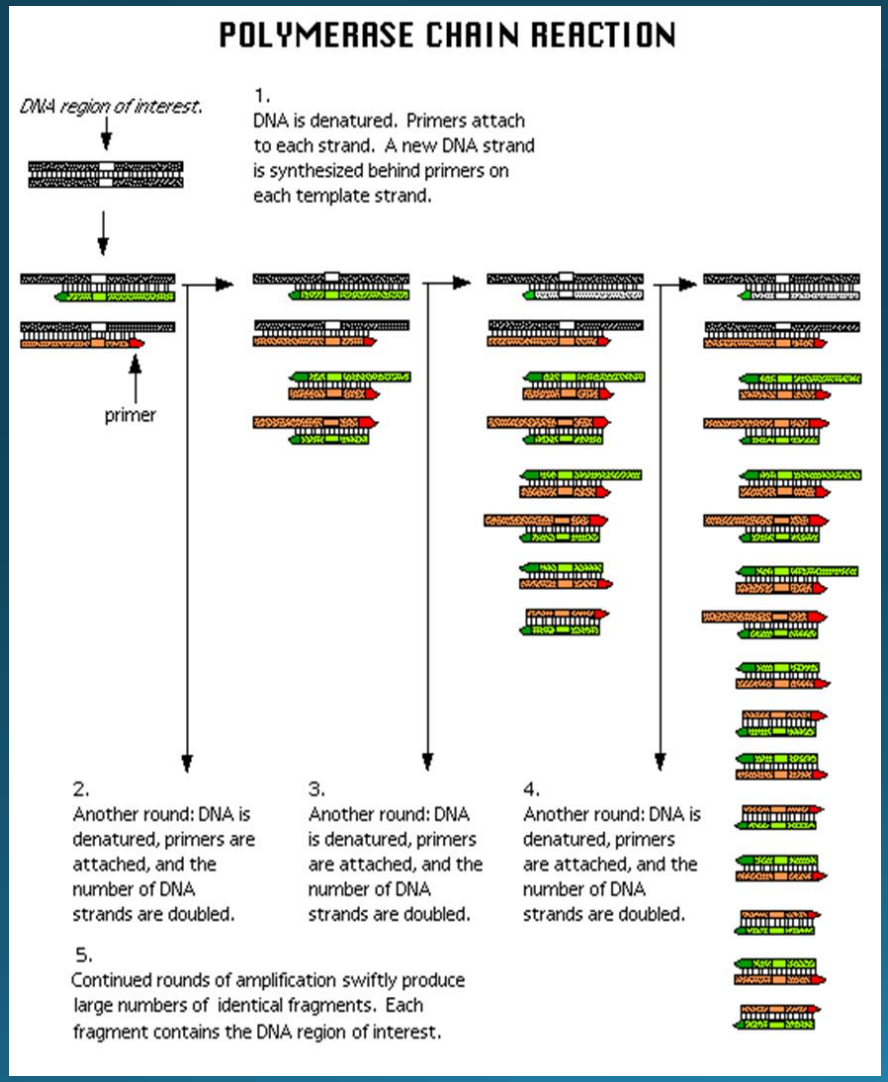
- Nucleic DNA was then isolated using Giagen DNeasy® Blood and tissue kit and verified usingNanoDrop® Spectrophotometer.
- Primers were taken from previously published research.
- The extracted DNA was amplified using a Polymerase Chain Reaction (PCR) and visualized using 2% Agarose Gel Electrophesis.
- After visualization, program GeneScan was used to analyze 64 samples at 1 microsatellite locus.

NanoDrop® Spectrophotometer

- The NanoDrop ® Spectrophotometer ND-1000 is used to quickly and accurately determine the purity/concentration of the DNA.
- By taking a ratio of the intensities: 260/280 = 1.8 ratio of DNA is perfectly pure.

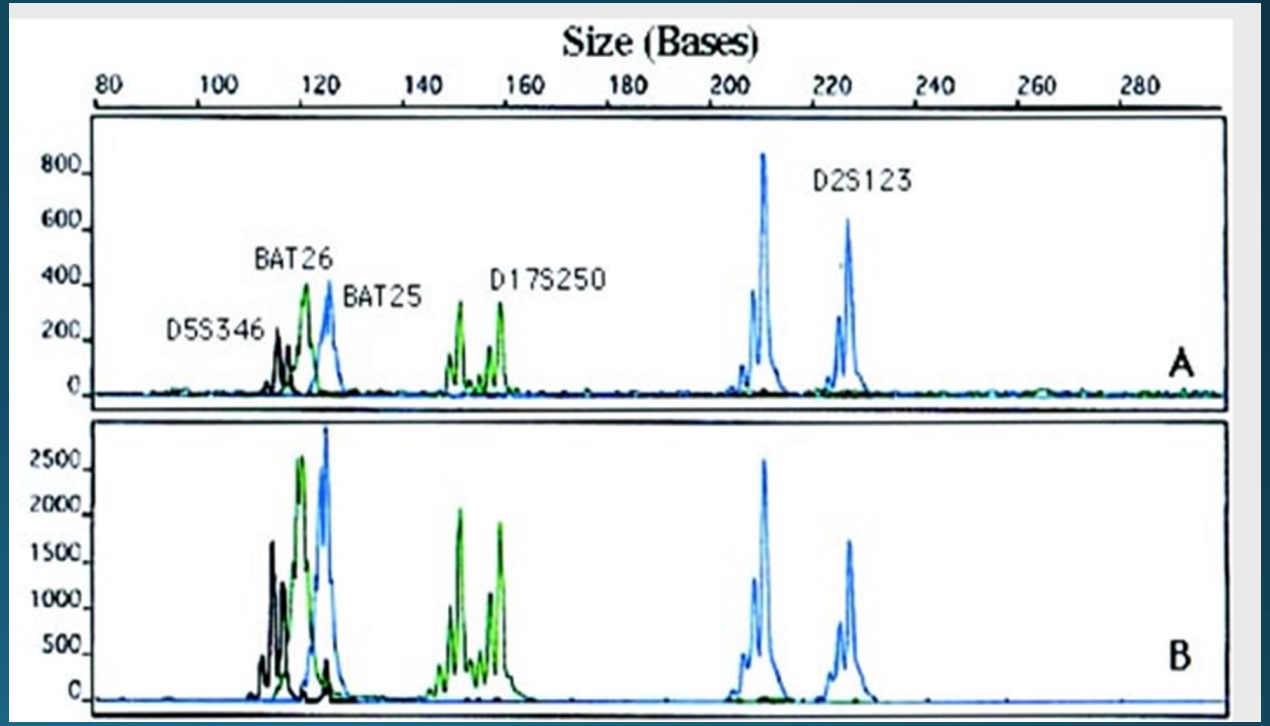


Polymerase Chain Reaction (PCR)



Fragment Analysis of Microsatellites

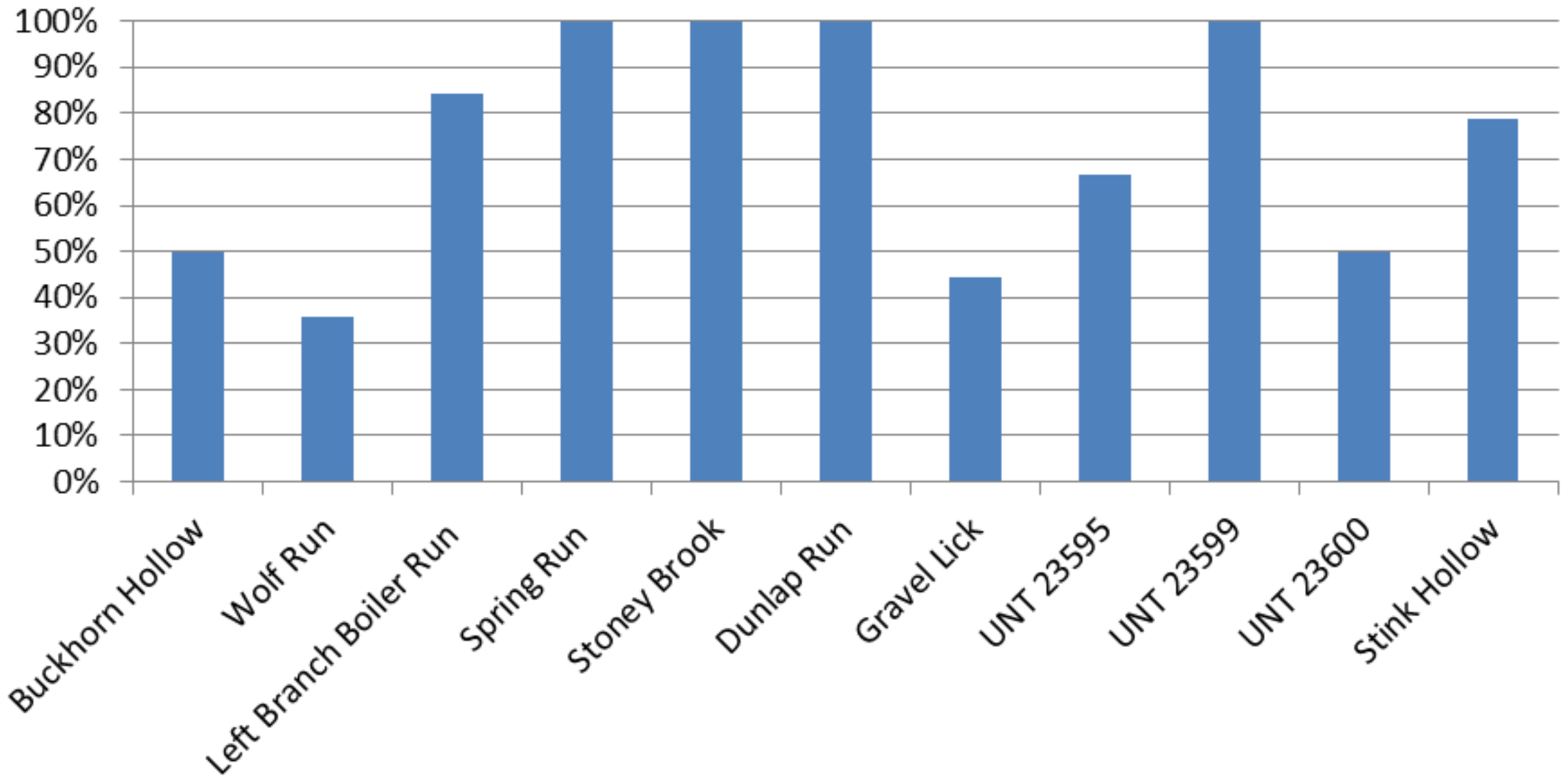
- ABI Prism GeneScan program was used in order to analyze the frequency that a specific microsatellite locus appears in each sample.
- By looking at the peaks, we were able to determine whether the sample was homozygous with one peak or heterozygous with two peaks.



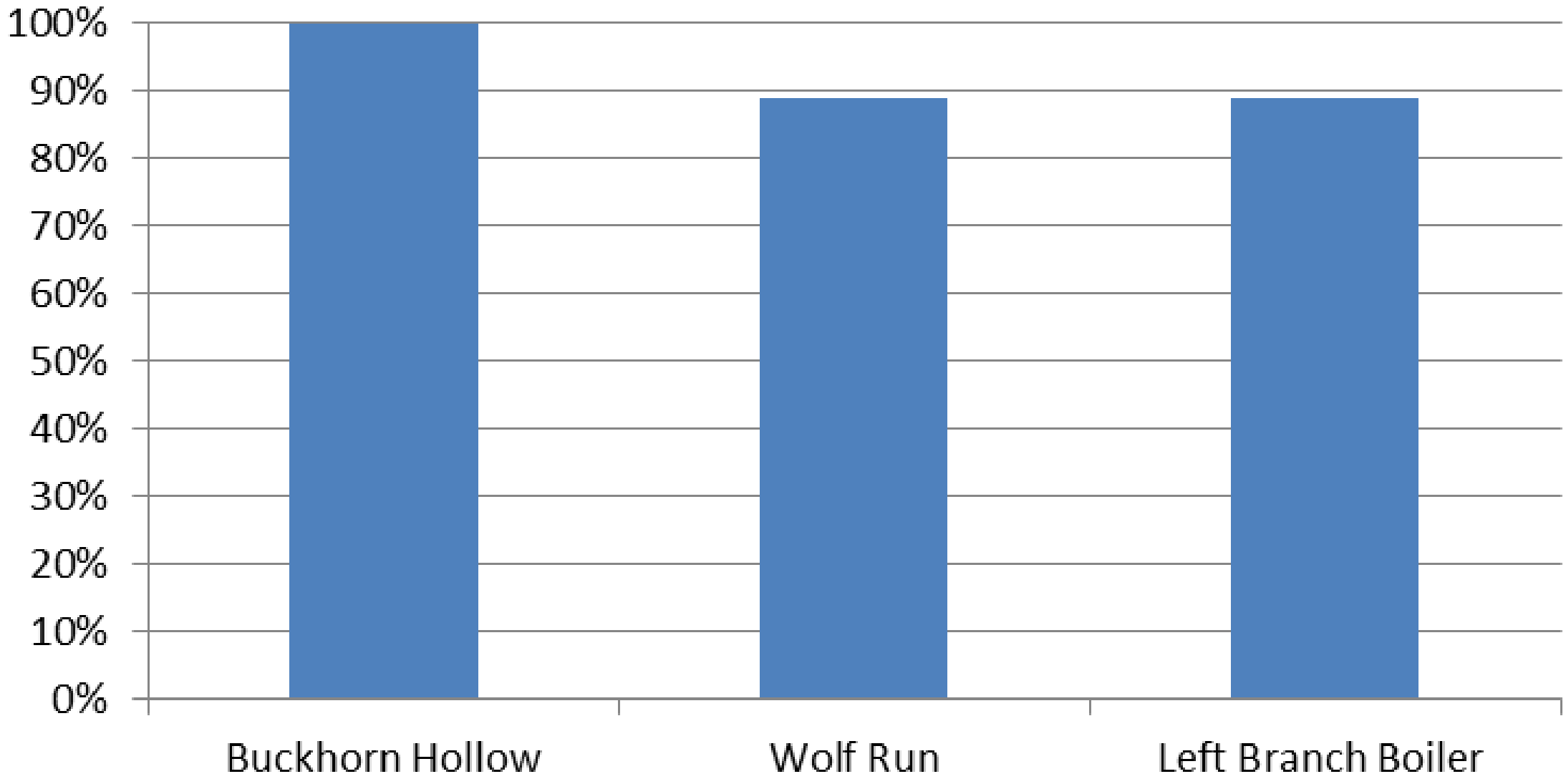
Results

- The initial focus of this research was to optimize the primers and DNA microsatellite production through the PCR process. This was accomplished by running gradients with PCR components.
- Once the primers were optimized, the GeneScan program showed signs of potential isolation due to high frequency of homozygosity in the 128 samples that have currently been analyzed.

% Homozygosity for Primer SFO-8



% Homozygosity for Primer SFO-12



Conclusions and Future Work

- Our preliminary results suggest that there maybe isolation in the streams that were tested due to a high frequency of homozygosity.
- Due to the limited number of samples currently evaluated, we are unable to determine if the streams are truly isolated.
- With all the primers now being optimized and ready for gene scan, we will be able to focus on performing fragment analysis using four primers sets on multiple of fish samples collected over the past three years. This will allow us to look at different streams and compare results with different primers in order to verify if isolation of these brook trout populations has taken place.