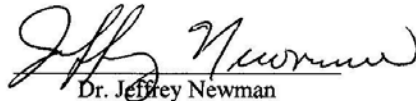# Microhabitat Selection and Genetic Analysis of Trout in Two Pennsylvania Streams

Presented to the faculty of Lycoming College in partial fulfillment of the requirements for
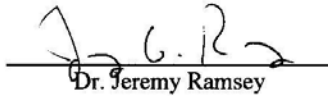Departmental Honors in Biology

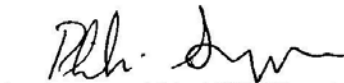Lori A. Smith
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Introduction

Within Pennsylvania, there exists a variety of threats to stream ecosystems. Agricultural activities, urbanization, and natural gas exploration are a few examples. The possible repercussions that these threats can have on streams are exponential including effects on aquatic life, water quality, and habitat (Forbes et al. 1994). In a recent occurrence, a spill of hydraulic fracturing fluid on a natural gas drilling pad in Dimock, Pennsylvania subsequently caused the killing of fish from a nearby stream. In addition to the stream, the spill also affected wetlands, another important habitat source for aquatic communities (Lustgarten 2009). Unfortunately, there are many other threats that have the possibility of producing similar negative impacts on stream ecosystems. Furthermore, the importance of conserving, sustaining and restoring stream ecosystems for the benefit of aquatic communities has been a long term goal for several state and federal agencies within Pennsylvania. The close relationship between habitat availability and trout population dynamics has long been identified (Fausch et al. 1988). It has been shown that necessary habitats such as minimum stream flows, low-velocity and deep refuge, foraging sites, overhead cover, and spawning gravels are vital in order to maintain healthy trout populations (Fausch et al. 1988). One example of the importance of this relationship can be seen at Big Bear Creek in Lycoming County, Pennsylvania (Lutz 2007, Montgomery 2008).

Big Bear Creek, a tributary to Loyalsock Creek, is a third order stream nestled in a 17 m² watershed. The stream itself runs approximately 5.2 miles, and has been in part the property of the Dunwoody Sportsmen's Club since 1884. The club purchased the property in order for its members to take advantage of the exceptional fishing the stream had to offer. However, around the 1920's a decline in the stream's trout population began to occur. In order to compensate for the loss, the Dunwoody Club opened a hatchery on the property and began stocking trout. This

effort was successful in rebuilding the population for some time, but was not a permanent solution.

In addition to the decline in population, the stream suffered extreme agitation to its habitat in 1972 from Hurricane Agnes and in 1975 from Hurricane Eloise. In response to the severe high flow conditions during these events, the banks of the stream eroded, depositing large amount of sediment into the stream and widening the banks. Other setbacks, such as the construction of a dam downstream near the mouth at Loyalsock Creek and the destruction of the 100 year old dam in the upstream portion of the stream led to further sediment deposition (Holmes, 2004).

The problem with sediment deposition is that it transforms the stream into a shallower and wider one, and changes the substrate of the stream from large gravel and cobble to fine sediment. Another repercussion of shallow water is in increase in current, and the combination of these effects produce unfavorable habitat for trout. Trout prefer slower, deeper water in order to reduce energy exerted in fighting current (Hayes and Jowett, 1994).

In order to compensate for loss of trout habitat, the Dunwoody Sportsmen's Club in cooperation with the U.S. Fish and Wildlife constructed a large, 4 mile, habitat restoration project on Bear Big Creek, between 1999 and 2001. These habitat restoration structures are a component to a process known as Natural Stream Channel Design (www.keystonestreamteam.org; Rosgen 1996). The purpose of Natural stream channel stability is achieved by allowing the stream to develop a stable dimension, profile, and pattern such that, over time, channel features are maintained and the stream ecosystem neither aggrades nor degrades (Rosgen, 1996). As part of a Natural Stream Channel Design, habitat restoration structures are constructed into the stream. Through this implementation, improvements to stream

bank stabilization, habitat for fish and benthic macroinvertebrates, and water quality can be accomplished (Rhodes 2008). In addition to the structures, the Dunwoody Club ceased stocking practices following construction in an attempt to produce self sustaining wild trout populations within the stream.

Through several projects completed by Lycoming College Clean Water Institute students, monitoring of the stream's progress prior to and after construction has been recorded (Appendix A). Overall, the studies document the benefits and improvements of the habitat restoration project on Big Bear Creek (Rhodes 2008). Shortly after construction ended, benthic macroinvertebrate density was shown to have improved to 3-5 times preconstruction numbers (Smith 2001). High benthic macroinvertebrate densities are vital to sustaining a healthy stream ecosystem because they provide food for trout and other fish species, which directly contributes to the improvement of habitat. In addition, Brook Trout (*Salvelinus fontinalis*) and Brown Trout (*Salmo trutta*) populations appeared to be improving in the immediate years following the construction (Holmes 2004). However, in the most recent study completed by Nicole Rhodes in 2008, electrofishing results suggested that the goal of increasing self-sustaining wild brook trout populations is not being fully met (Rhodes 2008). Populations of brown trout highly exceeded brook trout populations, leading to an uneven distribution between the two competing populations. In 2007, mean fish density per hectare was found to be 309 (#fish/ha) for brown trout and 78 (#fish/ha) for brook trout (Rhodes 2008). Substantial improvements to the habitat of Big Bear Creek have been made; therefore, results suggest the presences of other underlying reason for the uneven brook trout to brown trout distribution.

As stated previously, Big Bear Creek sustains both brook trout and brown trout populations. These species have been found to compete with each other for similar habitats.

Brown Trout, a non native trout species to Pennsylvania, will exhibit far more aggressive behavior in comparison to brook trout, the only native trout species to Pennsylvania. For example, Dewald and Wilzbach (1992) documented that brook trout's microhabitat location and vertical distribution has been found to shift in the presence of brown trout, and prey capture rates have been found to be higher for brown trout in trials where the two species were in the presences of one another. In addition, brook trout were also shown to lose weight in the presence of brown trout (Dewald and Wilzbach 1992). Moreover, brown trout can exceed 30 inches in length, whereas brook trout size ranges from 5 – 18 inches long (Stenier 2002). The combination of this size differentiation and the presumable aggressive behavior of brown trout may be a causative reason for the failure of wild brook trout populations to rebound at Big Bear Creek.

Another underlying reason could be the particular strain of brown trout that was stocked in Big Bear Creek. A strain is defined as an organism within a species that differ genetically in small ways from others in its species ("strain" 2011). Different strains of fish species are identified by their genetics and origin. Knowledge of the morphology of the brown trout that inhabit Big Bear Creek suggests that a particular strain of brown trout identified as Von Behr, may be the strain of brown trout in Big Bear Creek. Where in Ogdonia Creek, a third order stream located 9 miles from Big Bear Creek and also a tributary to Loyalsock Creek, may contain the stocked Loch Levan strain which is the main type stocked by the PA Fish and Boat Commission.

The Von Behr strain, or German Trout, was first introduced in the United States between 1883-1884, and first in Pennsylvania in 1886 (Stenier 2002). However, at the same time another strain of brown trout, known as Loch Leven, was also introduced. The morphology of these two strains is differentiable. The German brown trout is described as being brightly colored with

bright red spots, particularly on the lateral line, pronounced dark brown and black spots on the opercula and sides, black and white margins on the anal and dorsal fins, and rarely exceeding 12 inches in length. Whereas the Loch Leven brown trout is described as a silvery gray with black spots, no red ones, and can exceed lengths of 12 inches (Figure 1). Wild brown trout of Pennsylvania have been found to resemble the von Behr brown trout most frequently in morphology. However, morphology is not the only indicating feature of a species, and unfortunately, few measures were taken in keeping the two strains separate. This resulted in wide interbreeding and broad distribution of the two strains. Therefore, the North American brown trout could potentially be similar in appearance and life history to both strains (Bachman, in press).

Although morphology, life history and behavior are some characteristics that have been used in the past for classification, presently genetic DNA analysis is becoming the emerging tool in phylogenetic studies (Pechenik 2010). Genetic comparisons can be made through several methods, such as looking at the number and configurations of chromosomes, evaluating fine difference in enzymes, and evaluating DNA both directly and indirectly. The DNA genome of trout and salmon has about three billion nucleotides. Genetic DNA analysis typically results in five hundred to two thousand nucleotides being analyzed at a time. Therefore, this analysis relies on making inferences from a small portion of the genome (Behnke et al. 2002). Genetic DNA analysis is highly beneficial because it provides researchers with specific information about particular individuals. For instance, all humans appear similar because of common traits possessed by all humans. However, because of certain DNA that is specific to each individual, humans are also dissimilar. Through genetic analysis, the sequencing of DNA allows researchers to find those specific markers in DNA that can distinguish individuals across species.

This study will compare habitat and trout populations on the restored Big Bear Creek and the non-restored Ogdonia Creek. There were three main objectives of this study and they are as follows: 1.) to determine microhabitat selection of brown versus brook trout, and determine habitat selection of trout within Natural Stream Channel Design stream versus the non restored stream; 2.) to access genetic variation of brown trout among two populations (ie. Big Bear Creek and Ogdonia Creek); 3.) to assess water quality and habitat conditions, as well as fish, benthic macroinvertebrate, and periphyton densities.

Methods

The study area encompassed six sites from two third order streams within the Loyalsock Creek watershed. Three sites from Big Bear Creek (Natural Stream Channel Design stream) and three sites from Ogdonia Creek (non-restored) were selected to serve as the study area (Figure 2). The study sites were selected based on previous research that was completed. Each of the six sites were marked and measured as 200-meter sections. In a study completed by Gregory et al. (2002), a study site reach of at least 150-meter was found to be necessary when comparing trout population estimates; therefore these sites comply accordingly with previous research. Site numbers from Big Bear Creek correspond to past site number used on previous studies completed along the same stretch of stream (Gregory et al. 2003). Physical, biological and chemical data were collected at each of these sites. Trout microhabitat surveys were completed on the lower sites of Big Bear Creek (sites 16, 11), and on the lower sites of Ogdonia Creek (sites 1, 2). Brown Trout adipose fin clippings for DNA analysis were collected at site 2 and site 16 at Big Bear Creek, and site 2 and site 3 at Ogdonia Creek.

Big Bear Creek

Site 2 is the furthest site upstream, located just below the Dunwoody Club's pond, and directly after the first structure. Habitat structures within this section include four J-Hook structures and three Cross Vein structures. This site lies approximately 200-meters from the Dunwoody road and is surrounded by a well-established riparian zone.

Site 11 is located about 600 meters downstream from site 2 and begins with a log Cross Vane, the first structure added in 1999. This structure is also located just above a bridge known as the Red Bridge. This site also contains four rock Cross Vanes structures and two rock J-Hook structures. A construction road runs along this site which historically has resulted in massive depletion of vegetation from the riparian zone because of clear cutting trees. To combat this issue, the riparian zone was replanted and it has since begun to rebound.

Site 16 is the furthest site downstream and closest to the mouth of Big Bear Creek. This site is located about 150 meters from site 11 and includes two Cross Veins and one J-Hook. The road is not more than 50 meters from the road along the length of this site. At its closest point the road is approximately 15 meters from the road.

Ogdonia Creek

Site 3 is the furthest site upstream and its upper limit is at the border of Shrewsberry Township and Hillsgrove Township. From the township line, the site extends 200 meters downstream. The site is surrounded by heavily forested riparian buffer.

Site 2 is located approximately two miles downstream from site 3. This site begins at the Wyoming State Forest boundary and extends 200 meters upstream. The road is no more than 30 meters and no less than 15 meters from the stream. A single portion of the stream is used as a

passage way for vehicles to get to the other side of the stream. However, the site has a well established riparian buffer along the length of the stream.

Site 1 is the furthest site downstream and the closest to where Ogdonia Creek meets Loyalsock Creek. This site begins at the confluence of Kettle Creek, Sullivan County, with Ogdonia Creek and extends upstream to a bridge just below the Wyoming State Forest Boundary.

Physical, Biological and Chemical Assessment

Physical data was collected using habitat assessments completed in accordance to EPA's Rapid Bioassessment Habitat Assessment Protocol (Plafken et al. 1989). These assessments are visual estimates that rely on a researcher's interpretation of twelve habitat parameters from which a score of 0-20, indicating the lowest to optimal and highest to optimal, respectively, is assigned to each parameter (Kratzer, 2000R). Microhabitat composition measurements were taken on approximately 600 meters on Big Bear Creek and Ogdonia Creek seperately using the basinwide variation method described by Dolloff et al. (1993). As the surveyor walked upstream the length of habitat units was measured with a tape measure. Each habitat unit was classified as riffle, run, or pool according to Bisson (1984). The width of the stream was visually estimated and on random habitat units, actual stream width was measured to calibrate visual width estimations (Dolloff et al. 1993). The total area for each habitat unit was calculated by multiplying the distance walked by the estimated stream width.

Physiochemical data were collected using grab samples in 500-mL bottles between August and February. Immediately following collection, the samples were stored on ice and analyzed with twenty-four hours. In the lab, parameters of nitrate nitrogen (ppm), nitrite nitrogen

(ppm), total phosphate (ppm), orthophosphate (ppm), pH, and alkalinity (ppm) were analyzed. A HACH 5000 and HACH 2500 spectrophotometer were used to measure nitrate nitrogen, nitrite nitrogen, total phosphate, and orthophosphate. Alkalinity and pH were analyzed using an OAKTON 510 series pH meter and titration with 0.02N sulfuric acid. In order to obtain alkalinity, the pH of a 100mL of sample was recorded, and then 0.02N sulfuric acid was titrated into the sample until the pH was near 4.5. The amount of 0.02N sulfuric acid (mL) was then multiplied by a value of ten to obtain an alkalinity value. Field measurements of temperature (ºC), dissolved oxygen, conductivity (µS) and total dissolved solids (ppm) were taken. Temperature and dissolved oxygen measurements were taken using a YSI 55 DO meter, and an OAKTON CON 410 series meter was used to take conductivity and total dissolved solid measurements. Temperature (ºC) data over a five month duration was also assessed at Big Bear Creek using a HOBO XT temperature meter. This meter was fixed in the stream, and programmed to take a temperature reading every two hours for five months.

Benthic macroinverbrates samples were collected along all three sites from both streams. Kick samples were completed using a D frame kick net and diversity and water quality were assessed. Up to one meter in front of the net, placed downstream from the surveyor, was disturbed and invertebrates were collected on the net. All invertebrates collected at each of the sites were preserved in 70 percent ethanol. The individual site samples were then combined collectively for each stream and 200 individuals from each stream combination were later identified to genus. Invertebrate densities were assessed at each of the six sites using surber samples. Surber samples were collected using a one square foot surber sampler. Two separate surber samples were collected from two separate riffle areas within each study segment. Similarly, these samples were preserved in 70 percent ethanol and were later counted in the lab.

Macroinvertebrate population density and diversity was assessed using the Rapid Bioassessment protocol II (Plafkin et al. 1989).

Fish population surveys were conducted once on all six sites between August and September 2010 utilizing one Smith-Root model LR-45 backpack electro-shocker, and one Smith-Root model 1500 backpack electro-shocker using pulsed-DC. Two 200 meter passes with the electro-shocker were completed on each of the six sites in order to assess trout populations and total fish populations. For each fish collected during electro-fishing, fish were identified and length measurements (cm) were taken. Population densities were determined using Micro-Fish software (Penn State, 1998). Weight measurements were determined using Pennsylvania Fish and Boat Commission length/weight linear regression equation (Personal communication, Bob Weber PFBC biologist 2011).

Periphyton densities were assessed at the central site in both streams. Periphyton were collected using diatometers which are constructed using Carolina Blueboxes that have 6X8cm rectangle removed from either side and five microscope slides placed in the center of the box (Plafkin et al 1989). The diatometers were removed from the stream after a six week incubation period and placed in a Copeland Jar filled with Carosafe. Diatoms were counted and identified according to genera and densities calculated.

Microhabitat Surveys

Microhabitat surveys were conducted through a series of snorkeling surveys. Snorkeling is a preferred method for assessing microhabitat selection of fish because generally fish are not disrupted during snorkeling, thus microhabitat selection is easily observed (Pert et al., 1997H.)

Surveys were conducted between 12:00EST and 5:00EST from September through October. A 200 meter reach was snorkeled among two sites at both streams.

Each snorkeling session began downstream and progress upstream for the entire length of the site. When two individuals surveyed a reach, the surveyors snorkeled in two straight lines. However, when one individual snorkeled a zigzag pattern was used. Once a trout was observed, the species and length of the fish to the nearest 100 mm was recorded, fish elevation above the substrate was estimated and later measured. Immediately following an observation, a fluorescent yellow rock with a basic fish bobber attached was placed at the focal point of the fish (ie. the position of the fish's eye). Trout that were observed but their exact location was unable to be determined were not used in calculations of microhabitat.

After snorkeling a 200 meter reach, wetted stream width (m), depth (m) at the estimated fish's position, water column velocity (m/s), focal velocity (m/s, measure of the water velocity at the position of the fish's eye), and distance to nearest cover (cm) were measured. A Swiffer Model 2100 velocity meter was used to measure water velocity.

Data was analyzed on SPSS Statistics 17.0 using an Independent t-test statistic and the Wilcoxon Rank Sum test to determine if there was a significant difference in habitat selection among brook trout and brown trout, and among trout for the two varying streams. Both tests were conducted to ensure the results did not vary between the two tests. An independent t-test is a comparative means test that assesses differences in means among two unrelated groups. The Wilcoxon Rank Sum test functions by converting primary data into ranks and orders to obtain a test statistic. This type of test is most ideal to use when limited data points are available (Sullivan 2010).

Genetic Analysis

A total of ten adipose fin clippings were taken from brown trout within each study stream for a combined total of twenty samples. The analysis was completed at the U.S Fish and Wildlife Northeast Fishery Center's Population Ecology Branch located in Lamar, PA. Genomic DNA was extracted from adipose fin clip tissue using the Purgene DNA extraction kit (Gentra Systems, Inc., Minneapolis, MN) following the manufacturer's guidelines. The isolated DNA was resuspended in 100 μl of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

The selection of primers for microsatellite analysis was based on previous research completed by the Northeast Fishery Center. Their selection of primers was based on a study performed by Lerceteau-Kohler and Weiss (2006) and Tim King, whom they had personal contact with. Fourteen primers were selected for analysis: *Str*15, *Str*60, *Str*73 (Estoup et al. 1993); *Ssa*85 (O'Reilly et al. 1996); *SsoSL*438 (Slettan 1995); *Ssa*408Uos, *Ssa*410Uos (Cairney et al. 2000); *SSsp*2213, *SSsp*2216 (Paterson et al. 2004); *Ssa*D190, *Ssa*D71 (King et al. 2005); *Sfo*C86, *Sfo*C115, and *Sfo*D75 (Tim King). All loci were combined into three multiplexes for PCR amplification and electrophoresis depending on fragment size and fluorescent label.

Each 15 uL PCR consisted of 2.0 μl of genomic DNA extract, 1.0 X PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl), 2.5 mM $MgCl_2$, 0.25 mM each dNTP, 0.1-0.25 μM of each primer (forward primer fluorescently labeled with FAM, NED or HEX; Applied Biosystems, Foster City, CA), 1.2 units of Taq polymerase (Promega Corporation, Madison, WI), and deionized water added to achieve the final volume. The amplification cycle for all loci consisted of an initial denaturing at 94 °C for 2 min; 35 cycles of 94 °C denaturing for 45 sec, 56 °C annealing for 45 sec, 72 °C extension for 2 min; and a 5 min extension at 72 °C. An ABI Prism 3100®™ Genetic Analyzer (Applied Biosystems, Foster City, CA) was used for capillary

electrophoresis. Genotypic data was analyzed and scored with Genescan 3.7.1 Analysis software and Genotyper 3.7 Fragment Analysis software (Applied Biosystems).

Statistical analysis of genetic data was accomplished through a series of several programs and the presence of different alleles was based on different fragment sizes. Genepop (Version 3.2; Raymond M. & Rousset R. 1995) was used to test for conformance to Hardy-Weinberg Equilibrium. In addition, allelic heterogeneity between populations was assessed using a pairwise estimate which estimated allelic frequency differences among the two populations. Sequential Bonferroni adjustments (Rice 1989) were used to determine statistical significance for all multiple comparison tests. Geneclass (Version 1.0.02) was used to conduct a maximum likelihood assignment test was used to determine the probability of an individual being classified back to their original population (Crounuet J-M et al., 1999). Genetic distance or allelic variation between the populations was calculated using chord distance through the program BIOSYS-1 (Selander RB, 1989).

Results

Physical, Biological and Chemical Assessment

The EPA's Rapid Bioassessment for habitat assessment (following Plafkin et al, 1989) yielded optimal scores, indicating healthy habitat conditions at both Big Bear Creek and Ogdonia Creek. Table 1 compares the habitat assessment scores of each of the three study sties contained within both streams. The site found to be the most optimal, with optimal being a score of hundred percent, was Site 11 at Big Bear Creek with a score of 87%. Furthermore, the two sites being the least optimal were Site 2 and Site 3 at Ogdonia Creek; both had a score of 76%.

Comparatively, similar habitat scores were obtained by both study streams, indicating the presence of similar habitat condition.

The basinwide variation method for the determination of microhabitat measurements yielded similar microhabitat ratios between the three types of microhabitats and among the two study streams (Dolloff et al. 1993). Mean and standard deviation of microhabitat ratio is displayed in Figure 3. Within the three sites of Big Bear Creek mean riffle area was 402.62 m², mean pool area was 219.33 m², and mean run area was 453.51 m². This resulted in a percentage ratio of 37.4% for mean riffle area, 20.3% for mean pool area, and 42.3% for mean run area. Within the three sites of Ogdonia Creek mean riffle area was 443.01 m², mean pool area was 390.64 m², and mean run area was 592.34 m². This resulted in a percentage ratio of 31.1% for mean riffle area, 27.4% for mean pool area, and 41.5% for mean run area.

Physiochemical data for each of the six study sites among Big Bear Creek and Ogdonia Creek is summarized in Table 2 and Table 3, respectively. As seen in Table 2, pH values were within 6.08-7.76 for Big Bear Creek. Furthermore, alkalinity values for August 2010 were 6.0-7.0 ppm among the three sites. Conductivity, the measure of the water's ability to conduct an electrical current, was between 21.7μS and 41.7μS. Total dissolved solids (TDS) is the measure of the dissolved solids in the water. At Big Bear Creek, TDS ranged from 8.3 ppm to 20.9 ppm, which is considered an acceptable range. Among the three sampling events, orthophosphate levels were between 0.07 ppm and 0.23ppm, and phosphate levels were between 0.19 ppm and 0.53 ppm. Nitrate levels ranged from 0.9ppm and 1.2ppm, and Nitrite levels ranged from 0.002ppm and 0.005ppm. All these parameters were within acceptable values. Table 3 shows the chemical data collected at Ogdonia Creek from August to February. pH values were between 6.0 and 7.1 which are optimal values for pH. Alkalinity values were between 16ppm and 71ppm, the

lowest values were seen in August and the highest values in February. However, values were closer to optimal compared to Big Bear Creek. Unfortunately, equipment failure precluded conductivity and total dissolved solid values for February 2011 sampling. Conductivity values between 21.7μS and 30.15μS, and TDS values between 21.7ppm and 30.7ppm were found in August 2010. Orthophosphate levels ranged from 0.07ppm and 0.27ppm, phosphate 0.31ppm and 0.71ppm, nitrate 0.7ppm and 0.9ppm, and nitrite 0.002ppm and 0.005ppm among the three sampling events. In addition, results of five temperature monitoring can be seen in Figure 4.

Benthic macroinvertebrate densities (org/m²) from each of the two study streams are summarized in Table 4. The largest density of macroinvertebrates was 345.5 ± 313.2 (org/m²) found in September 2010 at Big Bear Creek. The smallest density of macroinvertebrates was 4.0 ± 1.41 (org/m²) found in August at Ogdonia Creek. The overall density results indicated healthy densities among both study streams. Macroinvertebrate densities change during certain times of the year, due to the reason that certain hatches occur at different times of the year. Result of diversity and water quality conditions were made using the Rapid Bioassessment Protocol II (Plafkin et al. 1989) and the Hilsenhoff Biotic Index (Hilsenhoff 1977). The Hilsenhoff Biotic Index score was 4.0 for Big Bear Creek and 3.8 for Ogdonia Creek, indicating very good water quality with the possible presence of slight organic pollution. Big Bear Creek total taxa were 19 with an EPT taxa of 11. EPT taxa are an indexes that represent the total taxa among stoneflies, mayflies, and caddisflies which are indictors of good water quality. Total taxa at Ogdonia Creek was 15 with a EPT taxa of 10, once again indicating good water quality. A complete macroinvertebrate taxa list is shown in Appendix A.

Total fish population densities and total trout population densities using the Micro-fish software. Total fish population estimates (# of fish/200m) and total trout population estimates (#

of trout/200m) for Big Bear Creek are shown in Figure 5. Diversity of fish species included brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*), and slimy sculpins (*Cottus cognatus*); the dominant fish species being slimy sculpins. Among trout, brown trout populations persisted over brook trout populations in a 2:11 ratio. The total fish population estimates (# of fish/200m) and total trout population estimates (# of fish/200m) for Ogdonia Creek are displayed in Figure 6. Diversity of fish species was greater at Ogdonia Creek than at Big Bear Creek. In addition to the three species found at Big Bear Creek, blacknose dace (*Rhinichthys atratulus*), longnose dace (*Rhinichthys cataractae*), smallmouth bass (*Micropterus dolomieu*), and fallfish (Semotilus corporalis) were also present. Brown trout populations persisted over brook trout populations at Site 2 and Site 3 in a 9:11 ratio. Between 2007 and 2010 at Big Bear Creek, brown trout populations increased by 14%, brook trout populations decreased by 8%, slimy sculpin populations decreased by 161%, and total fish species decreased by 88% (Table 7). At Ogdonia Creek between 2007 and 2010 brown trout populations rose by 15%, brook trout populations increased by 37% and total trout populations increased by 27% (Table 7). Biomass of trout per unit hectare was 19.46kg/ha at Big Bear Creek and 17.03kg/ha at Ogdonia Creek.

Results of periphyton densities (org/150mm) are shown in Table 5. Both study streams yielded healthy populations. The densities at Ogdonia Creek (468 ± 114 org/150mm) did persist over Big Bear Creek (320 ± 71 org/150mm). The most common periphyton identified was the diatom *Cocconeis sp.* (Figure 7). Other periphyton identified included *Synedral*, *Tabellaria*, *Amphora*, and *Cymbella*.

Microhabitat Surveys

Results of microhabitat surveys indicated that there was no significant difference between brown trout and brook trout habitat selection. In addition results indicated that there was no

significant difference between trout habitat selection between the two streams, Big Bear Creek and Ogdonia Creek. The mean and standard deviation of habitat selection among the parameters of fish depth (m), mean velocity (μ/S), focal velocity (μ/S) and distance to structure (m) are displayed in Table 6. The independent t-test statistic (α = 0.05) indicated no statistically significant difference among brown trout and brook trout habitat selection (Table 6). Similar results persisted in the Wilcoxon Rank Sum test (α = 0.05), the test statistic was greater than the tabular statistic among all parameters. Results of the Wilcoxon Rank Sum (α = 0.05) test indicated no statistically significant difference between trout habitat selection among the two study streams.

Genetic Analysis

The frequency of each allele at each locus in the two different streams is presented in Table 8. The observed heterozygosity of the two populations was similar to the expected thus samples showed conformance to Hardy-Weinberg Equilibrium. Individuals from Big Bear Creek had the highest number of alleles at locus *Ssa* 410 with fourteen alleles being present and heterozygosity being 0.885. Locus Sfo 115 was the least heterozygous with only one allele present and heterozygosity being 0.00. Mean heterozygosity per locus was 0.625 ± 0.066 for Big Bear Creek. Individuals at Ogdonia showed the highest number of alleles at locus *Ssa* 410. Nine alleles were present and heterozygosity was observed as 0.830. At locus Str 73 two alleles were present with heterozyogisty being 0.320. Mean heterozygosity per loci was 0.638 ± 0.042. Results of pairwise estimates (α = 0.05) indicated a significant difference (α < 0.0001) in allele frequencies among the two populations (ie. Big Bear Creek and Ogdonia Creek). A 90.00% assignment was attained through the maximum likelihood assignment test (Table 9). Eighteen out of twenty individuals correctly identified with their original population. This indicated the

probability of a removed individual having the same frequency of alleles as other individuals within the same population. The proportion of genes shared can be seen in Figure 8. Each individual shared the most similarities in genes with other individuals within their population. The two populations distinctly grouped according to their rightful populations.

Discussion

Physical, biological and chemical parameters collectively indicated overall healthy conditions at both Big Bear Creek and Ogdonia Creek. Habitat assessments conducted indicated optimal habitat conditions at both streams. These habitat conditions play a vital role in the health of stream ecosystems. In a study conducted by Warren and Kraft (2003), a decrease in trout abundance was seen as a direct result of a decrease in habitat by the removal of wood (Warren and Kraft 2003). Furthermore, two main issues that Big Bear Creek struggled with prior to restoration were severe bank erosion and channel widening. These have a large effect on trout abundance as well. In a study by Cooper et al. (1994) over widened streams had low mean water depth and were dominated by shallow, sandy habitats. This was compared to a reference stream that had greater mean water depth, contained more woody debris, and provided more deep pool habitats. Overall, fish size and fish species richness was found to be lower in widened stream in comparison to the reference stream (Cooper et al. 1994). Therefore, the addition of habitat structures to Big Bear Creek has likely been beneficial. Moreover, the ability to keep habitat conditions healthy at both streams is extremely important due to the repercussions these streams could face if habitat degradation began to occur.

In addition, microhabitats (riffle, run, pool) provide important environmental niches for macroinvertebrates and fish that inhabit a stream. Both streams showed an even distribution of

microhabitats (Figure 3). This even distribution is important in providing different species and

size class of fish with their respective habitats. Larger brook trout and brown trout have been

found to use deeper water than smaller fish (Patton 2005). In most cases, larger brown trout win

competitive interactions with smaller fish which results in larger brown trout assuming more

desirable feeding and resting positions, ie deeper water (Patton 2005). These deeper water

conditions occur within pool microhabitats. Pool habitats also provide trout species specifically

with colder more desirable temperatures. Optimal temperatures for brown trout are between 50-

60ºF and brook trout can only tolerate temperatures not exceeding 65ºF (Stenier 2002). During

the summer, water temperatures near the surface can exceed these needed trout temperatures.

Therefore, the ability of trout to seek habitats, which provide optimal temperatures, is important

to their health.

Water chemistry also plays a large role in health of stream as aquatic life is directly

affected by the conditions of the water. The results of water chemistry indicated acceptable

conditions at both streams. Alkalinity (ppm) and conductivity (μ/s) were the only two parameters

that were below optimal at Big Bear Creek. An alkalinity value greater than 20 ppm is

considered favorable for sustaining healthy fish populations. Limestone piles have been placed

along the stream at Big Bear Creek as a means of mitigating low alkalinity levels. As for

conductivity, to achieve optimal fish species diversity, a conductivity value greater than 100 μS

is optimal. Only Ogdonia Creek, however, had a greater conductivity value and therefore a

greater diversity of fish species. Seven species were found at Ogdonia Creek over three species

at Big Bear Creek. Therefore, the low conductivity at Big Bear Creek could be an explanation for

the lower species diversity seen, compared to that found at Ogdonia Creek.

Through the microhabitat surveys, brook and brown trout selected for similar habitats. This differed from previous research by Dewald (1992) that stated that brook trout's microhabitat location and vertical distribution shifted in the presence of brown trout (Dewald et al. 1992). One explanation for this result could be the size class of brook trout observed during microhabitat surveys. The majority of brook trout observed ranged from 15cm to greater than 20cm. This was converse to the brown trout observed which the majority ranged from 10cm to greater than 20cm. The lack of difference seen in microhabitat selection between the two species could be because larger brook trout may have increased ability at securing the more desirable habitats over smaller fish (Patton 2005). Therefore, larger brook trout can secure the habitats brown trout also do. In order to be more conclusive, more data should be collected a greater number of individuals, including all size classes of both species should be attained.

Also, a 30% decrease in brook trout populations and 14% increase in brown trout populations were found at Big Bear Creek (Table 7). Results from microhabitat surveys conducted could also explain this result. Through microhabitat surveys, brown trout and brook trout have been found to select for similar habitats (Table 6). In a study completed by Dewald (1992), prey captures rates, have been found to be higher for brown trout in trials where the two species were in the presences of one another. In addition, brook trout also lost weight in the presence of brown trout (Dewald et al. 1992). Since brown trout and brook trout compete for similar prey, the increase in brown trout abundance may have a direct effect on the abundance of brook trout because of their inability to out compete brown trout. Therefore, this may be a plausible explanation for the decrease in brook trout abundance seen at Big Bear Creek.

However, the overall goal of the Dunwoody Club to create a wildly reproducing trout population in Big Bear Creek can be seen through the genetic analysis of brown trout. A high

heterozygosity was found in both streams, specifically Big Bear Creek with a mean heterozygosity of 0.625 ± 0.066. High heterozygosity is directly correlated to the health of a population as it is the measure of variation within a population. This variation has been found to provide populations with the ability to adapt to an ever-changing environment (Cummings & Klug 2008).

The results of the genetic analysis were not conclusive in determining which strain, the Von Behr or Loch Levan strain, inhabits each study stream. To provide determination, samples from known Von Behr and Loch Levan strain trout would have to be taken for comparative analysis. Unfortunately, this goal could be difficult to meet because of the wide distribution and inbreeding that has occurred among brown trout of both strains (Bachman, in press). However, due to the distinct genetic variation seen between the two separate populations some predictions can be drawn. The high genetic variation among the populations indicates that two separate and distinct populations do exist. Moreover, if samples from known strain trout were acquired there may be a strong likelihood that prediction about each population may be met. This information may provide explanation for the decline in wild brook trout populations. As stated previously, it has been suggested that the Von Behr strain is more aggressive than other strains of brown trout (Bachman, in press). Therefore, wild brook trout may be less likely to out compete brown trout for resources and habitat.

In conclusion, monitoring of the progress of the habitat project at Big Bear Creek should continue. More data should be collected in order to gain a better understanding of the habitat selection of brook trout in relation to brown trout's selection. Furthermore, additional genetic determination of the strain of brown trout could provide the Dunwoody Sportsman's' Club, and

other organizations alike, explanation for the decline in wild brook trout abundance within sympatric brook and brown trout streams.

Acknowledgements

References

Bachman, R., in press. Brown Trout (Salmo Trutta). Trout.

Bisson, P.A. Nielsen, J.L., Palmason, R.A., Grove, L.E. 1981. A system of naming habitat types in small streams, with examples of habitat utilization by salmonids during low stream flow. 62-73. Armantrout, ed., Acquisition and utilization of aquatic habitat inventory information. American Fisheries Society, Western Division, Portland, Oregan.

Cooper C.M., Knight S.S., Sheilds F.D. 1994. Effects of channel incision on base flow stream habitats and fishes. Environmental Management. New York, NY: Springer-Vertag Inc.

Cronuet, J-M, Piry S, Luikart G, Estoup A, Solignac M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989-2000.

Cummings, M.R., Klug, W.S., Palladino, M.A. and Spencer, C.A. 2008. Concepts of Genetics. Salt Lake City, Utah: Pearson Education Inc.

Dewald, L., Wilzbach, M.A. 1992. Interactions between native brook trout and hatchery brown trout: Effects on Habitat Use, Feeding and Growth Transactions of the American Fisheries Society. Vol 121: Issue 3: 287-290.

Dolloff, C.A., Hankin, D.G., Reeves, G.H. 1993. Basinwide estimation of habitat and fish populations in streams. Asheville, NC: Department of Agriculture, Forest Service. Gen Tech. Rep. SE-83.

Fausch, K.D., Hawkes, C.L. and Parsons, M.D. 1998. Models that predict standing crop of fish from habitat variables: 1950-1985. Portland, Oregon: Department of Agriculture, Forest Service. Gen. Tech. Rep. 213.

Forbes, G.S., Majumdar, S.K., Miller, E.W., Panah, A.A., Schmalz, R.F. 1994. The Oceans: physical-chemical dynamics and human impacts. Easton, Pennsylvania: The Pennsylvania Academy of Science.

Gregory, S.V., Herlithy, A. T., Hughes, R. M., Kaufmann, P.R., Reynolods, L. 2003. Electrofishing Effort Requirements for Assessing Species Richness and Biotic Integrity in Western Oregon Streams. American Journal of Fisheries Management 23: 450-461.

Hayes, J.W., and Jowett, I.G. 1994. Microhabitat models of large drift-feeding brown trout in three New Zealand rivers. North American Journal of Fisheries management 14:710-725.

Holmes, N.T. 2004. The effects of Rosgen style trout habitat restoration on trout populations. Pennsylvania: Lycoming College.

Kratzer, J. 2000. Effects of Trout Habitat Restoration and Cessation of Stocking on Big Bear Creek. Williamsport, PA: Lycoming College.

Patton, K.A. 2005. Brook Trout and Brown Trout habitat use in two Pennsylvania streams. State College, PA: Pennsylvania State University.

Pechenik, I.A. 2010 Biology of the Invertebrates, Sixth Edition. McGraw Hill Higher Education. New York, NY.

Plafkin, J. L., Barbour, M.T. Porter, K.D, Gross, S.K., Hughes, R.M. 1989. Rapid Bioassessment Protocols. Washington, DC: US Enviornmental Protection Agency.

Raymond ,M., Rousset, R. 1995. GENEPOP (Version 1.2): Population genetics software for exact test and ecumenicism. Journal of Heredity 86: 248-249.

Rhodes, Nicole. Post Restoration Evaluation of Natural Stream Channel Design Project on Big Bear Creek. Williamsport, PA: Lycoming College, 2008.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225.

Rosgen, Dave. "Applied River Morphology." Colorado: Wildland Hydrology, 1996.

Selander, R.B., Swofford L.D. 1989. BIOSYS-1 conputer program for the analysis of allelic variation in population genetics and biochemical systematic. Release 1.7. D.L. Swofford, Illinois Natural History Survey, Champaign, Illinois.

Smith, Geoff. 2001. Colonization of Benthic Macroinvertebrates following Construction of Fluvial Geomorphology Structures. Williamsport, PA: Lycoming College.

Steiner, L. 2002. Pennsylvania Fishes: Chapter 15: Trout. 160-170.

"strain." *Dictionary.com Unabridged*. Random House, Inc. 23 Feb. 2011. <Dictionary.com http://dictionary.reference.com/browse/strain

Sullivan, M. 2010. Fundamentals of Statistics: Third Edition. Pearson Education Incorporated. Canada.

Warren, D.R., & Kraft, C.E. 2003. Development of large woody debris and debris dams in streams. Ithaca, NY: Development of Natural Resources, Cornell University.

Table 1: Habitat Assessment Scores among Big Bear Creek and Ogdonia Creek

Big Bear Creek			
Site #	#2	#11	#16
Epifaunal Substrate/Avaliable Cover	16	19	9
Embeddedness	16	18	12
Velocity/Depth Regime	17	19	15
Sediment Deposition	13	20	19
Channel Flow Status	11	19	13
Channel Alteration	13	8	18
Frequency of Riffles	19	17	17
Bank Stability (Left bank)	9	9	9
Bank Stability (Right bank)	9	8	6
Vegatative Protection (Left Bank)	9	10	4
Vegatative Protection (Right Bank)	9	10	5
Riparian Vegatatvie Zone Width (Left Bank)	10	10	10
Riparian Vegatatvie Zone Width (Right Bank)	9	7	7
Total	160	174	154
Percent from Optimal	80%	87%	77%

Ogdonia Creek			
Site #	#1	#2	#3
Epifaunal Substrate/Avaliable Cover	12	14	17
Embeddedness	18	18	16
Velocity/Depth Regime	17	19	14
Sediment Deposition	19	17	14
Channel Flow Status	9	8	10
Channel Alteration	20	15	18
Frequency of Riffles	19	19	16
Bank Stability (Left bank)	7	7	6
Bank Stability (Right bank)	4	5	8
Vegatative Protection (Left Bank)	10	8	8
Vegatative Protection (Right Bank)	8	8	8
Riparian Vegatatvie Zone Width (Left Bank)	9	4	6
Riparian Vegatatvie Zone Width (Right Bank)	9	9	10
Total	161	151	151
Percent from Optimal	81%	76%	76%

Table 2: Big Bear Creek Water Chemistry Data

Site #2			
Parameters	8/6/10	9/14/10	2/15/11
pH (lab)	7.76	N/A	6.08
Conductivity (ms)	36.1	37.8	29.3
Alkalinity (ppm)	7	N/A	9
Orthophosphate (ppm)	0.13	0.09	0.22
Total Phosphorous (ppm)	0.53	0.49	0.39
Nitrate (ppm)	0.8	0.9	N/A
Nitrite (ppm)	0.003	0.003	0.004
DO (ppm)	9.85	8.75	7.57
Temperature (°C)	14.9	11.8	0.2
TDS (ppm)	18.1	19.4	14.7

Site #11			
Parameters	8/6/10	9/14/10	2/15/11
pH (lab)	6.51	N/A	6.78
Conductivity (ms)	21.7	26.8	31.9
Alkalinity (ppm)	7	N/A	30
Orthophosphate (ppm)	0.11	0.12	0.23
Total Phosphorous (ppm)	0.45	0.19	0.24
Nitrate (ppm)	1	1.2	N/A
Nitrite (ppm)	0.005	0.003	0.005
DO (ppm)	7.96	8.4	5.84
Temperature (°C)	16.5	12.4	0.9
TDS (ppm)	10.9	13.6	8.3

Site #16			
Parameters	8/6/10	9/14/10	2/15/11
pH (lab)	6.65	N/A	6.58
Conductivity (ms)	41.7	38.7	35.4
Alkalinity (ppm)	6	N/A	65
Orthophosphate (ppm)	0.07	0.08	0.22
Total Phosphorous (ppm)	0.52	0.37	0.28
Nitrate (ppm)	1.1	0.9	N/A
Nitrite (ppm)	0.004	0.004	0.002
DO (ppm)	7.64	7.81	10.04
Temperature (°C)	16.2	12.3	1.0
TDS (ppm)	20.9	18.3	17.8

Table 3: Ogdonia Creek Water Chemistry Data

Site #1			
Parameters	**8/6/10**	**9/14/10**	**2/15/11**
pH (lab)	7.1	N/A	6.64
Conductivity (ms)	77.5	N/A	N/A
Alkalinity (ppm)	23	N/A	50
Orthophosphate (ppm)	0.09	0.1	0.27
Total Phosphorous (ppm)	0.71	0.59	0.22
Nitrate (ppm)	0.6	0.9	N/A
Nitrite (ppm)	0.005	0.004	0.002
DO (ppm)	8.72	8.53	6.70
Temperature (°C)	20.1	13.1	0.3
TDS (ppm)	23	N/A	N/A

Site #2			
Parameters	**8/6/10**	**9/14/10**	**2/15/11**
pH (lab)	6.95	N/A	6.95
Conductivity (ms)	43.3	N/A	N/A
Alkalinity (ppm)	21	N/A	65
Orthophosphate (ppm)	0.07	0.08	0.16
Total Phosphorous (ppm)	0.48	0.21	0.13
Nitrate (ppm)	0.4	0.9	N/A
Nitrite (ppm)	0.002	0.003	0.003
DO (ppm)	8.19	8.28	6.48
Temperature (°C)	20	13.6	0.1
TDS (ppm)	21.7	N/A	N/A

Site #3			
Parameters	**8/6/10**	**9/14/10**	**2/15/11**
pH (lab)	6.6	N/A	6.59
Conductivity (ms)	63.9	N/A	N/A
Alkalinity (ppm)	16	N/A	70
Orthophosphate (ppm)	0.12	0.08	0.18
Total Phosphorous (ppm)	0.31	0.21	0.19
Nitrate (ppm)	0.4	0.7	N/A
Nitrite (ppm)	0.002	0.003	0.002
DO (ppm)	8.44	8.49	7.23
Temperature (°C)	19.4	12.8	0.4
TDS (ppm)	30.15	N/A	N/A

Table 4: Benthic Macroinvertebrate Densities (org/m²) collected from surber samples - 2010

Macroinvertebrate Densities / m²		
Big Bear Creek		
	8/6/2010	9/14/2010
Site #2	173.5 ± 3.5	37 ± 24.0
Site #11	136 ± 138.6	151 ± 52.3
Site #16	4.5 ± 0.70	345.5 ± 313.2

Macroinvertebrate Densities / m²		
Ogdonia Creek		
	8/6/2010	9/14/2010
Site #1	4.0 ± 1.41	66.0 ± 18.3
Site #2	8.5 ± 6.36	32.0 ± 2.82
Site #3	12.0 ± 7.07	*

* Road Construction Precluded Sampling

Table 5: Periphyton Densities (org/150mm) collected from diatometers – 2010

Site Name	Big Bear Creek	Ogdonia Creek
Periphyton Density (orgs/150mm)	320.0 ± 71.43	468.0 ± 114.94

Table 6: Independent T-test statistic (α = 0.05) of Microhabitat Selection among Brook Trout and Brown Trout from both Big Bear Creek and Ogdonia Creek.

Mean +/- Std. Dev and α level			
	Brook Trout N = 12	**Brown Trout** N = 20	**P-value**
Parameter			
Depth (m)	0.362 +/- 0.126	0.389 +/- 0.185	0.6229
Mean Velocity (μ/s)	0.183 +/- 0.179	0.134 +/- 0.124	0.4159
Focal Velocity (μ/s)	0.155 +/- 0.236	0.095 +/- 0.155	0.4380
Distance to Structure (m)	0.350 +/- 0.272	0.400 +/- 0.465	0.6790

Table 7: Fish Density/hectare - 2010

Big Bear Creek

	Brown Trout	Brook Trout	Total Trout	Sculpins	Total Fishes
Site 2	307	70	377	456	877
Site 11	622	68	690	1096	1710
Site 16	149	43	192	N/A	N/A
Mean	359	60	420	776	1239
2007 Mean*	309	78	386	2032	2335
2002 Mean*	173	73	246		
% inc.(+)/dec.(-)	+ 14	- 30	+8	-161	-88

* 2002 data from Patton (2005), 2007 data from Rhodes (2007)

Ogdonia Creek

	Brown Trout	Brook Trout	Total Trout	Other Species	Total Fishes
Site 1	0	184	184	7920	8104
Site 2	296	256	552	2120	2672
Site 3	152	96	248	N/A	N/A
Mean	149	179	328	5020	5388
2002 Mean*	126	113	239		
% inc.(+)/dec.(-)	+15	+37	+27		

* 2002 data from Patton (2005)

Table 8: Allelic Frequency Table

He = expected heterozygosity
Ho = observed heterozygosity

	Big Bear Creek	Ogdonia Creek
Ssa 85		
(N)	10	10
113	0.65	0.55
115	0.3	0.3
117	0	0.15
119	0.05	0
He	0.511	0.616
Ho	0.7	0.7
Ssa 408		
(N)	8	9
208	0.125	0.056
212	0.063	0.111
236	0.118	0.222
240	0.313	0
244	0.188	0.056
248	0.063	0.389
260	0.063	0.167
He	0.858	0.797
Ho	0.875	0.889
Ssa 410		
(N)	10	10
187	0.1	0
207	0.05	0.15
211	0.05	0.05
215	0.05	0.25
219	0.05	0.1
223	0.05	0.15
231	0	0.1
235	0.05	0
247	0.1	0.2
251	0.05	0
263	0.05	0
271	0.05	0

279	0.05	0
283	0.25	0
He	0.932	0.874
Ho	0.9	1
SsoSL 438		
(N)	9	10
96	0.56	0.45
100	0.278	0.15
102	0.167	0
104	0.5	0.4
He	0.68	0.647
Ho	0.667	0.6
SSsp2213		
(N)	10	10
167	0.2	0.05
171	0	0.25
175	0.5	0.05
179	0.15	0.05
183	0.15	0.25
187	0.35	0.2
191	0	0.05
195	0.1	0.1
He	0.821	0.58
Ho	0.8	0.8
SSsp2216		
(N)	9	10
137	0.389	0.3
141	0.389	0.5
145	0.167	0.15
149	0	0.05
153	0.056	0
He	0.706	0.668
Ho	0.667	0.5
SfoC86		
(N)	10	8
109	0.3	0.5
112	70	0.5

He	0.442	0.533
Ho	0.4	0.75
*Sfo*C115		
(N)	9	8
155	1	0.75
169	0	0.063
239	0	0.188
He	0	0.425
Ho	0	0.5
*Ssa*D71		
(N)	10	10
183	0	0.05
187	0.5	0
191	0.5	0
195	0.5	0.35
199	0.1	0.25
293	0.35	0
307	0.25	0.15
211	0.05	0.15
219	0.1	0.05
He	0.826	0.805
Ho	0.7	0.9
*Sfo*D75		
(N)	10	9
257	0.55	0.611
265	0	0.056
269	0.35	0.056
277	0.5	0.222
285	0	0.056
333	0.5	0
He	0.6	0.601
Ho	0.5	0.667
*Ssa*D190		
(N)	10	10
123	0.5	0.1
127	0.5	0
131	0.2	0

139	0.2	0
143	0.35	0.25
147	0.15	0.6
151	0	0.05
He	0.811	0.595
Ho	0.9	0.5
*Str*15		
(N)	10	10
218	0.45	0.3
220	0.35	0
222	0.1	0.5
224	0.05	0.5
226	0.05	0.15
He	0.695	0.668
Ho	0.7	0.5
*Str*60		
(N)	9	10
93	0.333	0.4
97	0.667	0.6
He	0.471	0.505
Ho	0.444	0.6
*Str*73		
(N)	10	10
142	0.75	0.2
144	0.25	0.8
He	0.395	0.337
Ho	0.3	0.4

Table 9: Maximum Likelihood Assignment Test indicating placement of brown trout individuals from both Big Bear Creek and Ogdonia Creek.

```
 1   LORI-001 [BIG BEAR CREEK]
 2   LORI-002 [BIG BEAR CREEK]
 3   LORI-003 [BIG BEAR CREEK]
 4   LORI-004 [BIG BEAR CREEK]
 5   LORI-005 [BIG BEAR CREEK]
 6   LORI-011 [BIG BEAR CREEK]
 7   LORI-012 [BIG BEAR CREEK]
 8   LORI-013 [BIG BEAR CREEK]
 9   LORI-014 [BIG BEAR CREEK]
10   LORI-015 [ BIG BEAR CREEK]

11   LORI-021 [OGDONIA CREEK]
12   LORI-022 [OGDONIA CREEK]
13   LORI-023 [OGDONIA CREEK]
14   LORI-027 [OGDONIA CREEK]
15   LORI-028 [OGDONIA CREEK]
16   LORI-030 [OGDONIA CREEK]
17   LORI-031 [OGDONIA CREEK]
18   LORI-032 [BIG BEAR CREEK]
19   LORI-037 [BIG BEAR CREEK]
20   LORI-039 [OGDONIA CREEK]
```

18 individuals on 20 correctly identified (90.00%)

Individuals 1-10 are individuals that are from to Big Bear Creek, individuals 11-20 are individuals that are from Ogdonia Creek. Percentage indicates the proportion of individuals that correctly identified with their population.

Figure 1: Morphological comparison of brown trout strains.
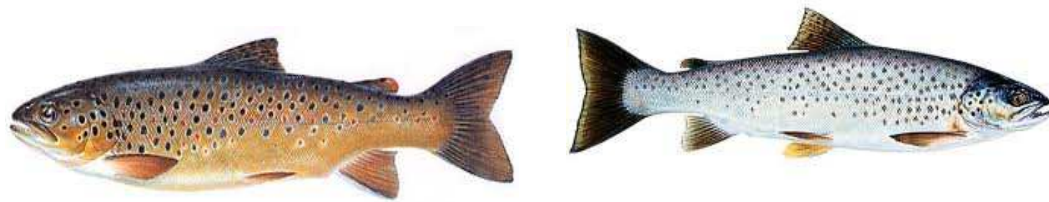
Von Behr   Loch Levan

* Pennsylvania Fishes (PA Fish and Boat Commission)

Figure 2: Land Map of Loyalsock Creek Watershed showing locations of Big Bear Creek and Ogdonia Creek.
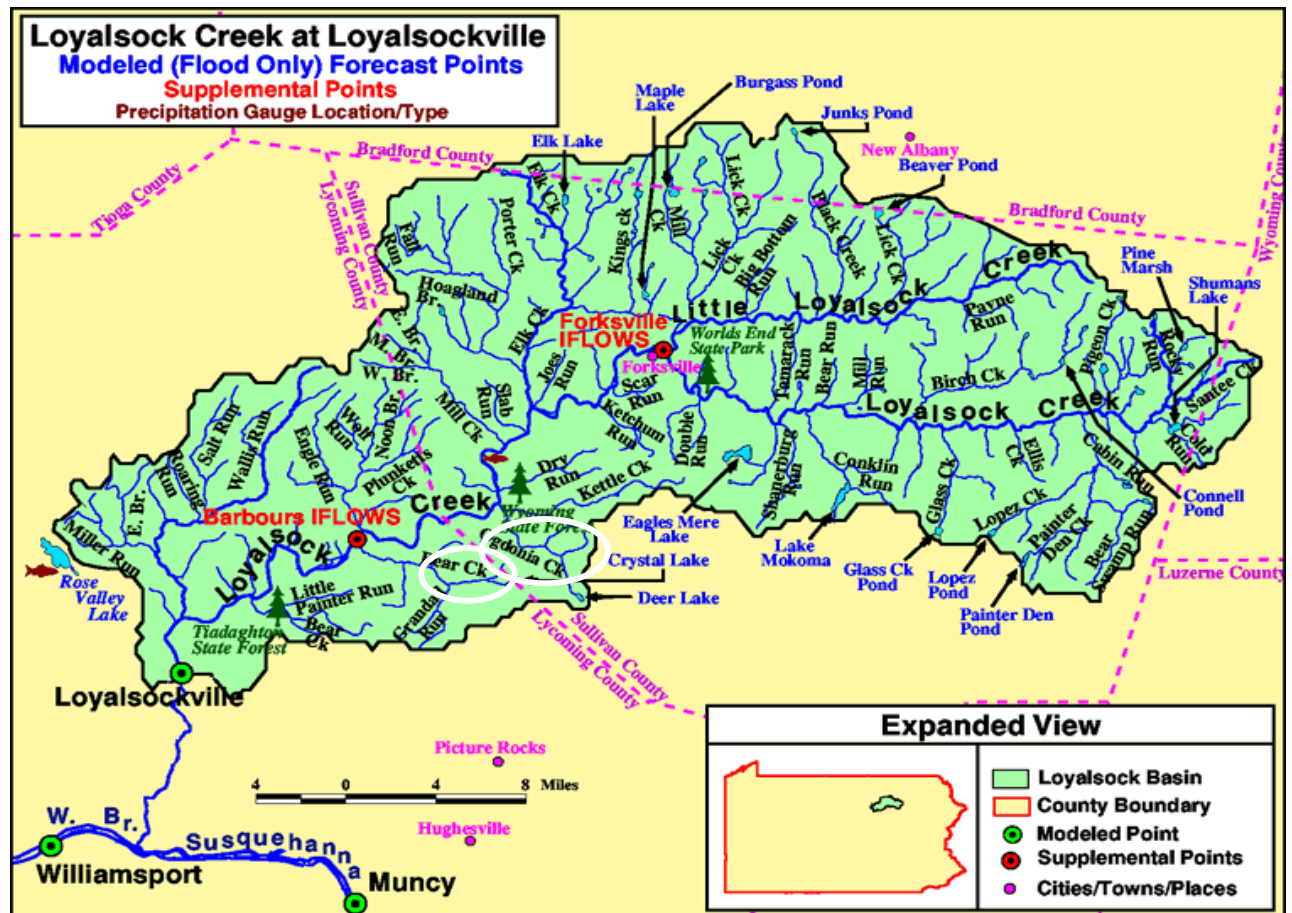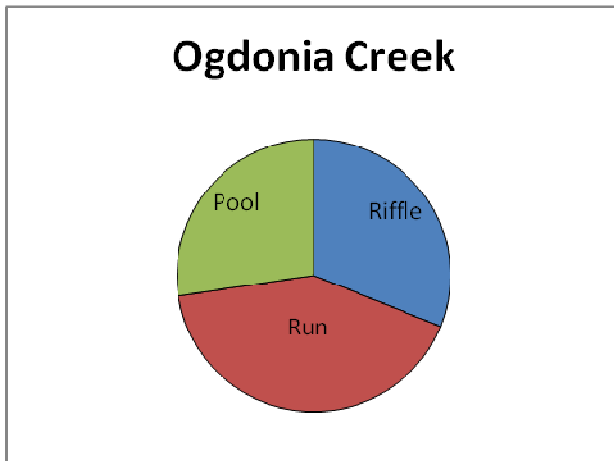
Figure 3: Mean ± Standard Error of Area (m²) of Microhabitats from 3 study sites on each creek. – Fall 2010
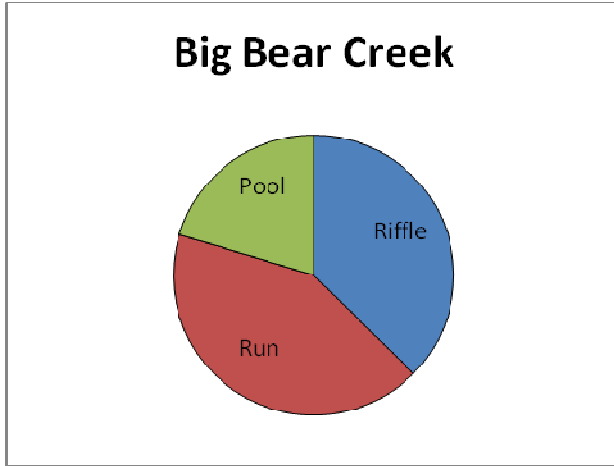
Big Bear Creek	
Riffle (m²)	402.62 ± 109.6
Run (m²)	453.51 ± 90.5
Pool (m²)	219.33 ± 39.5

Ogdonia Creek	
Riffle (m²)	443.03 ± 132.06
Run (m²)	592.34 ± 27.7
Pool (m²)	390.64 ± 94.3

Figure 4: Big Bear Temperature Data – August – December 2010
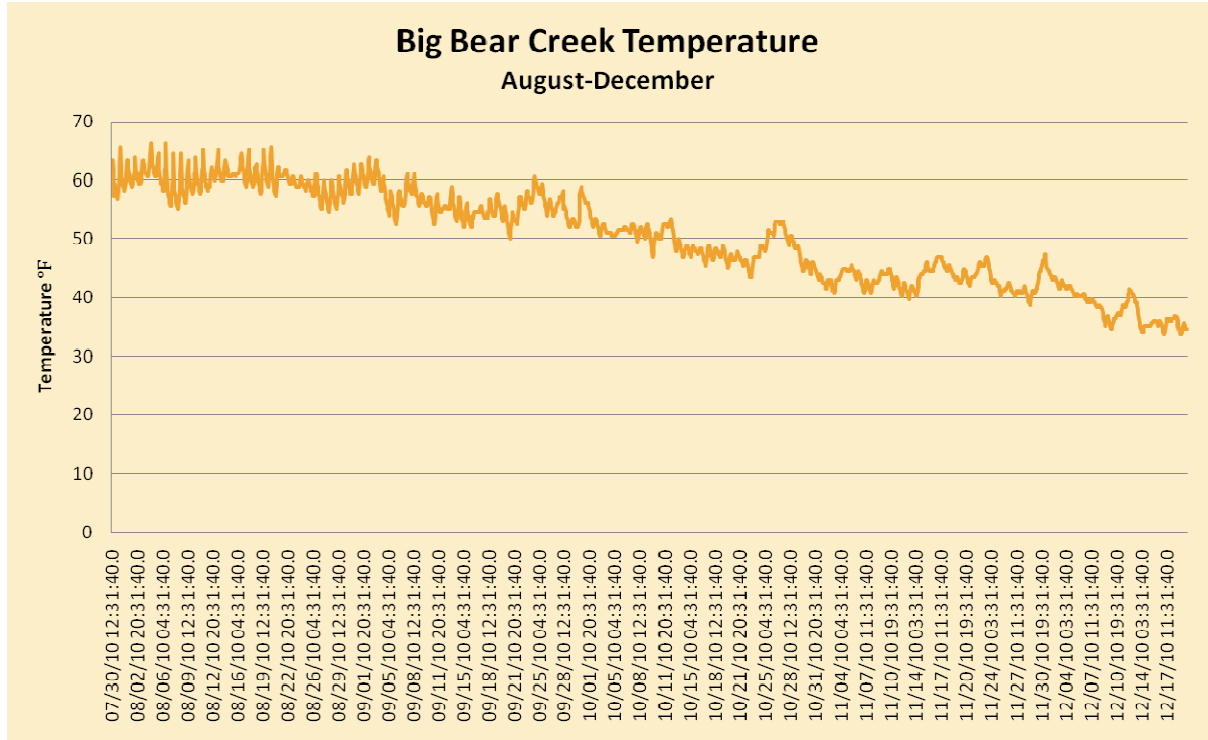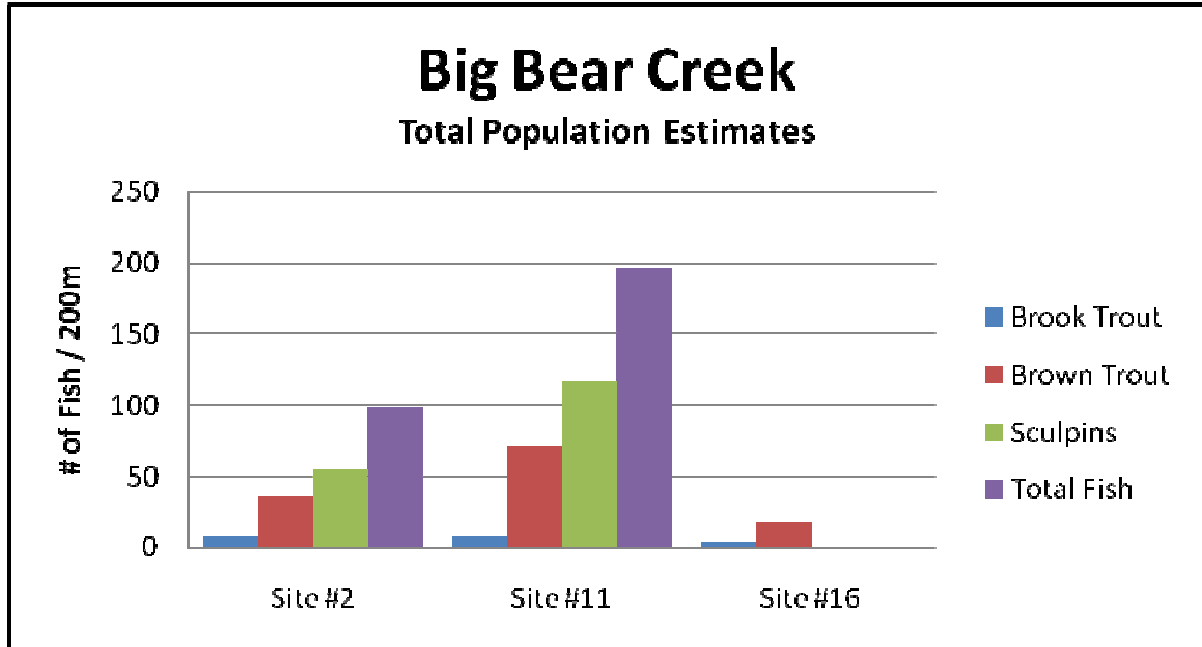
Figure 5: Fish Population Density at Big Bear Creek – 2010
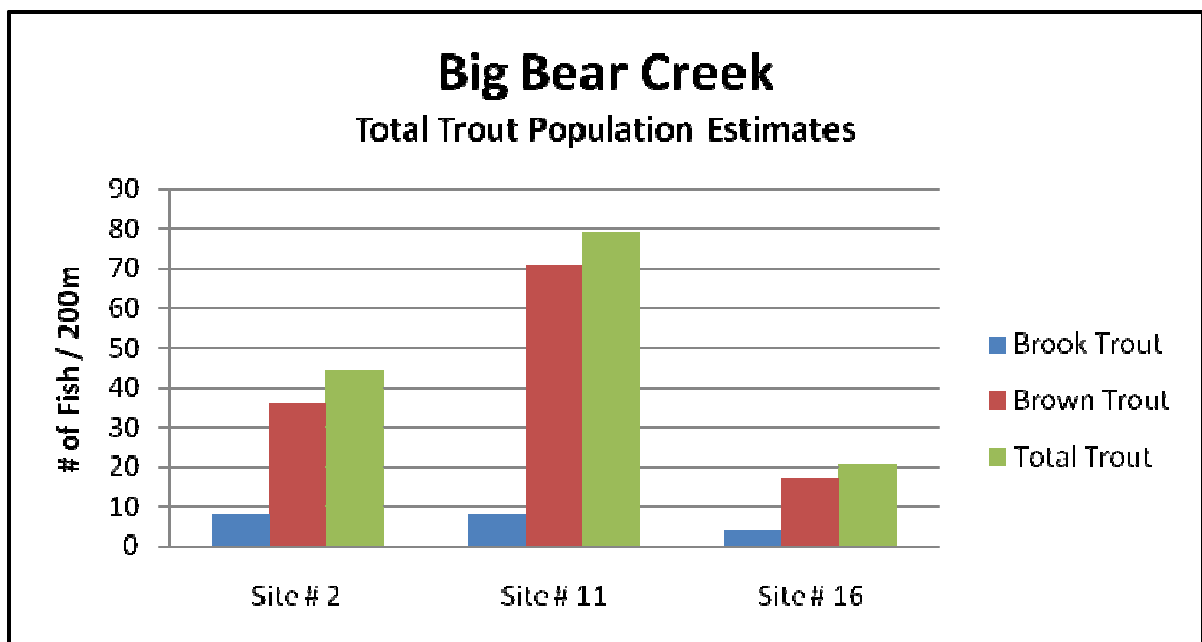
Figure 6: Fish Population Density at Ogdonia Creek - 2010
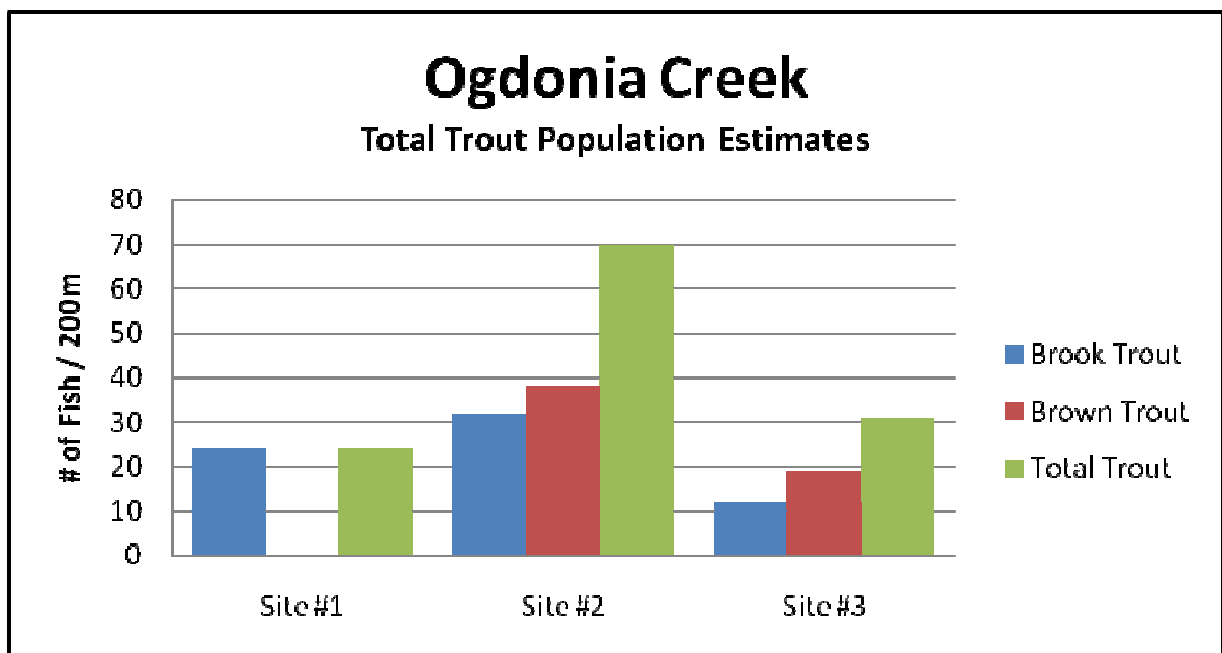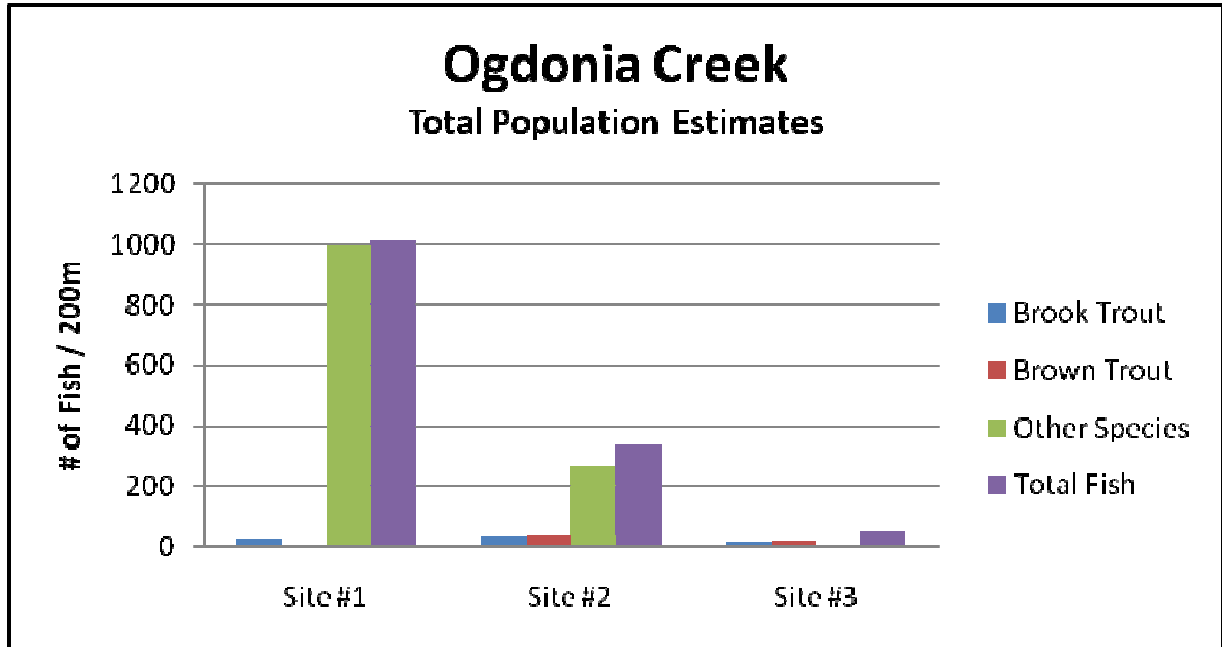
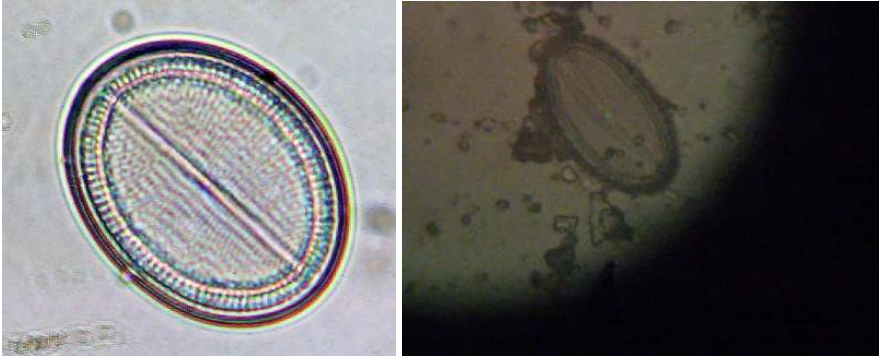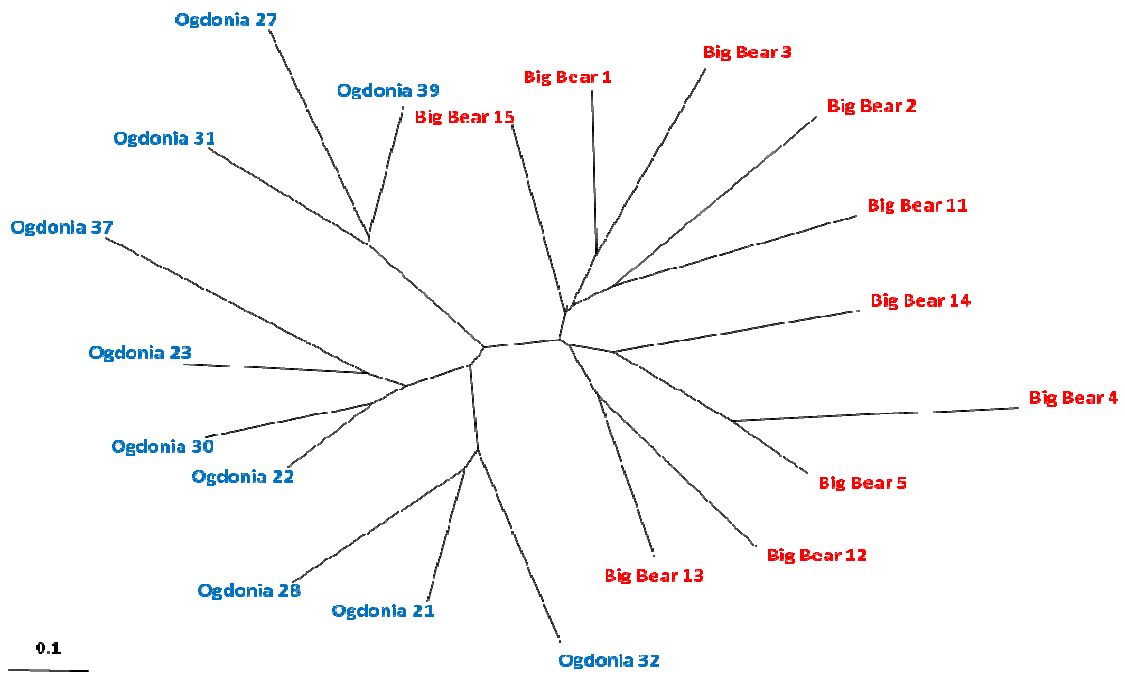**Figure 7: Common Periphyton Collected (*Cocconeis sp.*)**

Figure 8: Proportion Shared Tree indcating relatedness of brown trout individuals among all individuals from both Big Bear Creek and Ogdonia Creek.

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Appendix A: Total Taxa List of Benetic Macroinvertebrates at Big Bear Creek and Ogdonia Creek.

Big Bear Creek:
Annelida
    Oligocheata
Plecoptera
    Chloroperlidae
    Perlidae
    Perlodidae
Ephemeroptera
    Ameletidae
    Baetidae
    Caenidae
    Ephemerellidae
    Heptageniidae
Megaloptera
    Corydalidae
Trichoptera
    Brachycentridae
    Hydropsychidae
Coleoptera
    Elmidae
Diptera
    Chironomidae

Ogdonia Creek:
Decopoda
    Cambaridae
Plecoptera
    Capniidae
    Chloroperlidae
    Nemouridae
    Peltoperlidae
    Perlidae
    Perlodidae
Ephemeroptera
    Baetidae
    Heptageniidae
    Leptophlebiidae
Odonata
    Aeshnidae
Megaloptera
    Corydalidae
Trichoptera
    Hydropsychidae
    Philopotamiidae
Coleoptera
    Elimidae
    Psephenidae
Diptera
    Chironomidae