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Reintroduction of brook trout to the South River via Upwelling Springs

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Reintroduction of Brook Trout to the South River via Upwelling Springs

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Abstract:

Populations of brook trout, *Salvelinus fontinalis,* throughout Virginia mountain streams have seen a rapid decline due to warmer conditions, decreased oxygen levels, and changes in the main stem streambed (Hudy *et al.,* 2008). Current solutions have stocked more tolerant adult brook trout in the main stem rivers, which is costly and must be repeated yearly (Lennon, 1967). Finding an environment conducive for stocking brook trout eggs rather than adults would be ideal because they would return to this viable location to spawn, making them a self-sustaining population which is less costly and easier to implement. Upwelling springs connected to the main stem are potentially more conducive than the main stem due to lower temperatures, higher oxygenation, and less chemical runoff (Meehan, 1991).

Brook trout eggs were reared in upwelling springs connected to the main stem of the South River in Waynesboro, Virginia in order to create an inexpensive self-sustained population. Percent survival, occurrence of deformities, conductivity, and temperature were measured at four experimental sites and compared to two control sites. One of the experimental sites, City Springs, was the most viable of the experimental springs as it had the highest survival and the lowest occurrence of deformities. Another experimental site, Baker Box, was the least viable as it had the lowest survival and conductivity and highest severity of deformities. Overall, all of the experimental sites proved to be favorable for the reintroduction of brook trout, with the exception of the Baker Box site. Future experiments should rear the eggs directly in the sediment to see if sediment size in the springs is more or less conducive than in the main stem.

Introduction:

Declining populations of brook trout, *Salvelinus fontinalis*, throughout Virginia mountain streams has led to increasing focus on the species' reintroduction (Hudy *et al.,* 2008). Brook trout prefer cold, high water quality streams (Raleigh *et al.*, 1982), but are sensitive to changes in water conditions such as low oxygenation, sedimentation, and acidification (Grubb, 2003). Ecological deterioration such as warmer conditions, decreased oxygen levels, and changes in the streambed in the eastern United States has caused the decline of many brook trout populations. This decline is due to the fact that brook trout are unable to adapt to these changes during their egg stage (Hudy et. al. 2008). Stocking streams with juvenile and adult brook trout has become a temporary solution because trout in these stages are more tolerant of ecological deterioration such as a decrease in pH and an increase in UV exposure due to decreased vegetation (Lennon, 1967). Adult and juvenile trout are stocked annually, which increases the cost of intervention. Reintroduction programs that stock eggs with the goal of producing self-sustaining populations may be more permanent solutions.

Knowledge of brook trout lifecycle and reproduction is a prerequisite to reintroduction. At the beginning of the reproduction process, the female brook trout uses her caudal fin to create a redd, or a shallow nest in the stream bed (Everhart *et al.,* 1961). Females and males will release the eggs and milt respectively. Fertilized eggs fall into the redd and the female fills the hole with substrate (Roberts, 2000). If the water conditions are suitable, the fertilized eggs will hatch. After they have hatched, the eggs then feed off of a nutritious yolk until it is completely consumed, at which point the larval fish emerges and swims up through the sediment, leaving the redd in search of food and to begin its young adult life (Crossman, 1985). This is known as the "swim-up" stage.

There are multiple points in the lifecycle that can be inhibited by poor environmental quality. If the substrate is too fine, the egg has little chance of survival. The membrane surrounding the embryo is porous and susceptible to sediment clogs, which prevents oxygen from entering the egg and metabolic waste from being flushed away. Fine substrate also inhibits the ability of emerging trout to enter the stream after hatching because the embryos can be entombed under the sediment (Frannsen *et al*, 2012).

The presence of discharging groundwater from deep water springs is a preference for brook trout selecting red (Warren, et al. 2012). Fine sediment is limited in deep groundwater spring environments. Upwelling springs tend to have ideal temperatures, less agriculture runoff and fewer contaminants more common in the main stems, and disperse metabolic waste more quickly (Meehan, 1991). If the springs are successful hatching sites, then brook trout will imprint on them and return to these sites to spawn. This process will lead to future generations, providing a potential solution for the reintroduction of brook trout in Virginia mountain streams.

Currently, brook trout are not spawning successfully in the main stem of the South River due to fine sediment size, which suffocates the eggs (Argent and Flebbe, 1999). Deep groundwater springs in the South River's immediate area pose an environment that may be conducive to increasing brook trout fecundity. However, each of the potential hatching grounds has unique water chemistry that may not allow brook trout to survive to the juvenile fish stage and may lead to deformities. While the water chemistry of the South River has been monitored over the years, there have been few studies investigating the potential for the groundwater springs of the South River to incubate brook trout eggs until they hatch. The goal of this study is to successfully rear brook trout eggs in upwelling springs located in Waynesboro, Virginia in order to create a more naturally sustained population. Suitability of each spring will be determined by comparing survival and deformity occurrences to those of the South River's main stem.

We hypothesize that the springs will be more conducive, determined by greater survival and less deformities, than the main stem rivers. If the springs are equally or more conducive for brook trout egg survival than the main stem rivers, this proposes a possible solution for the reintroduction of brook trout to Virginia streams. If the survival of brook trout eggs reared in South River springs is greater than or equal to the survival of brook trout eggs reared in the main stem control, then the springs are conducive for brook trout reintroduction. Similarly, if the occurrence of deformities in brook trout reared in the springs is less than or equal to that of brook trout reared in the main stem controls, then the springs are conducive for brook trout reintroduction.

Experimental:

To test if upwelling springs are viable for brook trout reintroduction, survival, deformities, temperature, and conductivity were measured at four springs in the South River's immediate area. These measurements were compared to those of two controls taken directly from the South River's main stem. This study was conducted as a course embedded research project in BIO459/559 Freshwater Ecology (taught by Dr. Christine May). Statistics were performed using strategies gained in BIO454 Biometrics (taught by Dr. Grace Wyngaard and Dr. Nusrat Juhan). Additional assistance came from Mr. Thom Teears at the Virginia Department of Game and Inland Fisheries (VDGIF).

Site Locations:

A total of six sites were used to rear eggs. The four experimental sites, Baker Box, Rife Loath, Coyner Spring, and City Spring, are located in Waynesboro, Virginia (Figure 1). There were two control sites; the Montebello State Fish Culture Station and two tanks filled with South River main stem water. These tanks were kept in the Bioscience vivarium at James Madison University, Harrisonburg, Virginia. All eggs were provided by the Paint Bank Fish Hatchery. The eggs at the Montebello State Fish Culture Station were reared in the main stem of the South River. Eggs at Rife Loath, Coyner Spring, and City Spring were reared in the springs. Eggs at Baker Box were reared in coolers, and water at Baker Springs was supplied using a siphon from an underground pump system.

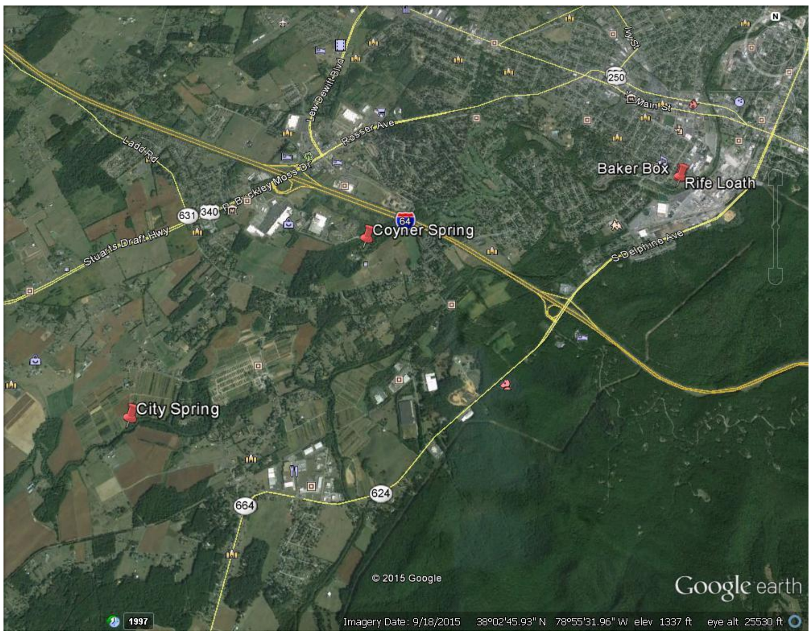

Figure 1: Four experimental sites located throughout Waynesboro, Virginia; labelled with a red pin. Baker Box and Rife Loath share a pin as they were positioned close to one another.

Hatch Box Setup:

Hatch boxes were used at experimental and control sites to rear eggs (Figure 2). Each site had two identical hatch boxes of nine compartments each labelled A-I. Each box had a screen lid zip-tied to cover the compartments and protect the eggs from tampering, predators, and sediment edition, and was tethered to a stationary object using a metal wire to prevent being swept away by the current. In late September 2015, eggs and milt obtained from Paint Bank Hatchery were hand mixed in baggies on site and stirred to ensure optimal fertilization. All eggs were assumed fertilized. Each of the nine compartments had 100 fertilized eggs, resulting in a total of 1800 eggs at each site.

After about two weeks, towards the beginning of October, all of the eggs died. To reestablish the experiment, we used late-stage eggs (eyed eggs). 30 eyed eggs reared at Paint Bank Fish Hatchery were placed in each compartment resulting in 540 eyed eggs at each site. Trays were covered with solid plastic lids in addition to the screen lids to prevent possible harm caused by UV radiation. Eyed eggs were used because they are at an older, less vulnerable stage, yet still acquire homing (Hudy *et. al.,* 2008).

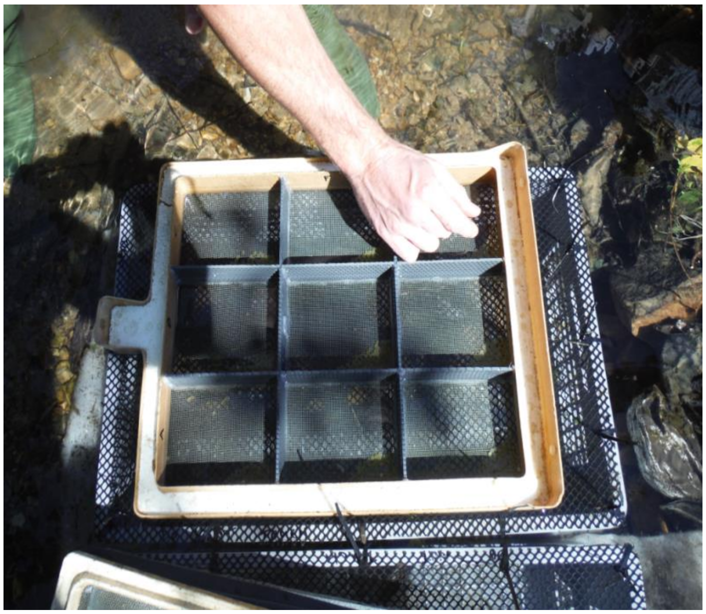

Figure 2: An example of one hatch box consisting of nine compartments. The screen lid for this hatch box is just out of the picture.

Hatch Box Maintenance:

Beginning when fertilized eggs were first put in hatch boxes in late September, 2015, hatch boxes were checked every three days for tampering and deceased eggs were removed to prevent the lethal spread of fungus. At this time, temperature and conductivity were recorded using a conductivity meter. These measurements were taken to possibly explain future discrepancies between survival and prevalence of deformities between sites. Dead eggs, identified by their foggy appearance, were discarded directly into ethanol. The number of dead eggs and corresponding compartment was recorded. As explained before, after all eggs died they were replaced with the 30 eyed eggs and the same procedure was repeated. Eyed eggs began to hatch and enter the yolk sac stage in late October, 2015. The number of dead eggs as well as dead hatchlings was recorded. Dead hatchlings as well as surviving juveniles were examined for deformities.

This procedure continued through mid-November, 2015 until the hatchlings consumed their yolk sac and entered the swim up. Once all hatchlings depleted their yolk sacs, any existing deformities were recorded and photographed for later classification. A final count of survival was recorded and all brook trout were released. Control hatch boxes were overseen using the same procedure by the manager of the Montebello Culture Station, Thom Teears.

Deformities:

Deformities in hatched fish, when present, were photographed and recorded as either craniofacial (i.e. jaw tissue reduction), skeletal (i.e scoliosis), or edema (i.e. fluid accumulation in the head region) (Holm *et al.,* 2005) (Figure 3). A craniofacial deformity is when the jaw is enlarged or reduced in size or the eyes are irregular in diameter or asymmetrical. A skeletal deformity is when the spine irregularly curves. Finally, an edema deformity is when excess fluid is accumulated in the body or head (Holm *et al.,* 2003).

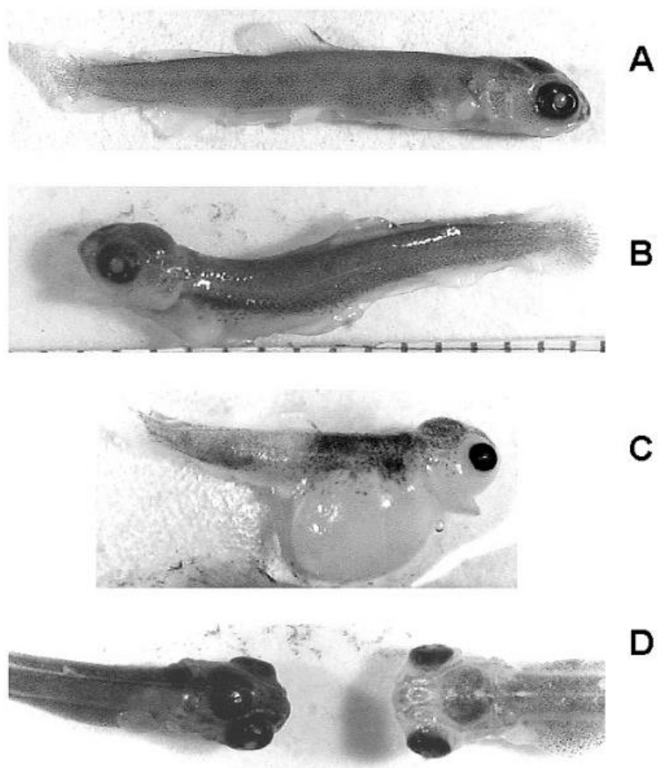

Figure 3: Deformities in rainbow trout fry. Picture A is a normal fry as well as the left fry in picture D, B has a skeletal deformity as seen by the curvature of the spine, C has edema as seen in the irregular accumulation of fluid, and the fry on the right in D has a craniofacial deformity as seen in the irregular spreading of the eyes (reproduced from Holm *et al.*, 2003).

Deformities were given a rating of lethality between 0-3 using a similar technique as used by Holm *et al* 2003. A score of 0 denotes no deformity, a score of 1 denotes minimal effects on survivability, a score of 2 denotes a deformity that affects ability to swim and/or forage but is still survivable, and a score of 3 denotes a deformity that was lethal. A Graduated Severity Index (GSI) (Holm *et al* 2003) was generated to compare not only the occurrence of deformities at each site, but their severity as well. This index uses the lethality ratings as well as count of deformities at each site to generate an overall severity index. Analyzing the severity of deformities provides deeper insight into viability of a spring than occurrence alone since one lethal deformity with a score of three is just as severe as three minor deformities with a score of one. Deformities follow the rule of quality over quantity.

Data Analysis:

All initial fertilized eggs resulted in 100% mortality, so tests were only performed using data from the eyed eggs. A Tukey's one way ANOVA test with a $p<0.05$ level was used to compare overall survival rates, deformity rates, conductivity, and temperature among experimental sites as well as between experimental and control sites. Additionally, a simple linear regression analysis was used to test if conductivity had a correlation with survival or deformities. Normality in each test was assumed.

Results:

Survival:

The eyed egg survival at the experimental Baker Box site was significantly lower than the Montebello control site according to a one way ANOVA test ($F(4,5)=8.92$, $p=0.017$). This difference was the only statistically significant difference in eyed egg survival (Figure 4). Survival of eyed eggs ranged from 61.5% at the Baker Box site to 96.9% at the Montebello control site, with the City Spring site having the highest survival among the springs of 83.2%.

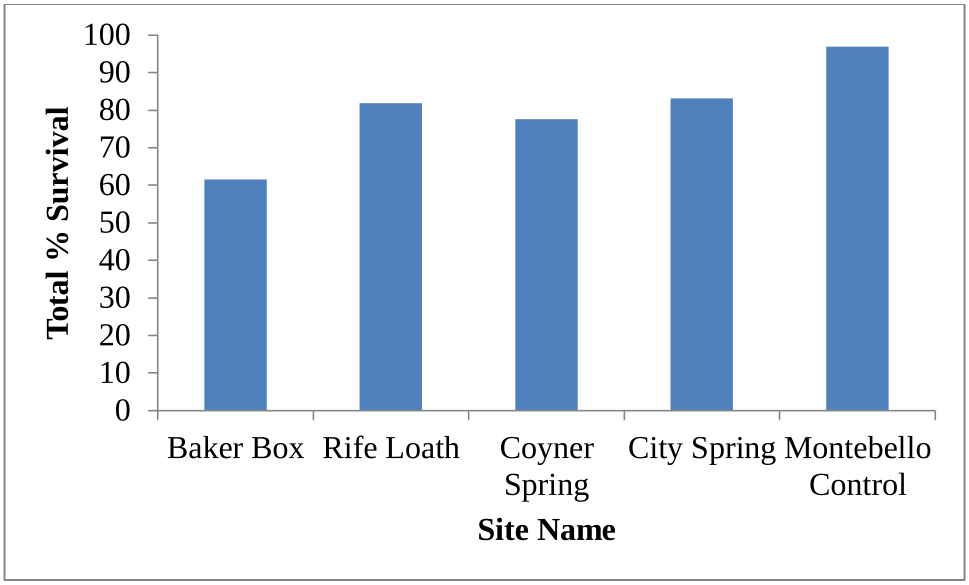

Figure 4: Percent survival of eyed eggs to hatchling release at experimental and control sites.

A simple linear regression analysis was performed to see if a relationship exists between survival and conductivity. Results indicate that approximately 78% of brook trout survival can be explained by conductivity. There is a positive relationship between conductivity and percent survival among brook trout (Figure 5).

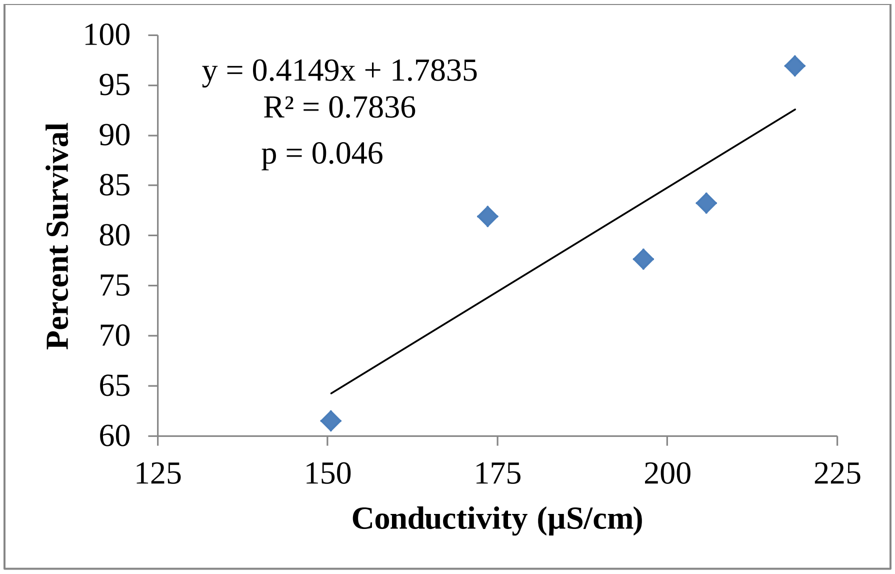

Figure 5: A plot of conductivity versus percent survival including results from a simple linear regression analysis. Only five data points are plotted because conductivity was not measured at the Montebello control.

Deformities:

The occurrence of deformities ranged from 0.0% at the Main Stem control to 3.7% at the Baker Box site, with the City Spring site and the Coyner Spring site sharing the lowest occurrence of deformities at 1.5% among the springs (Figure 6). However, there was no significant difference in occurrence of deformities among the experimental sites or between the experimental sites and the control site ($F(4,5)=1.57$, $p=0.313$).

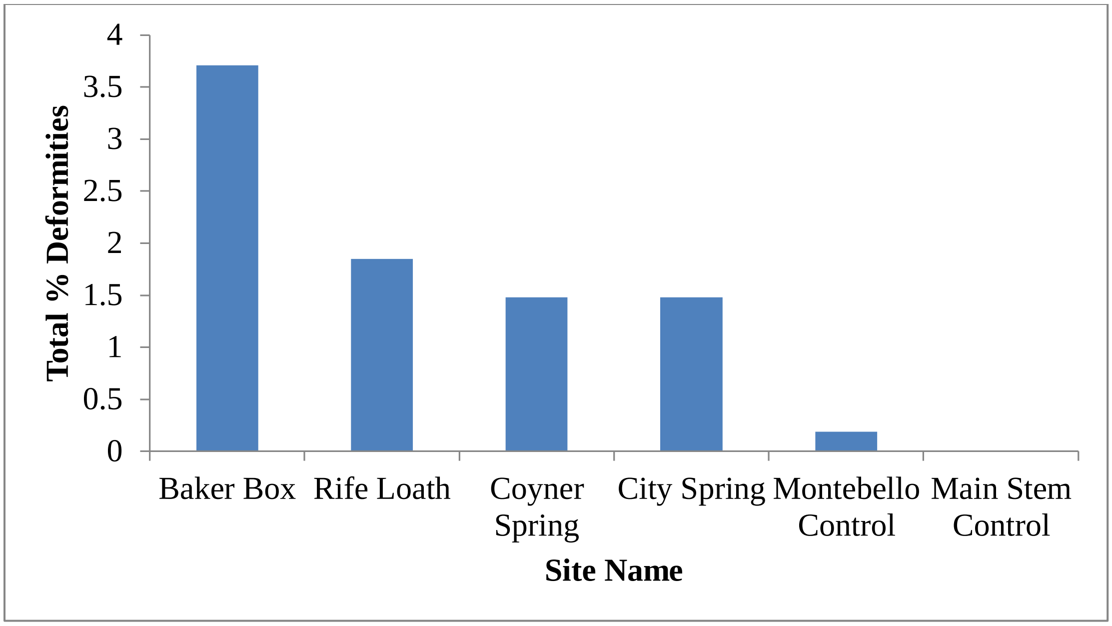

Figure 6: Total percent deformities at each site including dead and surviving hatchlings.

The Graduated Severity Index is a three dimensional representation that demonstrates severity of each type of deformity at each site. This figure has the site name on the x-axis, the type of deformity on the y-axis, and the severity measurement on the z-axis. Severity is a unit-less measurement with increasing values representing an increase in severity of the deformity. The GSI demonstrated that Baker Box had the highest severity of deformities with skeletal being the most abundant, the main stem control had the least overall, and among the experimental sites City Spring had the lowest (Figure 7).

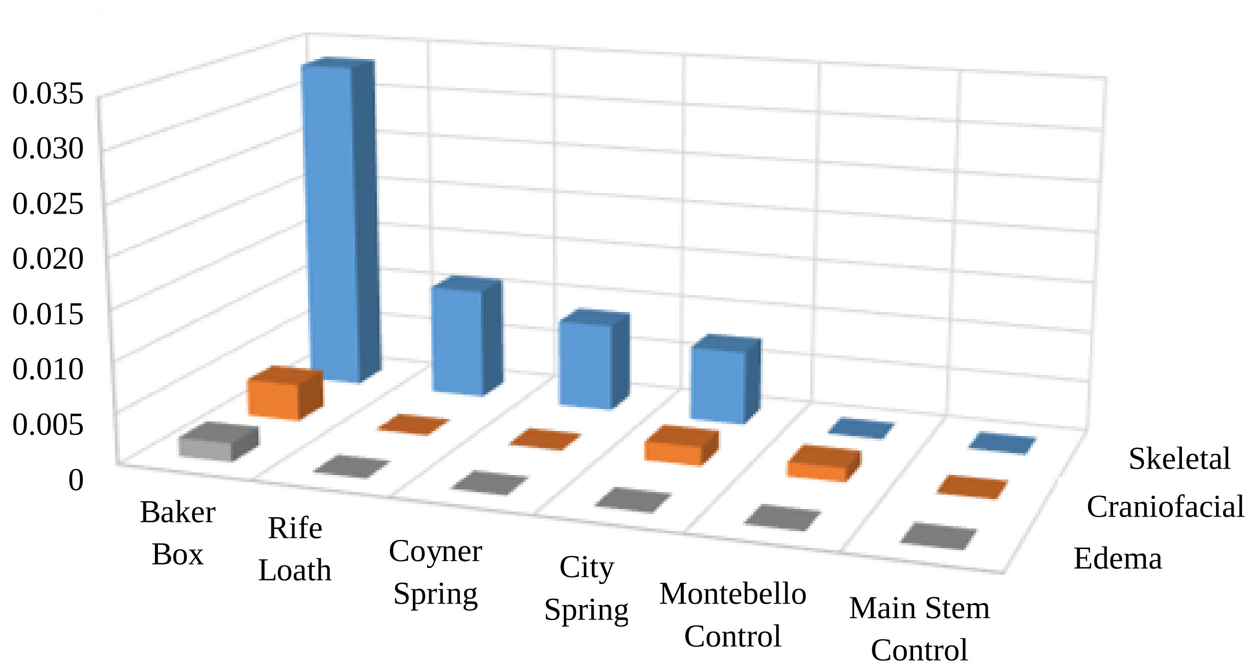

Figure 7: Graduated severity index (GSI) scores for total deformities at each site (x-axis) and types of deformities (y-axis) scored by severity. The z-axis is a unit-less measure of severity with increasing value representing increasing severity of the deformity.

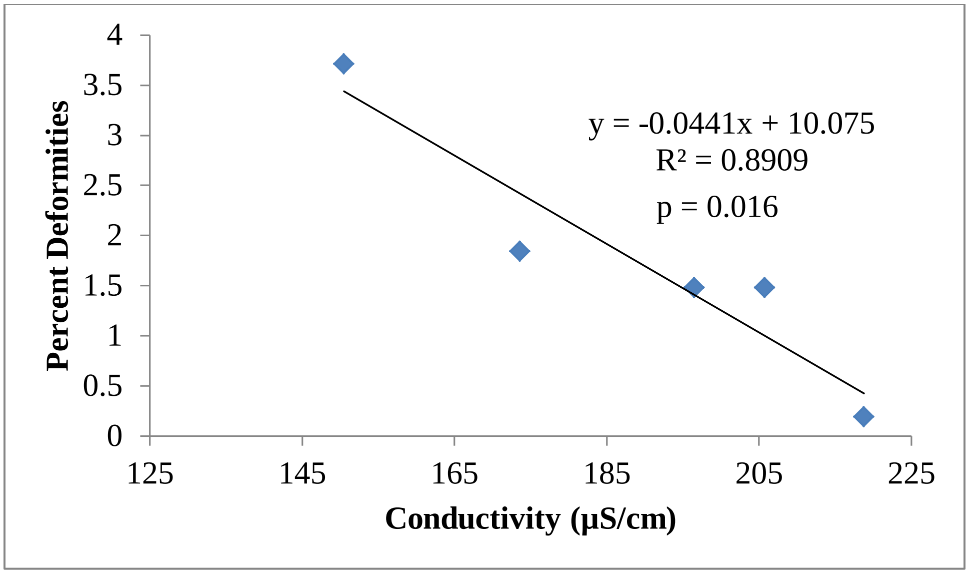

Figure 8: A plot of conductivity versus percent deformities including results from a simple linear regression analysis. Only five data points are plotted because data for conductivity were not collected at the Montebello control site. Each point represents averages found at each site.

Temperature:

There was a significant statistical difference in temperature between the experimental and control sites, but not within the experimental or within the controls according to a one way ANOVA statistical test ($F(5,19)=51.73$, $p<0.0001$). Water at the Baker Box, Rife Loath, Coyner Spring, and City Spring sites had similar temperatures of approximately 13°C. Likewise, the Montebello site and the main stem control site had similar water temperatures of approximately 10°C (Figure 9).

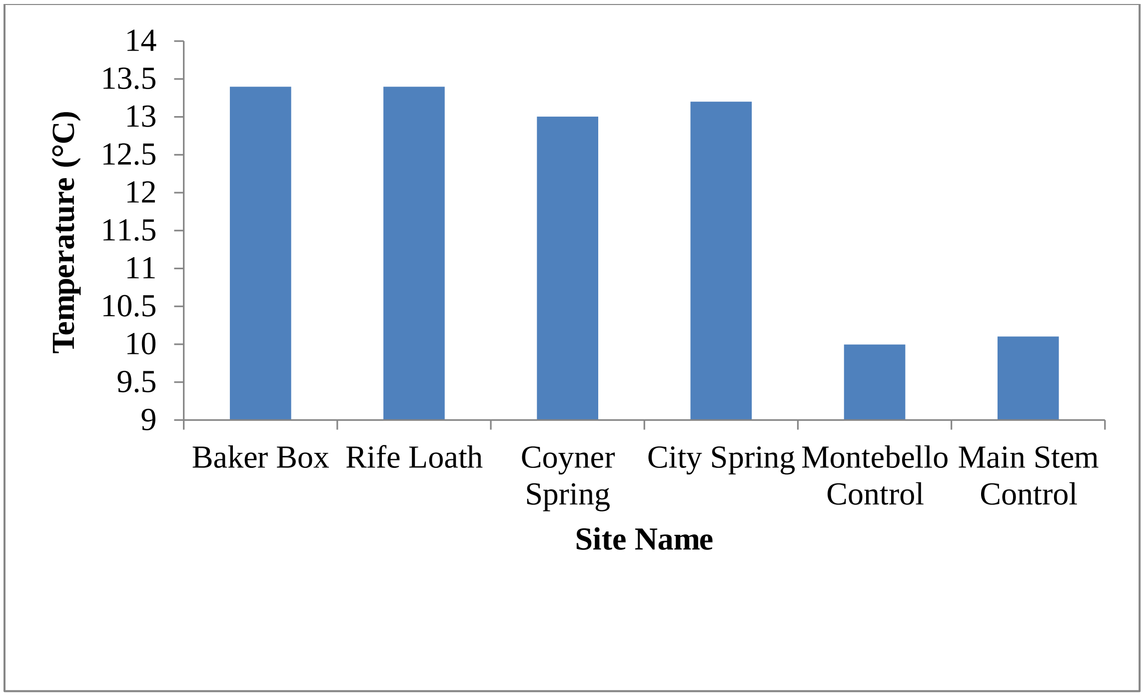

Figure 9: Average temperature at experimental and control sites.

Conductivity:

There was a significant statistical difference in conductivity at each site, including experimental and control sites according to a one way ANOVA statistical test ($F(4,16)=270.49$, $p<0.0001$). Data for conductivity at the Montebello control site were not taken. The main stem control site had the highest conductivity while the Baker Box experimental site had the lowest. Of the experimental sites, City Spring had the highest average conductivity of 206 µS/cm (Figure 10). Additionally, survival (Figure 5) increased with conductivity while deformities (Figure 8) decreased with conductivity.

A regression analysis was also performed to see if a relationship exists between deformities and conductivity. Results indicate that approximately 89% of brook trout deformities can be explained by conductivity. There is a negative relationship between conductivity and percent deformities among brook trout (Figure 8).

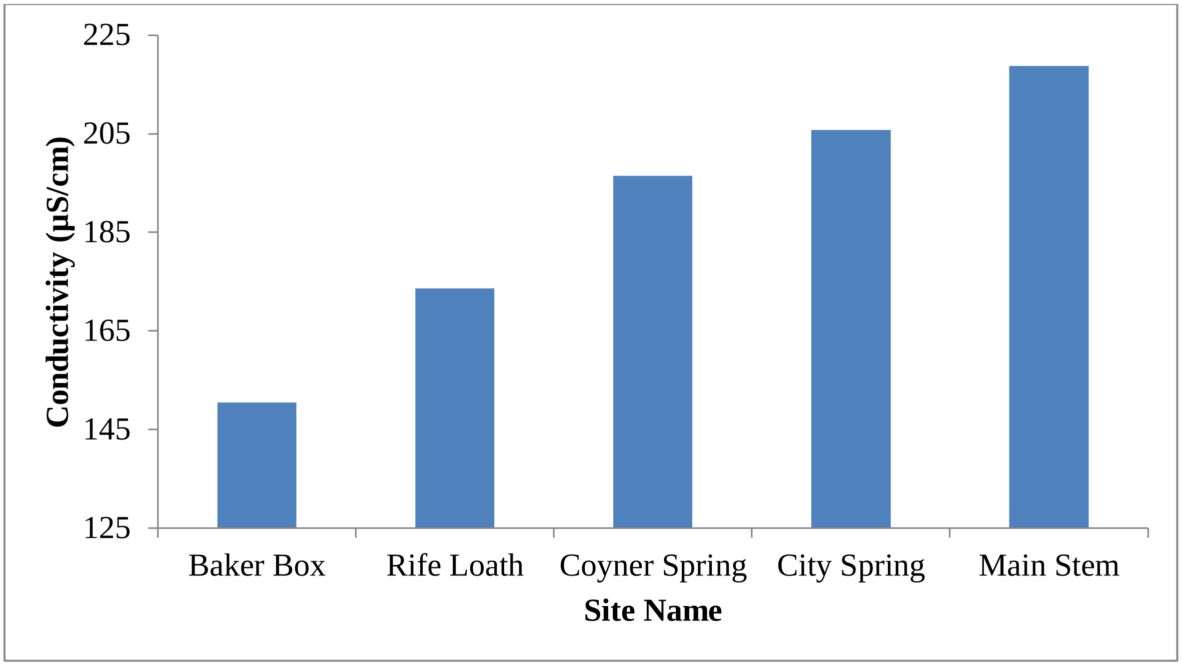

Figure 10: Distribution of conductivity at each site. The Montebello control's data were not included because conductivity measurements were not taken.

Discussion:

General:

Results indicate the springs are not conducive for early stage eggs due to the 100% mortality. However, there was no statistical difference in eyed egg survival at three of the four experimental sites when compared to the controls, with Baker Box having significantly lower survival. This result suggests three of the four sites are conducive for brook trout eyed egg survival. There were no statistical differences in deformities between experimental and control sites, which indicates that all experimental sites are conducive for brook trout in terms of deformities. Temperature at each experimental site was significantly lower than the controls, but still within the ideal brook trout ranges (Cunjak, 1985). Finally, experimental conductivity was significantly higher at the City Spring site suggesting it is the most conducive for brook trout reintroduction.

Survival:

One hundred percent mortality of the freshly fertilized eggs at each experimental site indicates that the upwelling springs are not conducive to young brook trout eggs. Other studies have found high mortality rates particularly among the early egg stage, which decreases as the fish get older (Dahlberg, 1979). Additionally, mortality has been attributed to low pH (Trojnar, 2011), low calcium levels (Ingersoll, 1989), and high UV radiation (Zagarese, 2001). The mortality in our study could have been a result of UV radiation as the hatch boxes were not covered until eyed eggs replaced the dead eggs. UV exposure has been shown to increase mortality in other fish taxa as a result of damaging DNA (Zagarese, 2001). In future experiments, plastic lids should be placed on the hatch boxes immediately after fertilized eggs are put in each compartment. Once lids were covered and eyed eggs replaced the dead eggs, survival increased at each site.

Survival at the experimental sites compared to the control sites showed that all experimental sites with the exception of Baker Box are conducive for brook trout. Based on survival the City Spring, Rife Loath, and Coyner Spring sites would be conducive for eyed brook trout eggs since there was no significant difference in survival between them and the control. The City Spring site had the highest survival among, though not significantly different from, the experimental sites, which suggests that it would be the most conducive for brook trout reintroduction. Baker Box, however, would not be conducive for brook trout since survival here was significantly lower than the controls. Three of the four experimental sites had survival rates similar to the controls', making these sites good locations for reintroduction of brook trout.

Deformities:

There was no significant difference in deformity occurrence between the experimental sites and the main stem control, thus any site is acceptable. However, it is still important to make comparisons among the experimental sites to determine which would be the most conducive. Brook trout eggs should be stocked at the most optimal site to ensure the best chance of survival. The Baker Box site had the highest number of and the most severe deformities making it the least conducive of the experimental sites based on deformity. On the contrary, City Spring would be the most conducive site since it had the lowest number of and least severe deformities. These results were consistent with the survival results.

Temperature:

Temperatures at the experimental sites were similar to each other, but significantly lower than the controls; however, the temperatures of the experimental sites were still within the optimal temperature range of brook trout (Cunjak, 1985). This evidence is consistent with the fact that most experimental sites had similar survival to the main stem control. Though water temperatures in the springs are different from the controls, they still lie within the optimal range for brook trout, so these springs are most likely still conducive for reintroduction of brook trout.

Conductivity:

Conductivity was significantly different among each site, being lowest at the Baker Box site and highest at the City Spring site. Our results are consistent with greater growth rates of larval gizzard shad fish, *Dorosoma cepedianum*, being found in areas of higher conductivity (Claramunt and Wahl, 2011). Lower measurements of conductivity prohibit growth in the larval fish stage as low conductivity is indicative of low nutrient load (Claramunt and Wahl, 2011). Survival and conductivity is known to fit a bell curve, so if conductivity is too high this can be detrimental as well (Claramunt and Wahl, 2011). The experimental sites, however, fit on the lower end of the bell curve where a higher conductivity is beneficial (Claramunt and Wahl, 2011). Therefore, based on conductivity measurements at each spring site, Baker Box should be the least conducive since it has the lowest measure of conductivity and City Spring should be the most. Again, this is consistent with the findings for survival and deformities.

The linear regression analysis tests demonstrated that conductivity has a significant relationship with both survival and deformities. As conductivity increases survival increases and deformities decrease. Therefore, the consistency that Baker Box is least conducive and City Spring is most conducive based on survival and deformities could be explained by the conductivity measurements at each of these sites. In search of future reintroduction sites, it might prove beneficial to first take measurements of conductivity as this is indicative of survival and deformities.

Water Chemistry Test:

Results may be explained by a water chemistry test that was performed at the springs in the year following our study. Water chemistry correlates with survivorship and the number of deformities in larval brook trout. The Baker Box site exhibited high levels of sodium ions which have been known to lead to deformities and decrease survival in brook trout (Phillips, 2011). The City Spring site had the lowest sodium ion levels. This is strikingly consistent with the low survival and high deformities the Baker Box site exhibited, as well as the high survival and low deformities at City Spring. Another consistency lies with the high dissolved oxygen concentrations found at City Spring and the controls, which demonstrated the lowest deformity occurrences and highest survival. Dissolved oxygen is known to determine the survival of brook trout embryos, and if levels are too low, the eggs can smoother to death (Raleigh, 1982). It may also prove to be beneficial to take a water chemistry tests when searching for a future site for brook trout reintroduction as measures of sodium ion and dissolved oxygen are indicative of survival and deformities.

Conclusion:

In conclusion, three of the four experimental upwelling springs are conducive for rearing brook trout given the similar rates of survival and deformities to the controls. Furthermore, of the experimental sites, City Springs is the most conducive given the highest survival and dissolved oxygen levels and the lowest sodium ion levels and rate of deformities. This provides potential for reintroduction of brook trout via stocking of eyed eggs, which is less costly than the current solution of stocking adult brook trout. Additionally, in search of future reintroduction sites, measures of conductivity, sodium ion levels, and dissolved oxygen should be measured before rearing as they are good indicators of brook trout survival and deformities.

Future experiments should attempt rear freshly fertilized eggs again, however cover them immediately so UV radiation is not a factor. Additionally, it is now known that the springs' water chemistry is conducive for brook trout eggs, but it is still unknown whether the sediment at these springs is conducive. A future study should test the survival of brook trout eggs reared directly in the sediment to see if the sediment would be conducive for brook trout survival, and therefore brook trout reintroduction.

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