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1 Introduction

Monitoring the health of a natural environment is often regarded as the first step to ensuring its conservation, maintenance, and stability [1]. Often increases in biodiversity correspond with increases in the health of an environment and serve to establish environmental resilience [2]. According to the diversity-stability hypothesis, this increase in resilience originates from the variability of species and the idea that ecosystems with a greater number of species, and hence greater variability, will be less likely to suffer from a selection event because some species will survive [1]. The main purpose of this investigation involves reporting the biodiversity of Anglers Park in Danville, Virginia USA (36°33'50"N, 79°21'03"W) over an eight year period (2008 - 2015) as calculated based on the population sampling of four different tetrapod species: *Anaxyrus americanus* (American toad), *Desmognathus fuscus* (dusky salamander), *Eurycea cirrigera* (southern two-lined salamander), and *Nerodia sipedon* (northern watersnake). The secondary purpose of this investigation relates to the relationship between resilience and biodiversity. Specifically, this study will detail the contrast between the biodiversity of Anglers Park before and after the Dan River coal ash spill incident.[1]

Anglers Park is situated along a contaminated segment of the Dan River. The spill undoubtedly harmed the river ecosystem in this area which in turn likely impacted the surrounding stream and forest ecosystems [4]. As such the event serves as an environmental perturbation from which a resilient ecosystem would recover. The data from this study will help determine the extent of this recovery.

The question of interest then is, generally, *how does the biodiversity of Anglers Park change among the four aforementioned species from 2008 to 2015?* The null hypothesis, H_0, suggests that there is not any change while the two-tailed research hypothesis, H_1, suggests the contrary. Analysis of the data is conducted in hopes of validating H_1 which would, by contradiction, invalidate, H_0. From this setup, the independent variable is the year data collection took place, and the dependent variable is the corresponding index of biodiversity. The controlled variables include the general location of the species sampling, the species sampled, and the time of year (mid to late Spring).

1.1 Background of Location and Species

Anglers Park consists of a 178062 square meter public zone situated in the Piedmont region of Southern Virginia. This area is characterized by temperate deciduous forests with a particularly humid climate year round. The average temperature is approximately 14.4° C and the area receives about 1.14 meters of rainfall annually [5]. Several streams flow through the park and, hence, vernal pools often appear around the park area. The park itself is owned and maintained by the City of Danville and it remains open to the public for recreational activities such as fishing, biking, hiking, and picnicking [6]. The following species are commonly found inhabitants of Anglers Park; a brief description is provided for each species to avoid future ambiguities.

- *Anaxyrus americanus*. Commonly known as the American toad, *A. americanus* undergoes early development in freshwater ponds. Once in adulthood, they reside in dense vegetation and remain active mainly during night. They feed on insects and small invertebrates including worms, spiders, and ants. The toads spend much of their time in water, absorbing the liquid through their skin to avoid dehydration. Currently, the species is not threatened and remains common throughout its inhabited areas [7].

- *Desmognathus fuscus*. Commonly known as the dusky salamander, *D. fuscus* is an amphibian species that feeds on small invertebrates such as spiders, beetles, and worms. The organism is semiaquatic [8] and spends most of its early development in water. The salamanders typically reside in wooded habitats with sufficient water resources nearby and can often be found around streams and rivers.

[1] In 2014 a power plant owned by Duke Energy spilled over 80,000 tons of coal ash and more than 27 million gallons of coal slurry into the Dan River [3]

Currently, the species is classified as *least concern* by the International Union for Conservation of Nature and Natural Resources (IUCN) [9].

- *Eurycea cirrigera*. Commonly known as the southern two-lined salamander, *E. cirrigera* inhabits areas around small streams in wooded areas [10]. It feeds on small organisms such as insects, crustaceans, and spiders. The IUCN lists the organism's conservation status as *least concern* [11].
- *Nerodia sipedon*. Commonly known as the northern watersnake, *N. sipedon* is a reptilian species that typically inhabits water-flooded areas including the grasses along the edge of flooded meadows and marshes along with the areas around streams and small rivers [12]. It preys on amphibians, crustaceans, and insects in addition to other snakes and turtles. As with the other organisms, the conservation status of *N. sipedon* is of *least concern* according to IUCN [13].

All four of the species are commonly found in Anglers Park, as the habitat of each is well-suited for the habitat provided by the park.

2 Methods

2.1 Survey areas

In May 2015, the author and his biology class participated in an annual "BioBlitz" at Anglers Park, during which the students and their teacher, J. Gibson, hiked through various trails around the park, collecting and identifying vertebrate species. The activity lasted 2.5 hours and the 11 group members covered 2309.45 m of trails. Gibson kept the documentation of the findings during this period and stored it with data from past surveys. He granted the author access to this information in order to conduct the following investigation. Each record provided information on the date, duration, number of members, and time of day in addition to a count of each species observed. The study area involved traversing the trails of Anglers Park. Figure 1 gives a view of Anglers Park from Google Earth. The area itself is outlined in green and the trail network is highlighted with white lines. The Dan River is outlined in yellow. Note that this map is rotated 90° clockwise, so true north is directed east in the picture. All surveys began at the main parking lot found near the south west corner of the park. Members of the surveying group then progressed, together through the trail system, often making a loop and eventually returning to the main parking lot. In figure 2, the parking lot is marked by a balloon and the area of Anglers Park is shaded green. This map is oriented so that true north is directed to the northern border of the image.

2.2 Data collection and Ethical Precautions

During surveys, if any group member found a live organism, he or she would report it to the designated recorder who would then identify the species and document it on a chart. The organism would then be returned to the exact location from which it was found. Occasionally, organisms would not even be handled, but rather spotted, identified, and documented. During all studies, group members took special care not to inflict any harm on observed animals and ensured that the park remained exactly as it had been before the survey took place. The surveys were always supervised by an adult who ensured that proper safety practices took place. These practices included the avoidance of poisonous animals and unknown areas of the park. The surveys from 2008, 2010, and 2015 each involved 10-15 participants. Each survey effectively produced a sampling of various species' populations in Anglers Park. This sample can be scaled accordingly to produce a population size estimate for that particular year. These particular years provide the most relevant and complete data out of the seven surveys conducted from 2008 to 2015. Consequently, the following analysis will apply exclusively to data from these three target years.

Figure 1: *Anglers Park from Google Earth outlined in green. Dan river outlined in yellow.*

2.3 Standardization

The author tracked the exact trails surveyed during 2015, but was not present during the previous studies to record the same data. Thus, the trail systems surveyed during 2008 and 2010 are unknown. To compensate for this lack of information, we make several assumptions, standardize the information from 2015's survey, and apply such metrics to the previous years. In 2015 the group covered 2309.45 meters of trails. Assuming that 11 members thoroughly cover a width of 5 meters as they progress, the total sampling area is approximately

$$2309.45(5) = 11547.25 \ m^2 \tag{1}$$

This area was sampled in 2.5 hours suggesting that, in general, a group of roughly 10-15 members, can search an area at a constant rate of

$$\frac{11547.25}{2.5} = 4618.9 \ {}^{m^2}/_{hr} \tag{2}$$

Assuming this rate is applicable to other groups, we can use it to determine the sampling areas from the surveys in 2008 and 2010 based on their respective durations. For example, in 2010, the group spent 2.83 hours surveying Anglers Park. Assuming that they surveyed the area at a rate of 4618.9 ${}^{m^2}/_{hr}$ for this time period, then the total distance covered is given by

$$4618.9(3.83) = 17690.387 \ m^2 \tag{3}$$

The total area of Anglers Park is 178062 m^2, so this sampling area surveyed in 2010 represents

$$\frac{17690.387}{178062} = 0.0993 \tag{4}$$

or 9.93% of the total area of Anglers Park. These methods are applied to each year's survey. The information gathered this way is summarized in Table 1.
The Percent of Total figure gives the percent of Anglers Park that was surveyed during that particularly year. This value will be a key scaling factor in determining population estimates.

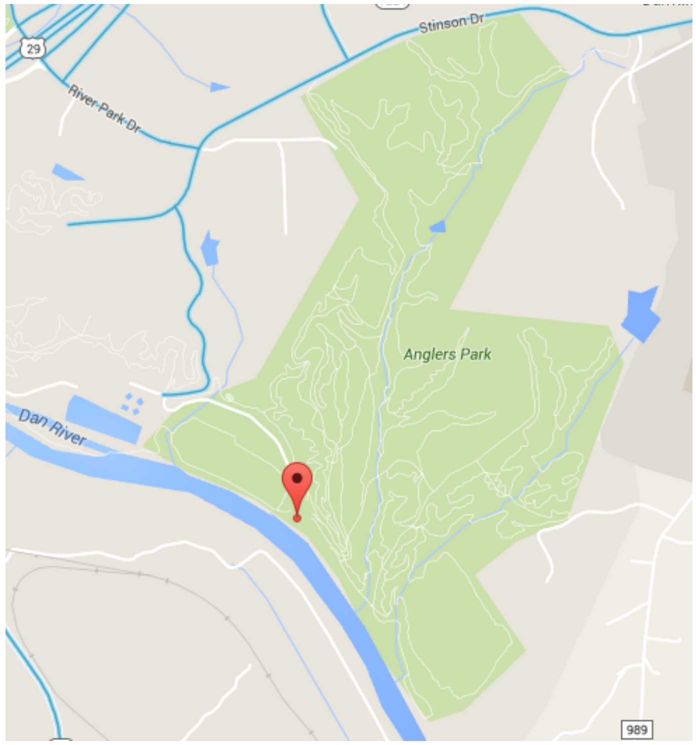

Figure 2: *Anglers Park from Google Maps shaded in green. Balloon marks location of parking lot.*

Table 1: *Survey Data*

	2008	2010	2015
Rate (m^2/hr)	4618.9	4618.9	4618.9
Duration ($hours$)	5.83	3.83	2.5
Sample Area (m^2)	26928.187	17690.387	11547.25
Percent of Total	15.12	9.93	6.48

3 Data and Results

The raw data for each survey is summarized in Table 2. The population estimate is calculated by dividing the total observed species count by the percent of the park's area sampled during that particular year. This relationship originates from the following observation where p_a gives the percent of the park's area sampled, p_e is the population estimate, and s_o is the number of species observed.

$$p_e(p_a) = s_o \iff \frac{s_o}{p_a} = p_e \tag{5}$$

Hence, the values from Table 1 are applied accordingly to determine p_e in Table 2.

The information from the table reflects mostly adult species counts along with some juveniles that were observed. Metamorphs were excluded. The population estimates (p_e) for each species are depicted as a triple bar graph in Figure 3. Note that in general, *D. fuscus* was observed most frequently and hence had the largest population sizes each year. In contrast, *N. sipedon* was observed the least and returned the smallest population estimates in both 2008 and 2015. This data and these trends are further analyzed by calculating the biodiversity from each year.

A diversity index provides information regarding the biological distribution and richness of species in a certain ecosystem. The Simpson index is popular in ecology for providing a standard measure biodiversity. The inverse Simpson index is simply the reciprocal of the primary index and provides a value in the range

Table 2: *Survey Information*

Year	Variable	*A. americanus*	*D. fuscus*	*N. sipedon*	*E. cirrigera*
2015	s_o	1	9	1	2
	p_e	15	139	15	31
2010	s_o	16	22	3	2
	p_e	161	222	30	20
2008	s_o	3	17	1	9
	p_e	20	112	7	60

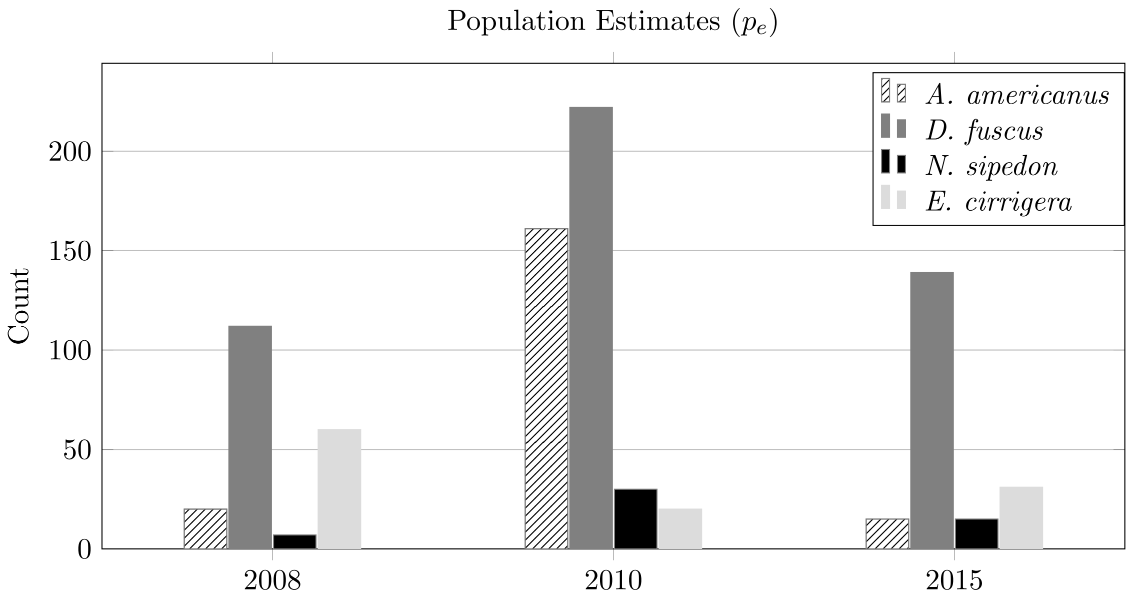

Figure 3: *The population estimates of the four species across three different surveys*

$(0, n]$ where n is the number of different species observed. A higher value corresponds with a higher level of diversity.

Simpson's index of diversity [14] is given by

$$D_{inverse} = \frac{N^2}{\sum (p_e)^2} \tag{6}$$

This calculation yields a representation of the ecosystems biodiversity relative to the observed species. It accounts for both the richness of a habitat and the evenness with which the species are distributed. Note that N denotes the total number of species. For example, the survey during 2008 would yield a value of 199 for N. The various data from the three target years is substituted for the appropriate variables in the formula to produce three unique indices. Before plotting these results, it is necessary to outline the assumptions related to conducting this computation. The following circumstances are assumed:

1. a group of 10-15 members search an area with a width of 5 meters as they progress through the park
2. each year, the observed species serve as an accurate representation of the population
3. the species are evenly distributed throughout the park's area
4. the group searches an area at a constant rate each year
5. recorded data accurately reflects observed data

6. the four species can provide an accurate representation of the park's biodiversity
7. each group searched similar environments within Anglers Park
8. each year abiotic factors (e.g. variable climate) did not significantly affect observed species (in other words, the potential of observing active organisms was constant)

These assumptions embody a number of major uncertainties that were not verified during the surveys due to the nature of the data collection. They also summarize the potential confounding variables that could affect the results of the data analysis. Regardless, the indices should be useful in determining the change in biodiversity over time. Such a comparison provides more accurate results because it remains unaffected by a number of assumptions. For example, the inaccuracies that may affect the scaling process are avoided by performing a comparative analysis of the results.

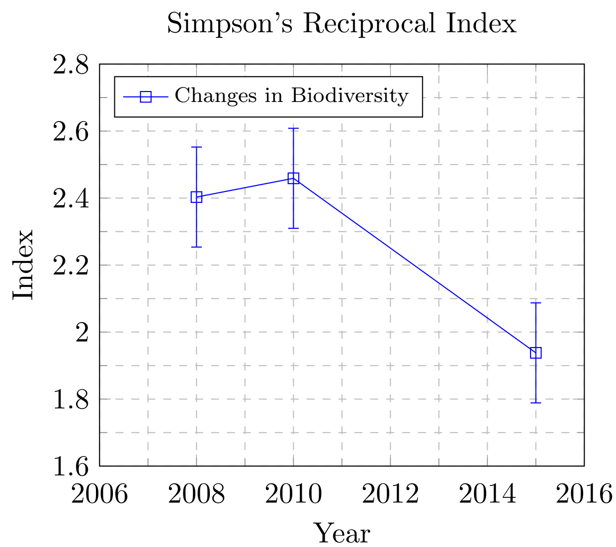

Figure 4: *The changes in the biodiversity of Anglers Park with standard error bars.*

If the diversity of the park partially manifests itself in the diversity of the four reptilian and amphibian species, then the Simpson's Reciprocal Index provides an approximate representation of the biodiversity of Anglers Park. Hence, Figure 4 depicts the trend in the biodiversity of Anglers Park from 2008 to 2015 by plotting the computed Simpson's reciprocal indices.

Table 3: *Results of regression analysis*

Regression Statistics	
Multiple R	0.9293
R Square	0.8636
Adjusted R Square	0.7272
Standard Error	0.1493
P-value	0.2408

This trend is further understood by performing a regression analysis. The results of this regression analysis are outlined in Table 3.

4 Discussion and Conclusion

From Figure 4, the biodiversity appears to increase from 2008 to 2010, then decrease from 2010 to 2015. The standard error bars demonstrate the range of possible values of a particular measure by taking into account potential variability and uncertainty in the data. By visually analyzing the graph and its error bars, one can determine the relative significance of each measure. Namely, since the range of error from 2008 overlaps the range of error from 2010, the two are statistically indistinguishable. In contrast, since the range of error from the year 2015 does not overlap either of the other two ranges, it is statistically *different* from the other two values. However, this visual test is inherently unreliable and cannot provide an accurate assessment of the data as a whole [15]. A regression analysis compensates for this uncertainty.

The regression analysis provides a description of the linear relationships among the points. It reports three R values that reflect the accuracy of a linear model applied to the data. The coefficient of determination or R square provides the percent of total variation in the dependent variable described by the independent variable. As Nagelkerke [16] describes it, the coefficient of determination reports "the proportion of variance explained by the regression model." For this investigation, the coefficient of determination is 0.8636 or 86.36% (Table 3) which suggests that around 86% of the variation in the biodiversity index is a result of the sampling year. This is a moderately strong correlation, but the p-value from the regression analysis is more pertinent because it relates to the original hypotheses, H_0 and H_1.

The p-value demonstrates the probability that the variation among the data points is a result of chance. More specifically, it assumes H_0 conditions and gives the probability of obtaining the observed results under such conditions [17]. From the regression analysis, the p-value is computed in relation to all the data. Thus, it does not specify any source of the variation. The p-value of 0.2408 (Table 3), then, shows that there is a 24.08% probability of the changes in biodiversity being a result of random fluctuations. By all measures of significance, this p-value requires us to accept H_0 because the variation in the data is not significant. As a corollary, H_1 is rejected. This result is unsurprising for two reasons. First, from the years 2008 to 2010, the biodiversity changes by a small percentage (approximately 6%), thereby limiting the effects of the larger change from 2010 to 2015 on the significance of the data. Second, the study was limited by the data available for testing. With only three primary points, there simply was not enough evidence to draw significant conclusions. Nevertheless, the drop in biodiversity after 2010 appears significantly different from 2015 due to the vertical spacing between the standard error bars.

This observation likely emerges from a multifaceted problem. Since 2010 Anglers Park has received national recognition for its extensive mountain bike trail system, Anglers Ridge. Due to this third party attention including its classification as the best mountain bike trail system in Virginia, the park has become more popular and has attracted more tourists at an increasing rate [18]. As the natural environment receives the effects of extended use, it can experience negative ecological effects. For example, increased mountain biking trail construction and use are regarded as leading to resource degradation. They also affect the conservation of soil composition and vegetation integrity [19]. More recent studies have validated the environmental impacts associated with trail use which include wildlife disruption, soil compaction, water quality degradation, erosion, and vegetation loss [19, 20]. In some areas, research suggests that tourism and recreation (e.g. mountain biking and hiking) have directly impacted and often harmed biodiversity [21, 22]. The effects of expanding recreation activities often cause problems that cascade into causing secondary ecological effects. For example, soil compaction from repeated trail operation is linked with higher amounts of runoff and hence higher amounts of soil pollution [20]. Thus, the decline in biodiversity from 2010 to 2015 may have been partially caused by the park's trail development, burgeoning popularity, and increased tourism.

As mentioned in Section 1.0, a significant coal ash spill took place in 2014 that imparted negative ecological effects on the Dan River which borders Anglers Park [4]. The source of the spill has been traced to Eden, North Carolina where stormwater drainpipes released the hazardous material directly into the river. Since then, toxic wastewater has been found more than 70 miles downstream (passing through and beyond Danville) and includes heavy metals such as arsenic and copper [23]. The hazards cause environmental degradation from the thick blanketing of ash deposits, often several feet deep. It also remains a high-risk contaminant for aquatic and semiaquatic organisms including toads, salamanders, and snakes. Additionally, the heavy metal elements progress through food chains and often bioaccumulate in top-level consumers. Such bioaccumulation can diffuse the toxicity to terrestrial environments. Studies on the Dan River have assessed these effects and determined that the coal ash spill undoubtedly affected the flora and fauna of surrounding ecosystems, including Anglers Park [23, 24]. Hence, the coal ash spill impacted the biodiversity of Anglers Park; however, the extent to which this accident caused the decrease in biodiversity from 2014 to 2015 remains elusive.

Similar case studies have been published [25, 26, 27] that survey the diversity of a group of species in a certain area. However, to the author's knowledge, no study has previously been published regarding the biodiversity of Anglers Park. Nevertheless, similar investigations of biodiversity often include a method of collecting species data, the analysis of this data, and the connection between the statistical results and the environment that was studied. As such, the biodiversity of an ecosystem is often a key component of monitoring its health [26, 27]. Consequently, this study provides an overview of the relative health of Anglers Park since 2008 but could still be improved in a number of ways.

4.1 Improvements and Significance

This study could be improved by including additional data to analyze changes in biodiversity over time. This way, the changes from year to year could perhaps provide whole, statistically significant trends. Also, with more data, the populations of more species would be incorporated into the calculation of biodiversity, thereby yielding more accurate indices. For future surveys, the group members could spend a fixed amount of time searching a predetermined section of Anglers Park. The increased consistency of the surveys would increase the reliability of conclusions drawn from the data. Likewise, if the aforementioned assumptions are reduced (in other words, if the assumed statements are observed in each study), then the reliability of the conclusions would increase. Thus, another major source of improvement includes the collection and consideration of weather data since amphibians and reptiles are often sensitive to meteorological changes. Regardless of the improvements, three main conclusions can be gleaned from this study. They are summarized below.

1. The biodiversity of Anglers Park, as indicated by the four aforementioned amphibian or reptilian species, has not changed significantly overall since 2008.
2. The biodiversity has however decreased significantly since 2010.
3. The relative biodiversity of the park remains comparatively low over all the years of study.

The third point originates from the nature of the computation of Simpson's Reciprocal Index. In such calculation, the upper bound of the index is defined by the number of different species observed (in this case, four). Since a higher index corresponds with a higher level of biodiversity, the most biodiverse environment would provide an index value of 4. The fact that the indices from 2008 to 2015 oscillate around half that value shows that Anglers Park is only moderately diverse. From the diversity-stability hypothesis [1] and from the standpoint of accepting a causal link between the coal ash spill and the subsequent decline in biodiversity, this observation would explain the park's inability to quickly reciprocate from the environmental disaster. Ultimately, this study could be extended into future decades to assess the effect of events such as these while simultaneously allowing individuals to monitor the relative health of the sensitive woodland ecosystems so that they can respond accordingly during instances of biodiversity decline.

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