

## Minimizing the effects of Green Sunfish (*Lepomis cyanellus*) on native competition.

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### Abstract

Impacts of introduced species are not fully understood, but it is known that the spread of invasive species is causing a drastic decline in biological diversity. Aquatic systems are highly susceptible to impacts from exotic species. One such species, Green Sunfish (*Lepomis cyanellus*) is competing with native Sonora Mud Turtle (*Kinosternon sonoriense*) in tinajas associated with grassland systems. I attempted to lessen this threat by removing as many Green Sunfish as possible from tinajas in the Post Canyon region of the Appleton-Whittell Research Ranch. The captured Sunfish were then fed to the Mud Turtles in an attempt to mitigate the negative effects that the invasive species had on Mud Turtle diets. A total of 427 fish were eradicated. Linear Regression analysis indicates that the date of capture was a significant predictor of the number that were caught ( $p=0.011$ ,  $r^2= 0.212$ ) and the rate of capture gradually decreased over time, indicating that efforts of removal had diminishing returns. The correlation between turtles that were hooked and fish that were caught varies based on which statistical test was used. This indicates that the Sonora Mud Turtles may be responding to rapid removal of Sunfish by behaving more boldly towards the bait. This study provides insight on the behavioral interactions between Sonora Mud Turtle and Green Sunfish, although more detailed observations would be possible in a laboratory setting. Efforts to improve the native habitat were successful, but quantifying such success is difficult without population data, which could be an avenue for future research.

### Introduction

Rapid change in ecological conditions harms many aspects of natural systems. Although abiotic variation is most often examined, changes in biotic communities are equally important, and the spread of invasive species is causing a drastic decline in biological diversity (Clavero & García-Berthou E. 2005; Davis 2003; Pimentel *et al.* 2005). One means of slowing the detrimental effects of climate change is habitat restoration by removal of such invasive species (Grarock *et al.* 2013).

Out of all regions of the planet, grassland systems are one of the most threatened in terms of further proportional changes in biodiversity (Sala *et al.* 2000). Biological richness is

directly related to the abundance of non-native species (Siebert 2011; Garock *et al.* 2013), and in such a vulnerable area, it is imperative that we take preventative measures to help conserve this natural ecosystem.

Grasslands constitute a large part of Southeastern Arizona. One region in particular, the Appleton-Whittell Research Ranch of the National Audubon Society, has been invaded by a number of different exotic species. These include, among others, Lehmann Lovegrass (*Eragrostis lehmanniana*; McLaughlin and Bowers 2007), Boer Lovegrass (*E. curvula* var. *conferta*; McLaughlin and Bowers 2007), American Bullfrogs (*Lithobates catesbeianus*; Cogan 2012), and Green Sunfish (*Lepomis cyanellus*; Gardner 2012). Ranch efforts have mitigated the spread of both Lehmann and Boer Lovegrass, and American Bullfrogs are being actively removed wherever they are spotted. However, efforts to remove invasive fish have not been pursued diligently, even though it is a pressing matter.

The rate of freshwater extinctions in North America greatly surpasses the rate of terrestrial extinctions (Ricciardi 1999), and Miller *et al.* (1989) found that 40 taxa were extirpated within the past century. Also, 49% of endangered species listings for fish implicated nonnative invasives (Magnusson *et al.* 1998). On the Research Ranch, Green Sunfish are the primary invasive fish. They compete with many natives including the Gila Chub (*Gila intermedia*) and Sonora Mud Turtle (*Kinosternon sonoriense*).

Green Sunfish are small, brightly colored member of the *Centrarchidae* family. Native to Eastern North America, they are dietary generalists and consume many small invertebrates (Paulson and Hatch 2004). It has been documented that they also feed on other small fish, including Gila Chub, which is listed as endangered in New Mexico (Dudley and Matter 2000) and is threatened in Arizona. Previous efforts to remove Green Sunfish have been pursued with the conservation of Gila Chub in mind. Green Sunfish have even been known to outcompete members of the same family (Werner and Hall 1979). Because of their aggressive nature and

high reproductive efficiency, they have been implicated in the decrease of many native aquatic species (Dudley and Matter 2000; Bramblett and Fausch 1991; Sih *et al.* 2003).

Green Sunfish thrive in a number of environments, though they usually inhabit stream pools with vegetative cover with a wide range of flow rates (Stuber *et al.* 1982; Etnier and Starnes 2001). This habitat is present on the Appleton-Whittell Research Ranch and is shared by a number of native competitors, such as the Sonora Mud Turtle. After a cursory survey of the Post Canyon area of the Research Ranch in early June 2014, multiple dead Mud Turtles were observed. Although the cause of death was unknown, it was inferred that they died of starvation. Sonora Mud Turtles, like Green Sunfish, are dietary generalists (Becker *et al.* 2010). Becker *et al.* (2010) found that the diets of Sonora Mud Turtles were primarily based on invertebrates except in areas where they lived with Green Sunfish. The diet of turtles living in these areas was primarily plant-based. This implies that the presence of Green Sunfish was impeding the success of Sonora Mud Turtle foraging.

In certain areas of the Research Ranch, Mud Turtles and Green Sunfish live in the same habitat, occupying close to the same niche. It is unclear whether or not this state of invasion is due to a realized niche shift (i.e. the spread of non-native exotics, attributed to physiological advantages with widespread adaptive capabilities in the invaded range; Broennimann *et al.* 2007; Chase and Leibold 2003), due to a fundamental niche shift (i.e. the influence of large-scale ecological changes that cause certain species to spread; Holt *et al.* 2005), or due to both. Determining which would dictate the direction of restorative action to take. No data exists to support either claim, but because Green Sunfish were originally artificially introduced in private ponds by fishermen for sport fishing (Gardner 2012), it is evident that the presence of this species on the Research Ranch is due to its extraordinary physiological and reproductive advantage, rather than a large change in environmental conditions. As a result, its existence in previously undisturbed habitats puts native species, such as the Sonora Mud Turtle, at risk for

out-competition. Therefore, the removal of Green Sunfish would theoretically facilitate higher reproductive success of the Sonora Mud Turtle and prevent further harm.

The purposes of this study are multifaceted, although they are primarily conservation oriented. The first is to remove as many Green Sunfish as possible from pools in the Post Canyon region of the Appleton-Whittell Research Ranch. The Sunfish carrion would be used to augment the diets of Sonora Mud Turtles in an effort to mitigate the effects of the invasive competitor. Another goal of this study is to observe and document the changes in Sonora Mud Turtle behavior as a result of the addition of a food source. Efforts to give a native species a competitive advantage by removing a high proportion of exotics have never been attempted or documented before this study. The results could be used for conservation management practices at other related sites, possibly providing a low-budget alternative to costly and ecologically disturbing chemical eradication procedures.

### Study Site

This study was conducted in southeastern Arizona at the Appleton-Whittell Research Ranch of the National Audubon Society, a ecological conservatory whose primary mission is to allow natural processes to rehabilitate the natural order after intense human alterations. The study was carried out at a site known as Post Canyon. The canyon is at the cusp of two convergent habitats: the Madrean Mixed Grass Prairie and the Madrean Oak Woodland. Through it runs a stream bed that is flowing about 9 to 10 months of the year, beginning in the monsoon season. This fosters a riparian ecosystem, and many mammals and birds use it as a source of drinking water. In the dry season, the stream dries considerably, leaving multiple tinajas and rocky pool areas. Two dams were constructed in the 1950s. The study area was just below the lower dam, where 5 tinajas are filled year-round. The uppermost pool was located at 31°34'44.27 N 110°31'32.78W. Of the 5 pools, the deepest is about 3-4 meters and the

shallowest is about 2 meters deep. This allows for a wide range of breeding opportunities for Green Sunfish.

Each tinaja was assigned a number, from the dam downstream in reverse consecutive order (Pool 4 was the closest to the dam, Pool 3 was the second closest, etc; See figures 1-3). One tinaja was disregarded because of its limited size and absent sunfish population.



Figure 1. Pond number 1. This pond had the largest proportion of muddy shallows and therefore the largest proportion of juvenile fish. This pond was heavily targeted with minnow traps for this reason.

Figure 2. Pond number 3. This pond was deemed to be an ideal breeding ground because of its abundance of shadowed rocky areas.



Figure 3. Sonora Mud Turtles basking on the rock in Pond 2.

Once there is a high water event above the dam, the 5 tinajas are flooded and they all become an interconnected stream. In terms of aquatic species, these areas are inhabited by aforementioned Sonora Mud Turtles (*Kinosternon sonoriense*), Green Sunfish (*Lepomis cyanellus*), Mosquito Fish (*Gambusia affinis*), and a number of invertebrates that are a source of food for the larger animals. Multiple American Bullfrogs (*Lithobates catesbeianus*) have been sighted and exterminated within the past year.

## Methods

This study was conducted starting on June 12th 2014 and ending on August 6th 2014. Starting in early June, the study area was surveyed for any clues as to proceed. Because this study was primarily conservation oriented and done on a limited budget, population estimates for both the Green Sunfish and Sonora Mud Turtle were conducted on a visual basis only. Data were collected, under a Scientific Collecting Permit from the Arizona Game and Fish department, using power bait, a fishing pole, and two minnow traps. The size of each captured fish, the pool in which it was caught, and the number of mud turtles that were hooked were recorded. The turtles that were hooked were gently unhooked and released into the pool at which they were caught. The fish were humanely exterminated and then distributed among the stream beds for the Sonora Mud Turtles to eat (Figure 4). The feeding habits and other behavioral data were gathered through visual monitoring in an attempt to discern a pattern that relates the frequency of caught fish to the rate of Mud Turtle



Figure 4. A Sonora Mud Turtle feeding on a recently found catch.

nibbles, or other such observations. The study was conducted on various days at different times of day, typically in the midmorning (8:00 through 11:30) or afternoon (1:00 through 3:30). Sampling was usually successful, but extended periods of time with no activity (surfacing, biting, etc) were recorded.

Because the objective of this study was to catch the greatest number of fish (rather than gather experimental data), sampling procedures were influenced by the experimenter's own bias and judgement calls based on how to catch the most fish, as opposed to methodically fishing for X number of minutes at each pool every day to get an unbiased data set. This prevents exact replication, but the project was primarily conservation oriented rather than research oriented. Still, discrete data regarding the number of Mud Turtles that were hooked and the number of Sunfish that were eradicated could be analyzed for correlational patterns.

## Results

A total of 427 Green Sunfish were captured in the course of approximately 20 days of fishing and trapping. Of these, 158 were fished out of various pools and 269 were trapped using minnow traps. Out of the total number of fishing days, a total of 39 Mud Turtles were hooked and 2 infant Mud Turtles were found in traps, both of which were found after large monsoon rains. No turtles died as a result of their capture. Pond 1 had the highest number of caught fish (94 fished, 219 trapped) and Pond 4 had the lowest number of caught fish (1 fished, 0 trapped). Pond 1 also had the highest number of Mud Turtles hooked (15 total), most of which were hooked after large removal days. Mating behaviors of the Sunfish were observed in Pond 3 beginning on day 14, and fish were hooked that were bearing eggs shortly thereafter.

There were many visual observations regarding the behavior of Sonora Mud Turtles. After the carrion was dropped into each pool, a Mud Turtle would find it within 5 minutes. It would flee into the muddy shallows with its catch until another turtle showed interest, in which

case they would each tug at it until the victor swam away with its newfound meal. By the 5th day of data collection (after a total of 91 fish were removed), a large number of turtles were accidentally hooked, with 5 hooked on day 5 and 6 hooked on day 6. This was after a big collection, on day 4, in which 33 Green Sunfish were removed from various ponds. The Mud Turtles started being very aggressive to the brightly colored bobber (biting it) beginning on day 6 (Figure 5), and sightings decreased dramatically after the first flooding event of the canyon (day 16).



Figure 5. Typical aggressive behavior toward the fishing bobber

The length of caught fish ranged from approximately 8 to 22 centimeters (3 to 8 ½ inches), and the mean was about 12 centimeters (4.8 inches). The length of trapped fish ranged from 4 to 10 centimeters (1 ½ to 4 inches) and the average length was 7 centimeters (2.6 inches). Histograms of the frequency of size classes that were fished and trapped show a strong right skew.

The date of collection was found to be a significant predictor of the number of Green Sunfish that were captured, and as time went on, the rate of captures decreased (Linear Regression,  $p=0.011$ ,  $r^2= 0.212$ ). Removal efforts slowed dramatically after day 12 (Figure 8), with more than half of the captures recorded before that date. The date of collection was also a significant predictor of Sunfish removed from Pond 3 alone (Linear Regression,  $p=0.016$ ,  $r^2=0.191$ ). However, paradoxically, the rate of capture increased as time went on (Figure 9).

The date of collection was not a significant predictor of the number of Sonora Mud Turtles that were hooked (Linear Regression,  $p=0.679$ ). However, the number of captured Sunfish was positively correlated with the number of Sonora Mud Turtles that were hooked

(Pearson's correlation,  $r=0.316$ ). Using a different correlational model revealed a higher correlation coefficient (Spearman's correlation,  $r=0.410$ ).

## Discussion

A large number of Green Sunfish (*Lepomis cyanellus*) were removed from pools in the Post Canyon region of the Appleton-Whittell Research Ranch, and this effort helped to restore the habitat of affected Sonora Mud Turtles (*Kinosternon sonoriense*). Smaller fish were more likely to be caught than fished, which is to be expected, given that the minnow traps only had a entry diameter of about 4 centimeters (about 1.5 inches), and the hooks were too big to be swallowed by smaller fish. It is likely that the right skew shown in figure 6 is a product of this. That is, the strong right skew would be reduced if smaller fish were easier to catch. A histogram of the size classes of fished that were hooked and a random sample (of equal length) of fish that were trapped shows that the right skew was affected by something other than high numbers of trapped fish (Figure 7), possibly the low size range of trapped fish.

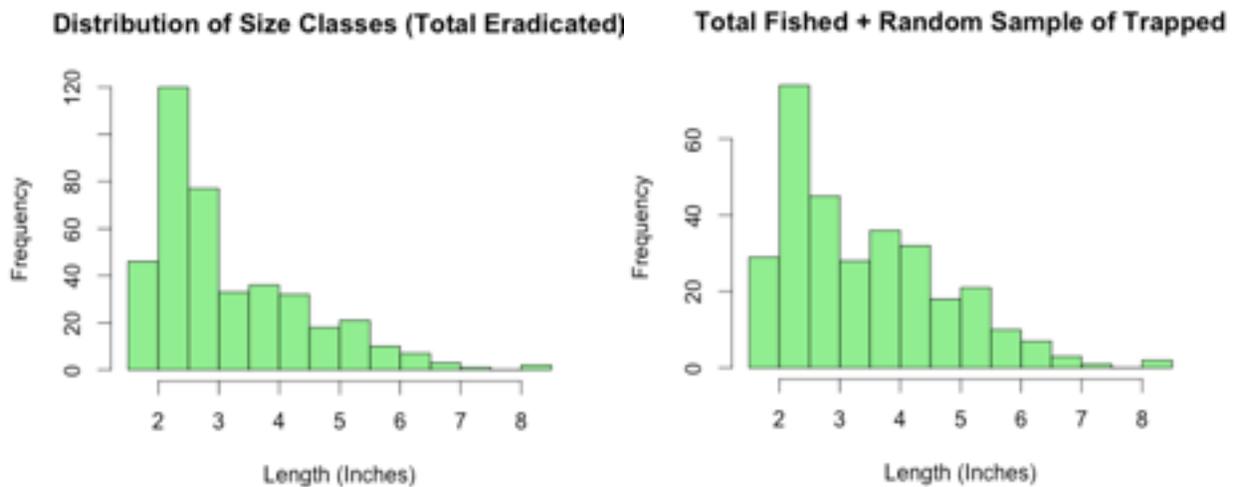


Figure 6. Histogram showing the frequency of size classes for the total amount eradicated. See Appendix I for SI units.

Figure 7. Histogram showing the distribution of all fish that were caught with a pole (158) and a random sample of 158 trapped fish. The shape of Figure 5 is mimicked closely, implying that proportionally high numbers of trapped fish are not the only cause of the strong right skew. Another cause may be the low range of size classes for the trapped fish.

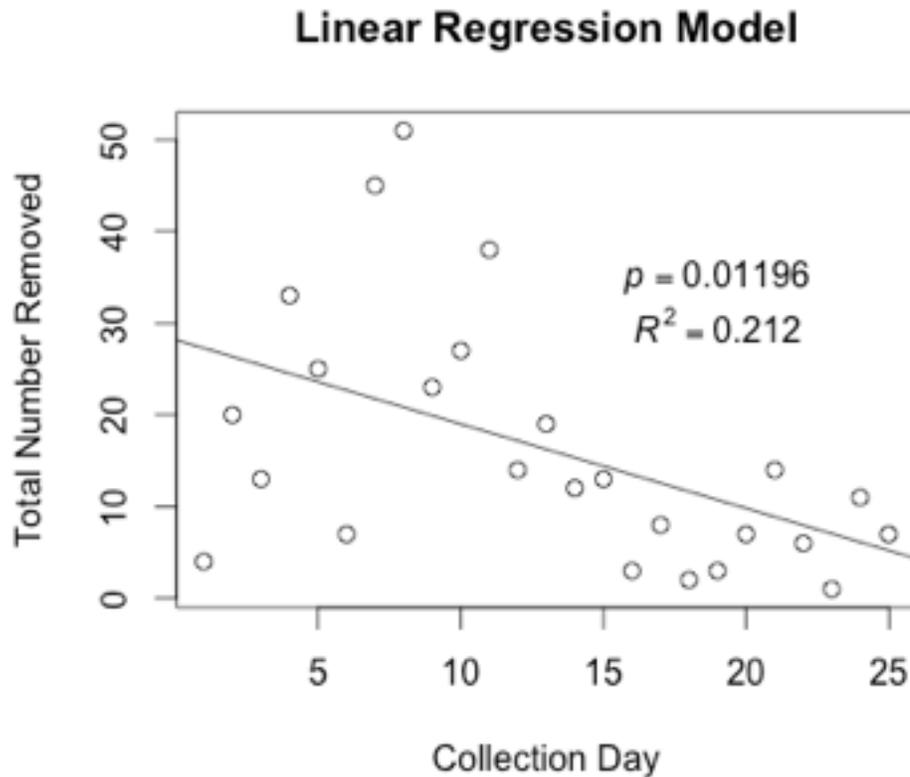


Figure 8. Linear Regression analysis of the amount of Green Sunfish removed versus the day of collection ( $p=0.011$ ,  $r^2= 0.212$ ). A decreasing hyperbolic trend is evident. See Appendix IV for log transformation.

Linear regression shows that the date of capture was a predictor of the number of Green Sunfish that were removed (Linear Regression,  $p=0.011$ ,  $r^2= 0.212$ ). Also, a hyperbolic trend is evident (Figure 8), and the rate of Sunfish removal gradually decreases. Therefore, the effort put into fishing has diminishing returns, and it is unlikely that complete eradication would be possible using the same sampling procedures. Log transformation of the total number removed shows a stronger trend (Linear Regression,  $p<0.01$ ,  $r^2= 0.233$ ; see Appendix IV).

Figure 9 shows that the previously mentioned trend is reversed in Pond 3 (Linear Regression,  $p=0.016$ ,  $r^2=0.191$ ; Figure 9). The rate of capture increases over time, starting at around day 13. Because sampling was not carried out in an unbiased manner, this could be due

to increased efforts of fishing. However, because mating behaviors were observed (i.e. males guarding their nests and acting aggressively towards other males), and because unsuccessful efforts to trap and fish at Pond 3 were carried out before day 13, it is likely that this trend is a product of the reproductive behavior of sunfish.

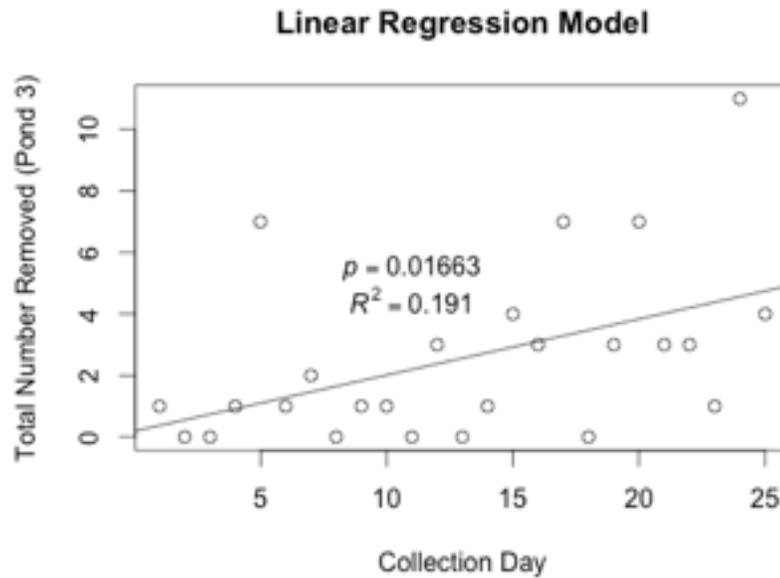


Figure 9. Linear Regression analysis of the amount of Green Sunfish removed from Pond 3. An increasing trend is evident. Although increased efforts of removal targeted this pond, reproductive behavior was observed before being targeted.

The correlational coefficient between the number of Green Sunfish that were removed and the number of Sonora Mud Turtles that were hooked varies based on which statistical test was used. Pearson's correlation yields an  $r$  value of 0.316 whereas Spearman's correlation yields an  $\rho$  value of 0.410. This implies that the relationship between these two variables is monotonic but not linear, and that the two variables are growing at different rates. This is indicative of the Mud Turtles' response to the changing ecosystem; a large number of Green Sunfish removed might have sparked an increase in Mud Turtle boldness and therefore amount of times hooked. This is supported by the fact that Pond 1 had both the highest number of

removed fish and the highest number of hooked turtles, even though it was not the largest. Also, the one day that a fish was caught at Pond 4, a total of 6 Mud Turtles were hooked during the same session. Early estimates placed the smallest concentrations of Green Sunfish in pond 4 because of its extreme depth and lack of shallow rocky and vegetative cover that are typical breeding grounds, and the high proportion of mud turtles caught to sunfish caught is a product of this. Becker *et al.* (2010) found that Sonora Mud Turtles are primarily carnivorous except in ponds where they are in competition with Green Sunfish. Evidence suggests that this is due to the aggressive behavior of the sunfish. It is likely that this correlation between Mud Turtles hooked and Sunfish captured is a product of that behavior, and decreased proportions of Sunfish increases the likelihood of turtles getting a chance to bite the bait. This trend would be more evident given a more rigorous and data oriented method of sampling, rather than sampling from a conservation standpoint.

The diminishing returns of removal efforts could be a product of a chemical alarm system that Green Sunfish are known to exhibit (Brown and Brennan 2004). It may be that the decreased aptitude to bite is a product of conditioning and selection. In such an enclosed area with a relatively high proportion of fish that escaped capture, the fish may be exhibiting classical conditioning between the presence of bait and the release of a chemical alarm system, but this decrease could also be indicative of a drop in population and therefore a reduction of the numbers of Green Sunfish that are willing to bite the bait.

Further research could document the interactions between Sonora Mud Turtles and Green Sunfish in a laboratory setting. Also, detailed analysis on the behavior of Green Sunfish associated with bait fishing and trapping would provide insight on the hyperbolic trends observed earlier. This analysis would answer the question of whether this trend was a result of classical conditioning, extreme population decrease, or behaviors associated with reproduction.

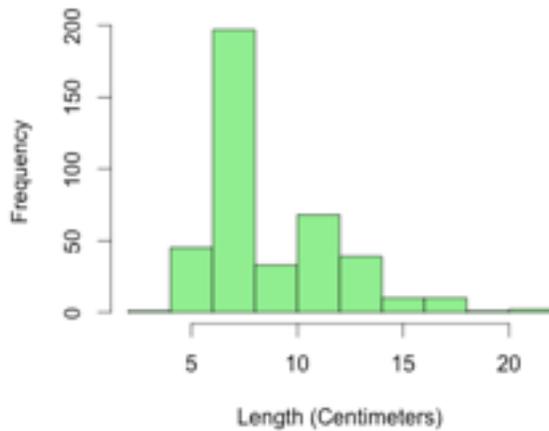
Conducting a multi-year capture-mark-recapture estimate of Mud Turtle population would provide insight on the effectiveness of this study.

#### Acknowledgements

I'd like to thank the Appleton-Whittell Research Ranch of the National Audubon Society for their invaluable support in the pursuit of this project. In particular, I'd like to thank Linda Kennedy, director, for her guidance and council and Roger Cogan, Conservation Coordinator, for his assistance in data collection and his herpetological knowledge. I'd also like to thank the National Audubon Society for providing the opportunity to pursue an Undergraduate Apacheria Fellowship for research at the ranch. Finally, I'd like to thank an anonymous contributor for providing the funds necessarily for the pursuit of this project. Your contribution made it possible to do a great service to the environment, and I hope this study makes it evident.

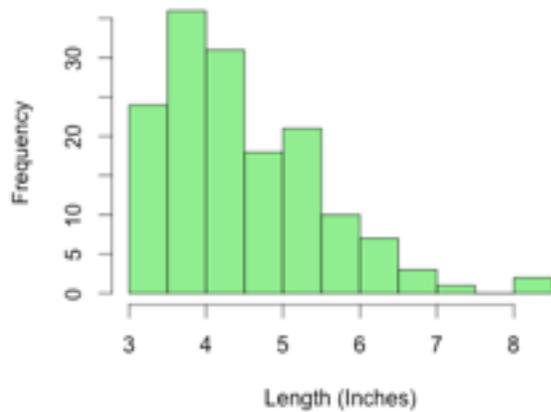
## Appendices

**Distribution of Size Classes (Total Eradicated)**



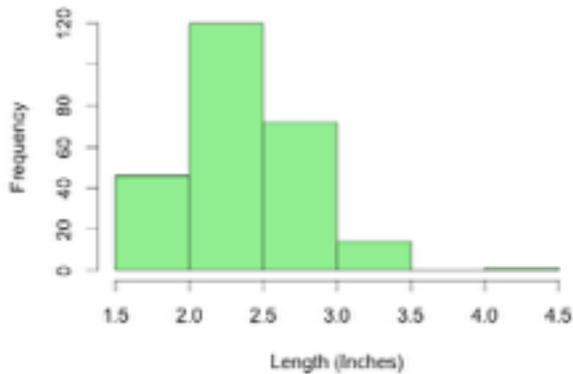
Appendix I. Histogram of size classes in centimeters. This graph was excluded from main body because the linear transformation of data warped the shape of the distribution.

**Distribution of Size Classes (Total Fished)**



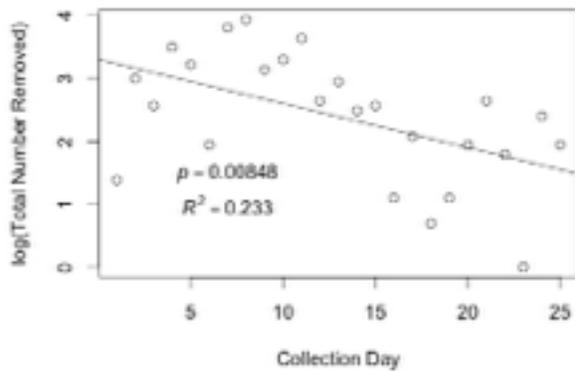
Appendix II. Distribution of the fish that were caught using a pole.

**Distribution of Size Classes (Total Trapped)**



Appendix III. Distribution of fish that were caught using minnow traps.

**Linear Regression Model**



Appendix IV. Linear Regression model showing the log transformation of the total number removed. This transformation better fits the assumptions of Linear Regression. However, it was excluded from the main body because the untransformed data illustrated the diminishing returns more clearly.

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