5-2011

A Study of Habitat Selection and Fluctuating Asymmetry of *Amybstoma tigrinum* at Henderson Island Wildlife Management Area in Jefferson County TN.

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A thesis

presented to

the faculty of the Department of Biological Sciences

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Science in Biology

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by

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May 2011

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Keywords: Fluctuating Asymmetry, Nonbreeding Habitat, Tiger Salamander

ABSTRACT

A Study of Habitat Selection and Fluctuating Asymmetry of *Amybstoma tigrinum* at Henderson Island Wildlife Management Area in Jefferson County, TN

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Studies were conducted on a population of tiger salamanders, *Ambystoma tigrinum,* at Henderson Island Wildlife Management Area in Jefferson County, TN. Tests were conducted to locate the nonbreeding habitat of the salamanders and to detect any difference in fluctuating asymmetry (FA) between larval populations in a large, permanent pond and an ephemeral wetland. Drift fences were installed with pitfall traps at selected locations around each pond to determine non-breeding habitat use by adults. Most adult salamanders were found using a blackberry (*Rubus* sp.) dominated old-field, a grassy field, and a shrub-grass mix field, which were all adjacent to the ponds. No statistical difference in FA between the 2 ponds.

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CHAPTER 1

INTRODUCTION

The population of tiger salamanders (*Ambystoma tigrinum*) at the Henderson Island Wildlife Management Area in Jefferson County, TN is the eastern most known population of this species in Tennessee (P. Wyatt and S. Dykes, personal interview, September 30, 2010). Individual specimens have been found at various cattle ponds across East Tennessee, but additional reproducing populations are unknown. The distribution of tiger salamanders in East Tennessee is very patchy. A unique characteristic of this population is that it is an island population that possibly could have been isolated for over 67 years following the Tennessee Valley Authority's (TVA) construction of Douglas Dam in 1943. The salamanders breed in 2 ponds. One pond is a larger, constructed pond used for fish-rearing. The other pond is a smaller, constructed pond apparently without fish. The tiger salamanders share the breeding and nonbreeding habitat with spotted salamanders (*Ambystoma maculatus)* at this location.

Fluctuating asymmetry (FA) is differing right and left side measurements of physical characteristics of individuals and is thought to be a measurement of developmental stability, i.e., the greater the degree of right side vs. left side measurements in an individual then the less stable development was in that individual (Palmer 1994). Many researchers consider FA to be a bio-indicator of environmental stress and has been investigated as such (Oxnevad et al. 1995; Bjorksten et al. 2001; Hogg et al. 2001; Jentzsch et al. 2003; Angelopoulou et al. 2009). There has been much criticism of FA as an indicator of environmental stress and some researchers have concluded it to be unreliable (Bjorksten et al. 2001; Hogg et al. 2001). Hogg et al. (2001) found in a literature review of 44 studies that 43.2% found no relationship between FA and experimental stress. Hogg et al. (2001) went further to say that this finding was independent of taxonomic group or the number or type of traits. Leung et al. (2000) has suggested using multiple traits in a FA analyses in order to strengthen the likelihood of detecting FA. Little information is known about predator-induced stress and fluctuating asymmetry, but it can be expected to increase FA (Stoks 2001). Stoks (2001) showed an increased level of FA in larval damselflies when raised with *Aeshna cyanea*, a predator of the damselflies.

Tiger salamanders have an aquatic larval stage with external gills that lasts approximately 2.5 to 5 months (Petranka 2010). The tiger salamanders on Henderson Island breed in 2 ponds. One of the ponds is also used as a fish-rearing pond for white crappie, *Pomoxis annularis*, at the same time the tiger salamander larvae are in the pond. The other pond is ephemeral, has no fish, and has considerable more vegetation in and around it. Measurements of 16 traits of larval tiger salamanders were used to examine potential predator-induced fluctuating asymmetry.

This population of tiger salamanders is a nongame species priority for the Tennessee Wildlife Resources Agency (TWRA) because it is an island population of the species isolated from other populations, because of the sporadic distribution of the species in East Tennessee, and because it is the only known breeding population in the region. Spotted salamanders are included in this portion of the study because they do share the habitat with this population of tiger salamanders. Both species breed in ponds from January through February but spend the remainder of the year in terrestrial habitat (Petranka 2010). In the nonbreeding season these salamanders are fossorial and primarily use small mammal burrows (Semlitsch 1983a; Madison 1997; Madison and Farrand 1998; Smyers et al. 2002; Regosin et al. 2003; Steen et al. 2006). It has not been documented that the spotted salamander can excavate its own burrows, but the tiger salamander apparently can (Madison and Farrand 1998; Semlitsch 1983a, 1983c). Petranka (2010) has described the tiger salamander as being found in a variety of habitats including bottomland hardwoods, open fields, brushy areas, grasslands, deserts, and both deciduous and coniferous forests. TWRA nongame managers have a particular interest in identifying this species terrestrial habitat usage at Henderson Island, which will allow for better management of this unique population during the nonbreeding season on this Wildlife Management Area (WMA) and others.

A soil survey map for Henderson Island was also obtained from Natural Resource Conservation Service (NRCS) because of the fossorial nature of both salamanders to see if there is a relationship between soil type and habitat preference. There are no previous studies of Abystomatid salamander soil preference, but Petranka (2010) suggested that "sandy or otherwise friable soils" should provide optimal habitat. Friable soils are described as sandy and will break apart easily when handled. The term loam is usually used to describe this soil type.

CHAPTER 2

MATERIALS & METHODS

Description of Study Site

Henderson Island Wildlife Management Area is located in the Jefferson County, TN portion of Douglas Lake. It has been isolated since TVA flooded the area with the construction of Douglas Dam on the French Broad River in March of 1943 (Tennessee Valley Authority 1949), and is only accessible by boat except for a couple of months of the year during lake drawdowns by TVA. The larger, constructed pond was created by TWRA in 1991 in a partnership with Ducks Unlimited to create a moist-soil impoundment for waterfowl usage (Ducks Unlimited; J. Mike, personal interview, September 30, 2010; and TWRA). The Pond was created by installing a levee and a water control structure. Since then, it is has been converted into a fish-rearing pond for white crappie and sauger, *Sander canadensis*, and has been drained to remove rough fish and vegetation each year to prepare for the fish stocking. This pond was first stocked with crappie in 2005. In 2009, the pond was used to raise crappie but it was not be drained. This pond does not contain woody vegetation in or near the pond but does contain emergent and shoreline, grassy vegetation such as broomsedge (*Andropogon virginicus*) and cattails (*Typha sp.*). This pond is referred to as pond 1. The smaller, constructed pond is located approximately 50 meters from pond 1. It is an ephemeral pond and is usually dry by June and starts to recharge with water in late winter. This pond has considerably more grassy and woody vegetation in and around it compared to the pond 1. This pond is referred to as pond 2. Both ponds were filled with water on November 13, 2008, by mechanical pumping. The salamanders should use pond 2 more than pond 1 not only because it has no fish in it but also because both species of salamanders prefer water with more vegetation for oviposition (Semlitsch 1983a; Madison and Farrand 1998)

On March 9, 2009 pond 1 was stocked with 35 brood stock white crappie. Between March 16 and March 20, 70 to 75 more crappie were stocked in the pond, bringing the total between 105 and 110 brood stock crappie. The survival rate and reproduction rate of the stocked crappie is unknown and so numbers could be higher or lower but should be considerably higher when accounting for the fingerlings produced.

Sampling Technique

Tiger salamander larvae were captured with seines and minnow traps. Pond 1 was seined on May 19, 2009. Pond 2 was also seined, but the technique did not work very well because of a large amount of vegetation in the water. The minnow traps were wrapped in aluminum foil with a glow stick placed inside and submerged. Twenty traps were used for 8 trap nights for a total of 160 trap nights for both ponds combined. The minnow traps were open May 21 through May 23, June 3 through June 6, and June 18 through June 21.

Captured salamander larvae were photographed. Weight was taken using a digital scale. Tail depth was recorded using dial calipers. All other measurements were recorded from digital photographs. Tail clippings were also taken for 2 purposes. First, it marked the individual as captured in case it was caught again, and so measurements were not taken a second time. Second, the tail clippings may be used for future research. This technique is discussed in more detail later.

For the nonbreeding habitat study, drift fences with pitfall traps were installed at selected locations around each pond. Each fence was assigned a unique number. Fences 1 through 4 and

10 were located around pond 1, and fences 5 through 9 were located around pond 2. The drift fences were placed within 5 meters of ponds. The bottom portion of the fence was buried approximately 10 inches deep so as to not allow salamanders to move under it. All fences had a standardized length of 100 feet. In late January of 2009, 600 feet of drift fence were installed in 6 sections with 8 bucket traps at each section. Four 100-foot sections were installed around pond 1, and 2 such sections were installed around pond 2. The total of 48 bucket traps were open for 30 nights for a total of 1,440 trap nights. On January 18, 2010 four more sections of drift fence were installed in 100-foot sections. Three of these sections were installed around pond 2, and one section was installed around pond 1. This added 31 bucket traps to the total count. In 2010, the total number of trap nights was 2,214.

Photographic Technique

Specimens were placed in a plastic container filled with water. Photographs of each specimen were taken from a vertical perspective using a 4 megapixel digital camera. To establish a measurement scale a piece of lab tape having a width of 19.5 cm was placed on the bottom of each photography container, as were 10 cm lines. A minimum of 20 photographs were taken of each individual to obtain useable images. Multiple traits were measured from the photographs using ImageJ 1.42v (Public Domain Imaging Software, National Institutes of Health, http://rsb.info.nih.gov/ij/). The traits measured were total length, snout-vent length, body width, head width, left and right hind limb width, left and right gill length, intraocular length, hand angle width, and width and length of all the digits on the right and left hind limbs. Gill length was standardized as the bottom gill (Figure 1). Measurements for the hind limb width were taken on the distal end (Figure 2). Body and head width were taken at the widest parts of the body and head (Figures 3 and 4). Hand angle width was taken from a notch on the proximal side of the first digit to the middle of the webbing between the fourth and fifth digit (Figure 5). If either of the fourth or fifth digits was missing, then the last digit and second to last digit was used for the endpoint of the measurement. Digit width was also measured at the widest part of each digit (Figure 5). For digit length, measurements were taken from the tip of the digit to the middle of the webbing on each side of the digit (Figure 6). For all digits except 1 and 5, there are 2 measurements labeled as A and B.

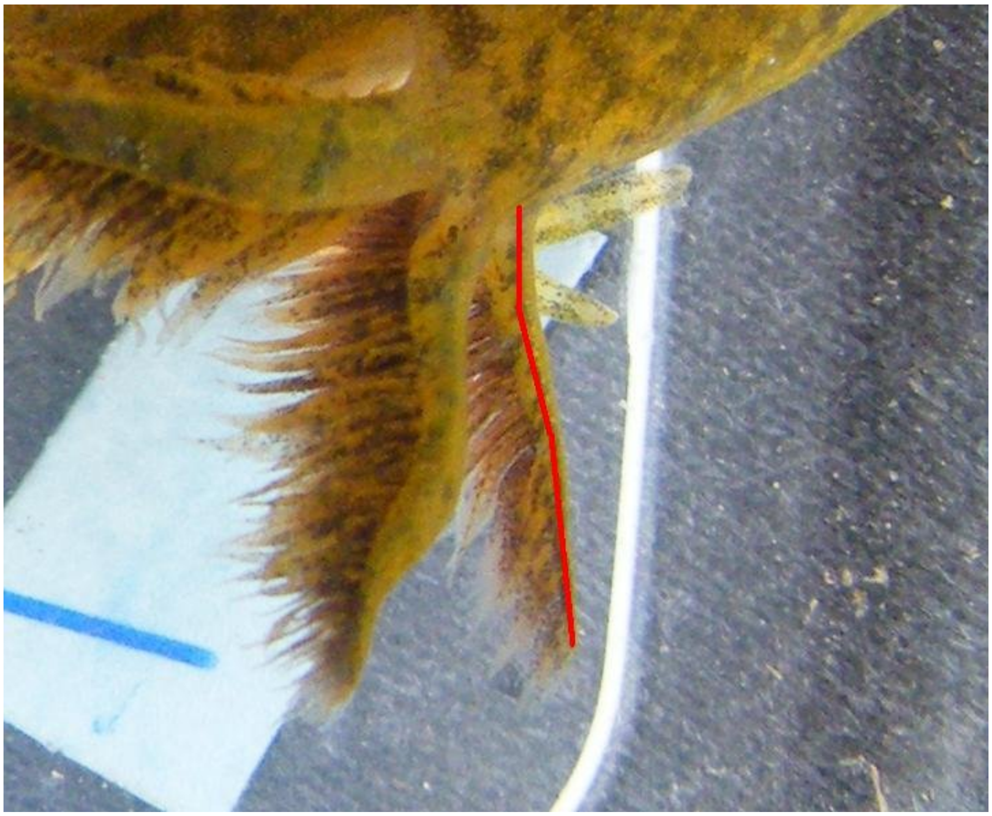

Figure 1 Gill length

Note: The red line marks the measured gill length of the bottom right gill.

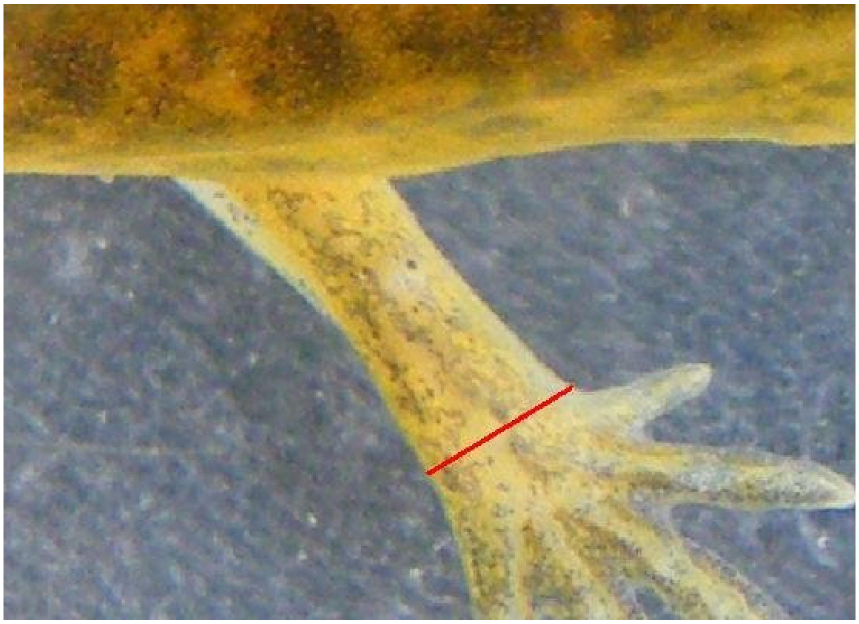

Figure 2 Hind limb width

Note: Red line marks the location where hind limb width was measured.

Figure 3 Body Width

Note: The red line marks the location where body width was measured.

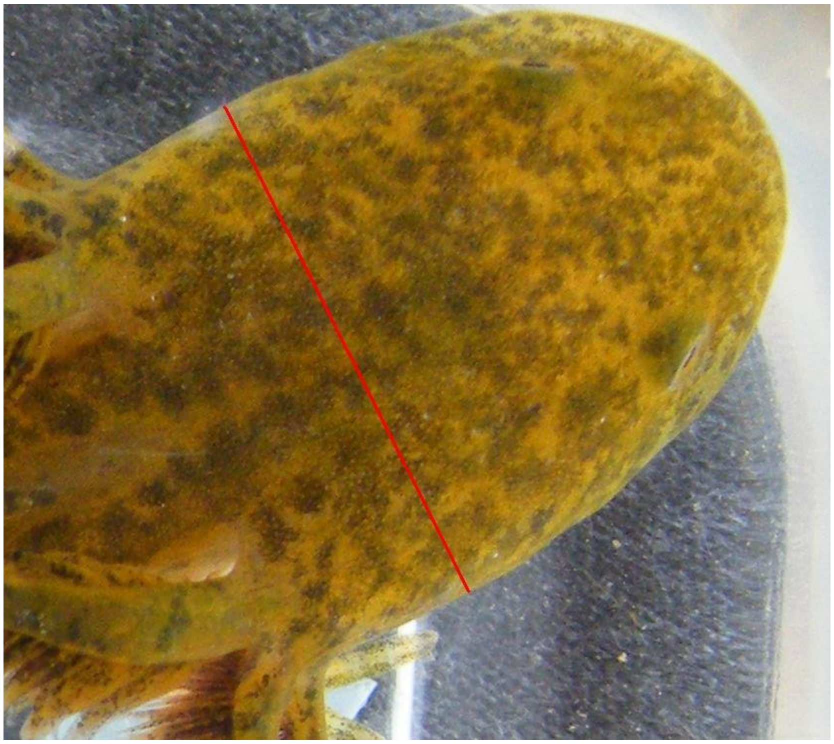

Figure 4 Head width

Note: The red line marks the location where head width was measured.

Figure 5 Hand angle width and digit width

Note: The red line marks the measured hand angle width and the blue line marks the measured digit width.

Figure 6 Digit length

Note: The red line marks the measured length A of the third digit and the blue line marks the measured length B of the same digit.

Statistical Analysis

Two right-left side traits were used for the statistical analysis of fluctuating asymmetry because I thought that these 2 measurements would be the most consistent in the photographs thus producing the least amount of error. The first trait I used was the length of the proximal side of the third digit on the hind foot referred to as L**R**D3A for the right side and L**L**D3A for the left side. The second trait used was referred to as the hand angle width and is noted as RAngleW for the right side and LAngleW for left side.

Before statistical testing for fluctuating asymmetry differences between the ponds, the data must meet some assumptions. One must first account for size differences by individual because the larval tiger salamanders were caught at different times over a span of 17 days. In order to account for this I used an FA index (Palmer and Strobeck 1986; Palmer 1994). I chose FA2 (Palmer 1994), which is |R-L)/((R-L)/2|, because this index accounts for size differences among individuals.

A second assumption is that traits that show FA are normally distributed because FA is defined as "a pattern of bilateral variation in a sample of individuals where the mean R-L difference is zero and variation is normally distributed" (Palmer 1994). The raw data (individual right and left side measurements) were tested for normality using the Shapiro-Wilk Statistic and the Kolmogorov-Smirnov Test.

Measurement error must also be accounted for in studies of FA. Repeated measures of both digit length and angle width were taken and the signed differences (R-L) were subjected to a fully nested ANOVA in order to reveal how much of the variability in measurements was among individuals and how much resulted from multiple measurements.

General Linear Model (GLM) analysis was then used to test for differences between ponds for both digit length and hand angle width. Before the GLM analysis was conducted, the results from an Oneway ANOVA were used. It was determined that there was no significant difference in hand angle width between ponds ($F=0.619$; $p=0.434$; Table 10), so it was not used in the GLM analysis. The GLM analysis tested the influence of several factors on the length of the proximal side of the third digit. The factors included pond number, snout-to-vent length (SVL), and the length of the proximal side of the third digit on the left hind foot.

CHAPTER 3

RESULTS

Fluctuating Asymmetry

Twenty-one larval tiger salamanders were captured and measured for the analysis of pond 1, and 44 salamanders were captured and measured for the analysis of pond 2. Twenty of the larvae for pond 1 were captured on May 19, 2009, and the other one was captured on May 22. In pond 2, 12 larvae were captured on May 22, 13 larvae on May 23, 3 larvae with 2 recaptures on June 4, 6 larvae on June 5, and 10 larvae on June 6.

The Shapiro-Wilk Statistic and the Kolmogorov-Smirnov Test showed similar results for both digit length and hand angle width (Table 3). Both tests showed that digit length in pond 1 was not normally distributed ($p<0.001$; $p<0.001$), and that digit length in pond 2 was normally distributed ($p=0.165$;$p=0.200$). Also, both tests showed that hand angle width was not normally distributed in both ponds (Pond 1: $p<0.001$,$p=0.002$; Pond 2: $p=0.004$,$p=0.009$). The nonnormal distribution is addressed in the GLM analysis.

When the Fully Nested ANOVA was used to assess measurement error we combined the ponds (Table 4). In digit length, 79.85% of the variability in the measurements (signed differences) was accounted for by differences between individuals, while 20.15% of the variability comes from repeated measurements of individuals ($p<0.001$). Angle width had similar results with 71.63% of the variability explained by differences from individual to individual, and 28.47% explained by differences in measurements for the same individual ($p<0.001$).

The initial General Linear Model (GLM) analysis showed that all three of the factors were significant (length of the digit: $p<0.001$; SVL: $p=0.001$; and Pond #: $p=0.014$; Table 5). However, when the studentized residuals were graphed by the predicted values, they were found to have a pattern (Figure 7). In order to account for this, the data is transformed using square root and logarithmic transformations, and it was found that SVL and Pond # were no longer significant in every combination of transformations. The combination that presented the best residuals graph (Figure 8) was a square root transformation of both the index value and the actual length of the digit ($p<0.001$; Table 6). The residual were then plotted with a histogram, and they were observed to be normally distributed (Figure 9). However, two outliers were found to still be affecting the residuals graph (Figure 10), so those 2 data points were removed, and the analysis was conducted again. Those two data points were from individuals 58W and 70W. The GLM was performed without transformations on all 3 factors, and again SVL ($p=0.236$) and Pond # ($p=0.339$) were found to be not significant (Table 7). The actual length of the digit was found to be highly significant ($p<0.001$); however, the residuals graph still had a pattern (Figure 11). Square root and logarithmic transformations were then implemented again, and once again the combination that presented the best residuals graph (Figure 12) was a square root transformation of both the index value and the actual length of the digit ($p<0.001$; Table 8). These residuals were also plotted with a histogram and observed to be normally distributed (Figure 13). Therefore, the only factor found to be significant in the difference between the ponds was the actual length of the digit.

Nonbreeding Habitat Usage

In 2009, 100 salamanders were captured. Of these 100 captures, 25 were tiger salamanders and 75 were spotted salamanders. The total number of salamanders caught in 2010 was 533. Tiger salamanders accounted for 63 captures and spotted salamanders accounted for 470 captures.

For the nonbreeding habitat portion of the study 2010 data were used for analysis because of the greater number of captures. Sixy-two salamanders were captured at pond 1 and 471 were captured at pond 2, suggesting that pond 2 was used more than pond 1 during reproduction (X^2=313.85; p<0.001; Table 9). This is probably due to pond 2 containing considerably more emergent and shoreline vegetation, and not size difference between the two ponds as pond 1 is considerably larger. Tiger salamanders were found to more frequently encounter fences 1, 2, 6, 8, and 10. The number of captures at each fence were 5, 6, 6, 27, and 9 respectively (Figure 7). When spotted salamander captures are included, the most encountered fences were 5, 6, 7, 8 and 9, which are all around pond 2. The number of captures at each fence were 91, 35, 71, 244, and 30 respectively (Figure 8). The total number of captures are represented in Table 1. The most encountered fences for both salamanders at pond 1 were fence 1 with 13 captures and fence 2 with 16 captures.

Table 1 Salamander captures

	2010			2009		
Fence #	Total	Tigers	Spotted	Total	Tigers	Spotted
1	13	5	8	30	17	13
2	16	6	10	12	5	7
3	11	1	10	3	1	2
4	11	1	10	7	0	7
10	11	9	2	n/a	n/a	n/a
5	91	3	88	45	2	43
6	35	6	29	3	0	3
7	71	2	69	n/a	n/a	n/a
8	244	27	217	n/a	n/a	n/a
9	30	3	27	n/a	n/a	N/a
Totals	533	63	470	100	25	75
Pond 1	62					
Pond 2	471					

Pond 2 captures are in Red type

Pond 2 was used more than pond 1, which was expected because of the greater amount of vegetation in and around the pond for oviposition (Semlitsch 1983a; Madison and Farrand 1998), as well as the fact that pond 1 contained fish. The most encountered fences at pond 1 were 1 and 2, while the most encountered fences at pond 2 were 7 and 8, which are adjacent to each other. The habitat closest to these fences, as well as fences 5 and 9, is an old-field habitat dominated by blackberry, *Rubus sp,* (Figure 14). Between the drift fences is a wet drainage channel that the salamanders may use as a migration corridor. The habitat near fence 6, which was another highly encountered fence, is a mixed shrub-grass field with a few trees. Another frequently encountered fence is fence 10, which occurs on the levee above a low-lying, grassy area (Figure 15).

Semlitsch (1998) conducted a literature review on 6 Ambystomatid salamander species and found that the average distance traveled from breeding sites was 125.3 meters. He assumed that this encompasses half of the population, and so 164.3 meters should encompass 95% of the population because this is the upper range of 95% confidence intervals. The nonbreeding habitats described in this study are found within 164.3 meters.

In the course of trapping, small mammals were also captured. It is notable that small mammal captures almost doubled from 2009 (23 captures) to 2010 (43 captures). The only difference in methodology between the 2 years was the 4 drift fences added in 2010. Three out of 4 drift fences added in 2010 were around pond 1 on the border of the previously described shrub dominated habitat. Small mammal captures for each species are listed in Table 2. The greatest change in capture frequency was the hispid cotton rat, *Sigmodon hispidus*, with one capture in 2009 and 14 captures in 2010.

Table 2 Small mammal captures

	Number Captured	
Species	2009	2010
American Deermouse *Peromyscus maniculatus*	6	2
White-footed Deermouse *Peromyscus leucopus*	6	8
Northern Short-tailed Shrew *Blarina brevicauda*	4	4
Least Shrew *Cryptotis parva*	0	8
Unknown Shrew *Sorex* sp	4	0
Woodland Vole *Microtus pinetorum*	2	7
Hispid Cotton Rat *Sigmodon hispidus*	1	14
Total	23	43

A soil map of Henderson Island from a soil survey conducted in 1983-84 was obtained from the Natural Resource Conservation Service (Soil Survey Staff). The soil unit that occurs where the salamanders are believed to be during the nonbreeding season is described as a Swafford Silt Loam (Soil Survey Staff). This is comparable with the sandy or friable soil described by Petranka (2010) as suitable for these species. Most soils occurring on the map are described as silt loams, but the other soils identified on Henderson Island have higher gradient slopes and are described as eroded, whereas Swafford Silt Loam is not an eroded soil.

CHAPTER 4

DISCUSSION

Fluctuating Asymmetry

The results of the GLM analysis suggests that the difference in fluctuating asymmetry between the 2 ponds can only be attributed to digit length and not SVL or Pond #. This difference is not caused by a difference in size between individuals because SVL was not significant. This difference is probably a result of measurement error. In the Fully Nested ANOVA, error was detected and estimated to be 20.15% for digit length. Because of the conservation status of this particular population, it was not possible to obtain permission to transport specimens to a laboratory setting for FA measurement photographs. All photography was performed in the field, which likely contributed to the level of observed measurement error. The present study is inconclusive on whether fluctuating asymmetry is present in this population of tiger salamanders because of the measurement error involved in the technique.

There should have been a difference between the 2 ponds because one pond had a stressor, the predator, and the other pond did not have this stressor. However, there could have been other variables between the 2 ponds such as water temperature or pH that could have caused similar stress in Pond 2 as the predator did in Pond 1. All of this is overshadowed by the possibility of measurement error associated with the measurements

Nonbreeding Habitat Usage

The results indicate that the tiger and spotted salamanders seem to be using the shrub dominated area south of pond 2, the grassy field east of pond 2, and the low-lying grassy area located between the two ponds. This agrees with previous findings by Petranka (2010) in his review of other studies, in that these salamanders can be found in a variety of habitats. The old-field area appears to be the area of greatest concentration of the salamanders. Also, they do appear to be using the wet drainage ditch as a migration corridor.

The fact that small mammal captures almost doubled from 2009 to 2010, adds to the conclusion that they are using the old field habitat. This is because most of the additional captures came at fences located on the edge of this area. Also, most of the additional captures were hispid cotton rats, which are burrowing mammals (Schwartz and Schwartz 2001), and the salamanders use burrows during the nonbreeding season. A special association between the spotted salamander and the northern short-tailed shrew, *Blarina brevicauda*, has been hypothesized (Madison 1997), but never anything with the hispid cotton rat, and never any relationship between the tiger salamander and any other burrowing mammal. However, it has been found that tiger salamanders were frequently predated upon, most likely by short-tailed shrews (Madison and Farrand 1998). The fact that hispid cotton rats are omnivorous but prefer herbaceous foods, (Schwartz and Schwartz 2001) could be a possible reason both species of salamanders and an abundance of hispid cotton rats were captured at the same fences. This could indicate a previously unfound relationship between these animals.

The soil map also supports the finding that this area is important salamander habitat during the nonbreeding season. All of the areas where capture data suggests the presence of salamanders in terrestrial habitat are characterized by the same soil type. This soil type is the Swafford Silt Loam, which is described as moderately well drained (Soil Survey Staff). This is a sandy soil that allows for easier burrowing by the tiger salamanders and the small mammals that are an essential component of nonbreeding habitat for both the tiger and spotted salamanders.

In conclusion it is recommended that TWRA maintain pond 2 as a fish free environment because large numbers of both the tiger and spotted salamanders migrate to this pond during the breeding season. It is also recommended that pond 1 be established as a fish free environment in order to provide more breeding habitat for these 2 species. If this pond cannot be established as a fish free environment, then it is recommended that draining of the pond to remove rough fish should stop, as this usually occurs after salamander eggs are deposited, and very few rough fish are ever found in the pond. Mowing of the drainage channel between the 2 ponds has been observed in previous years during the migration and breeding periods of both salamander species. This activity should stop and mowing and other potential disturbances should be limited to the extent possible during breeding migration periods from January through March. Mowing can cause soil compaction issues and eliminate cover.

The greatest concentration of both species of salamanders occurs in the blackberry-dominated old field habitat south of Pond 2. There is little historic information regarding habitat management at this site. Secondary succession has occurred with some managed burning. The frequency of burning is unknown, but it has not been burned since at least 2006 (D. Sams, personal interview, March 25, 2011). It is recommended that this area be subjected to prescribed fire on a rotation of 3 to 5 years during the fall season. This would slow succession and prevent canopy closure. The fall season should be the time of least salamander activity and so burning during this time period would be optimal. This burn should be a backing fire while the ground is slightly moist, which would be the least intense, and would cause the least amount of change in the organic matter in the soil, and provide the least amount of scorch to the soil (Wade and Lungford 1989). The primary access road on the island can be used as a control line to the south. The road to the east can act as a control line also, but this road may need to be prepared with a disk harrow. The control line to the north can be pond 2, but a disk line may also need to be established to connect to the east control line and the west control line. The west control line could be the old road bed located between the ponds, but it is recommended that pond 1 be established as a west control line with a disk line attaching it to the road that is the south control line. Establishing pond 1 as a control line would eliminate the need to mow the previously mentioned drainage channel between the ponds.

Burning this area should not affect the salamander population on the island as the southeastern United States has a long history of fire dating back to precolonial times and many amphibians may even be adapted to fire (Bailey et al. 2006), provided the recommendations of how and when to burn. The burn will also help the small mammal population, especially the hispid cotton rats, as fire (Masters et al. 2000) and a good understory (Diskson 2001) with very little canopy have been proven to be very beneficial to this species. This could be important as a great number of hispid cotton rat species were captured at the same fences as both salamander species.

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APPENDICES

APPENDIX A

Additional Tables

Table 3 Results of normality tests (Kolmogorov-Smirnov and Shapiro-Wilk)

		Kolmogorov-Smirnov			Shapiro-Wilk		
	Pond	Statistic	Df[a]	P[a]	Statistic	Df[a]	P[a]
Digit Length	1	0.15	132	0	0.919	132	0
	2	0.059	174	.200*	0.988	174	0.165
Hand Angle Length	1	0.102	132	0.002	0.892	132	0
	2	0.08	174	0.009	0.976	174	0.004

[a]Codes: Df, degrees of freedom; p, p-value.

Table 4 Variance components table for the fully nested ANOVA

	Source	Variance Components	% of Total[a]	StDev[a]
Digit Length	individual	0.322	79.85	0.568
	measure	0.081	20.15	0.285
	total	0.404		0.635
Hand Angle Width	individual	0.286	71.63	0.534
	measure	0.113	28.37	0.336
	total	0.399		0.631

[a]Codes: % of total, percentage of total variation accounted for by each source; StDev, standard deviation.

Table 5 GLM table with untransformed data

Dependent Variable:LD3A Average[a]					
Source	Type III Sum of Squares	Df[a]	Mean Square	F[a]	p[a]
Corrected Model	1.338	3	0.446	19.215	0.000
Intercept	0.015	1	0.015	0.625	0.432
L.LofD3A[a]	1.128	1	1.128	48.579	0.000
SVL[a]	0.275	1	0.275	11.854	0.001
Pond#	0.150	1	0.150	6.449	0.014
Error	1.416	61	0.023		
Total	4.922	65			
Corrected Total	2.755	64			

[a]Codes: LD3A Average, FA index value; DF. Degrees of freedom; F, F-ratio, p, p-value; L.LofD3A, length of digit; SVL, snout-to-vent length.

Table 6 GLM table with square root transformations

Dependent Variable:sqrtaverage[a]					
Source	Type III Sum of Squares	Df[a]	Mean Square	F[a]	p[a]
Corrected Model	3.243	1	3.243	152.357	0.000
Intercept	7.773	1	7.773	365.194	0.000
SqrtLD3A[a]	3.243	1	3.243	152.357	0.000
Error	7.854	369	0.021		
Total	51.603	371			
Corrected Total	11.097	370			

[a]Codes: sqrtaverage, square root transformation of the FA index value; Df, degrees of freedom; F, F-ratio; p, p-value; sqrtLD3A, square root transformation of the length of the digit.

Table 7 GLM table with outliers removed and no transformations

Dependent Variable:LD3A Average[a]					
Source	Type III Sum of Squares	Df[a]	Mean Square	F[a]	p[a]
Corrected Model	0.301	3	0.100	9.719	0.000
Intercept	0.063	1	0.063	6.076	0.017
L.LofD3A[a]	0.179	1	0.179	17.364	0.000
SVL[a]	0.015	1	0.015	1.434	0.236
Pond#	0.010	1	0.010	0.930	0.339
Error	0.609	59	0.010		
Total	2.410	63			
Corrected Total	0.910	62			

[a]Codes: LD3A Average, FA index value; Df, degrees of freedom; F, F-ratio; p, p-value; L.LofD3A, length of the digit; SVL, snout-to-vent length.

Table 8 GLM table with outliers removed and square root transformations

Dependent Variable:SqrtAverage[a]					
Source	Type III Sum of Squares	Df[a]	Mean Square	F[a]	p[a]
Corrected Model	2.584	1	2.584	126.526	0.000
Intercept	6.529	1	6.529	319.716	0.000
SqrtLD3A[a]	2.584	1	2.584	126.526	0.000
Error	7.495	367	0.020		
Total	49.453	369			
Corrected Total	10.078	368			

[a]Codes: SqrtAverage, square root transformation of the FA index value; Df, degrees of freedom; F, F-ratio; p, p-value; SqrtLD3A, square root transformation of the length of the digit.

Table 9 Chi-Square analysis for ponds

	Observed	Expected	(O^a-E^a)	$(O-E)^2$	$(O-E)^2/E$
Pond 1	62	266.5	-205	41820	156.924
Pond 2	471	266.5	205	41820	156.924
Sum	533	533			313.848

[a]Codes: O, observed value; E, expected value.

Table 10 Oneway ANOVA table for hand angle width

Hand Angle Width	Sum of Squares	df[a]	Mean Square	F[a]	P[a]
Between groups	.002	1	.002	.619	.434
Within Groups	.155	63	.002		
Total	.156	64			

[a]Codes: df, degrees of freedom; F, F-ratio; p, p-value.

APPENDIX B

Additional Figures

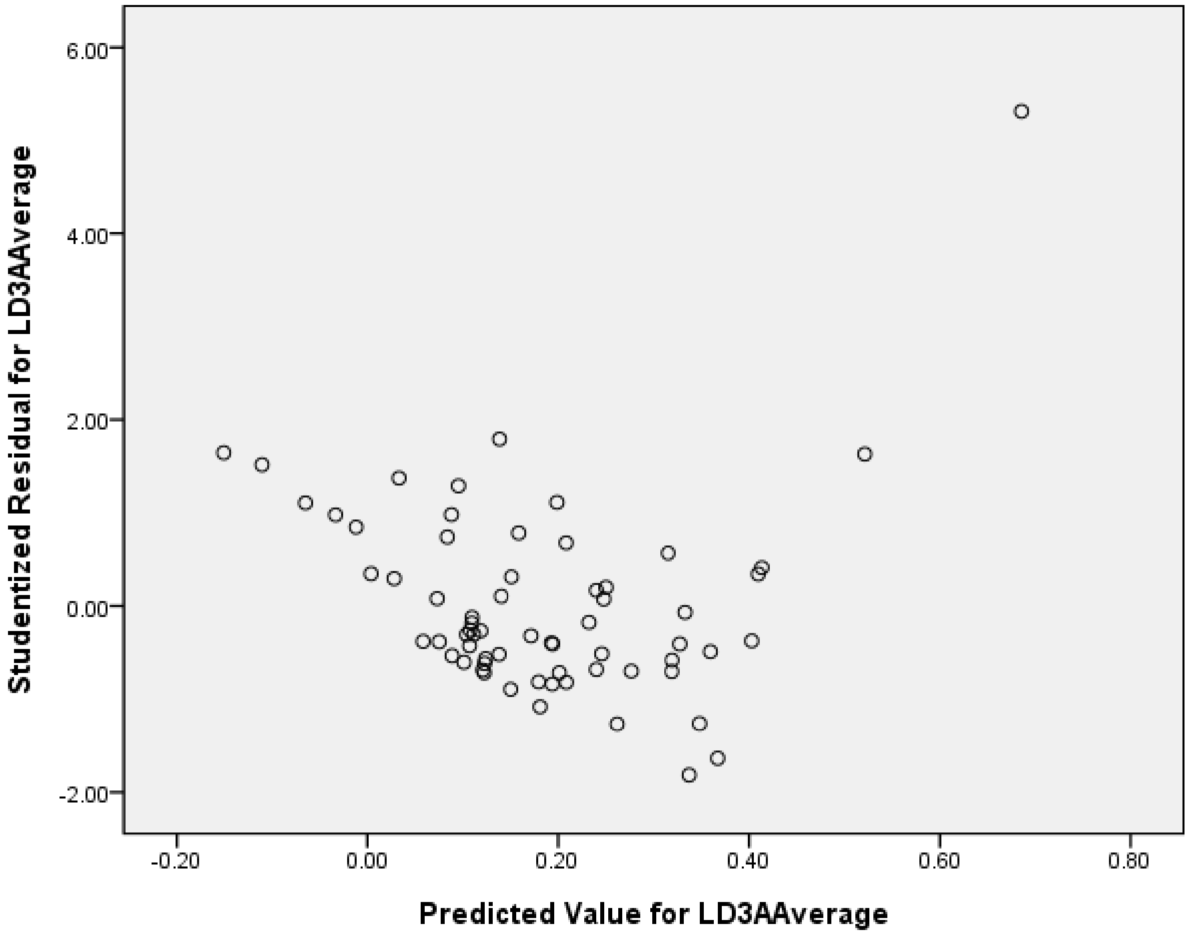

Figure 7 Residuals graph of GLM analysis in Table 5

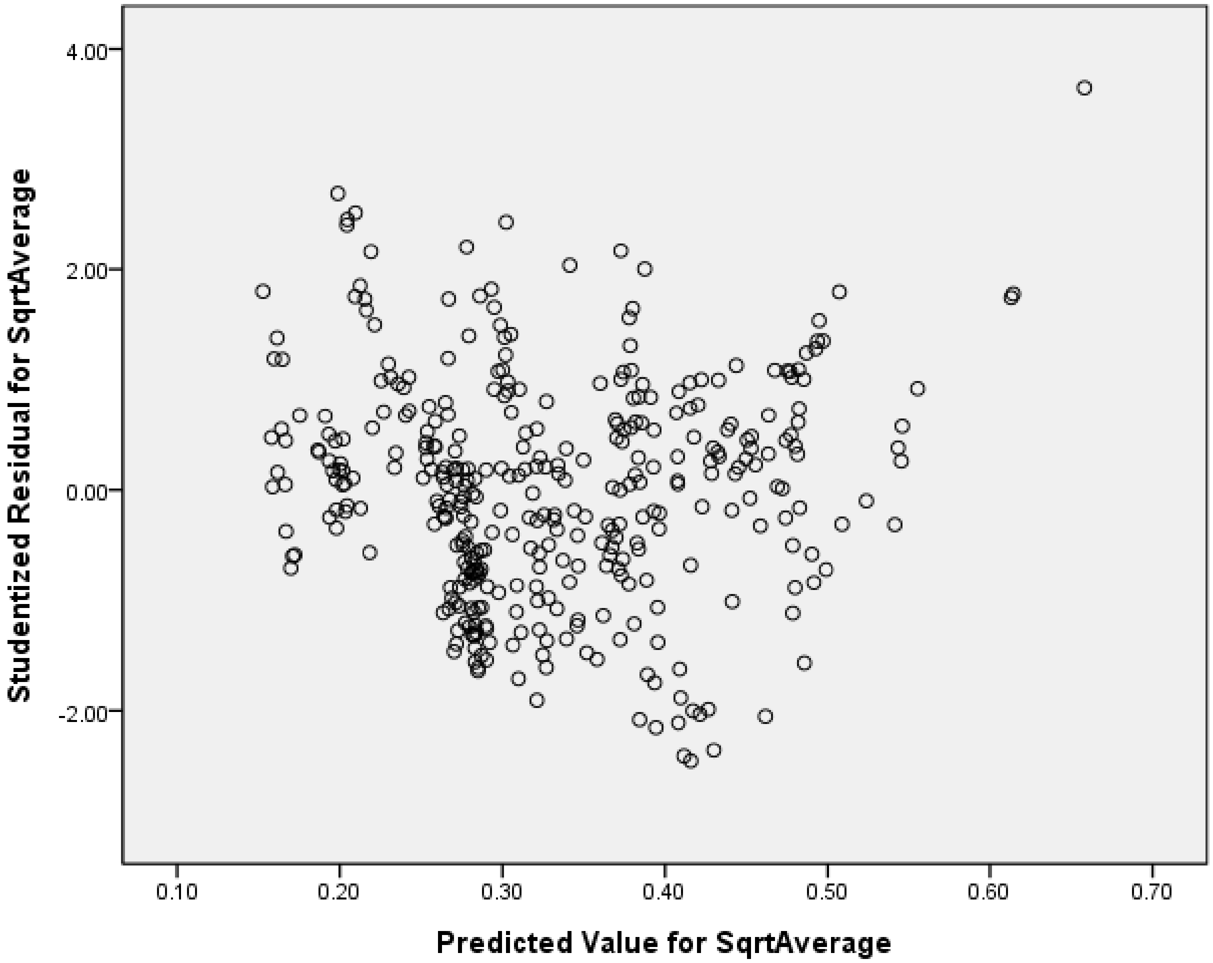

Figure 8 Residual graph of GLM analysis in Table 6

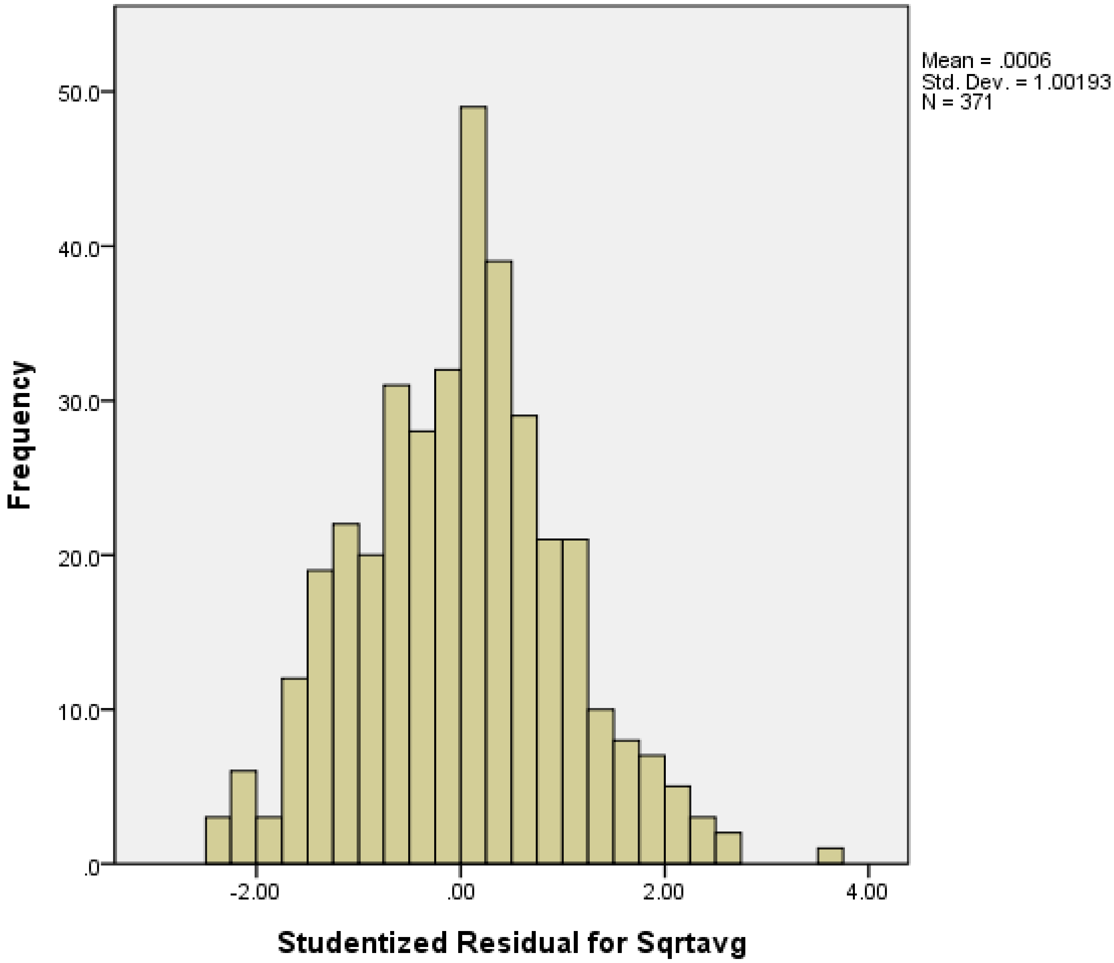

Figure 9 Histogram of the residuals of the GLM analysis in Table 6

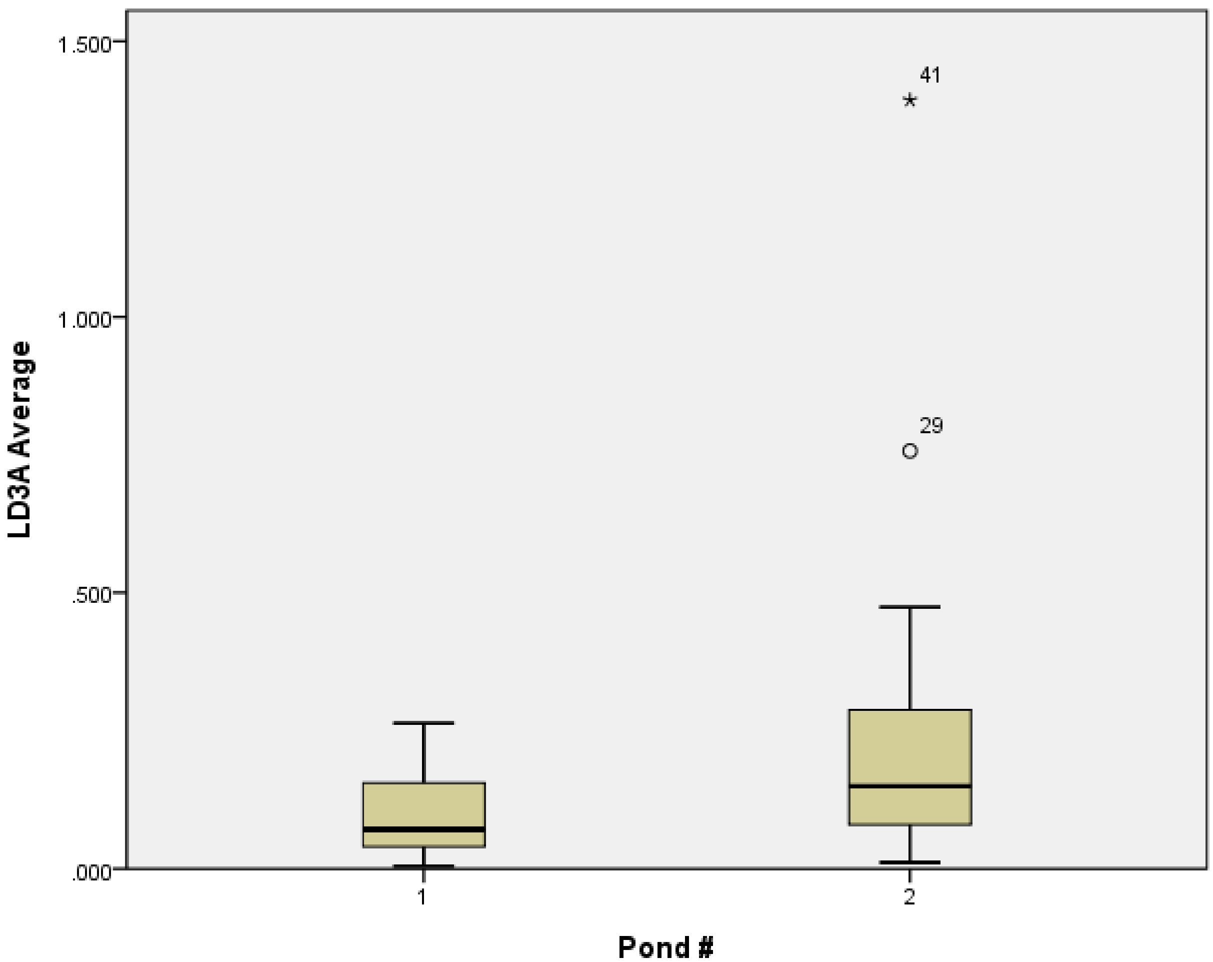

Figure 10 Boxplots of digit length data demonstrating outliers

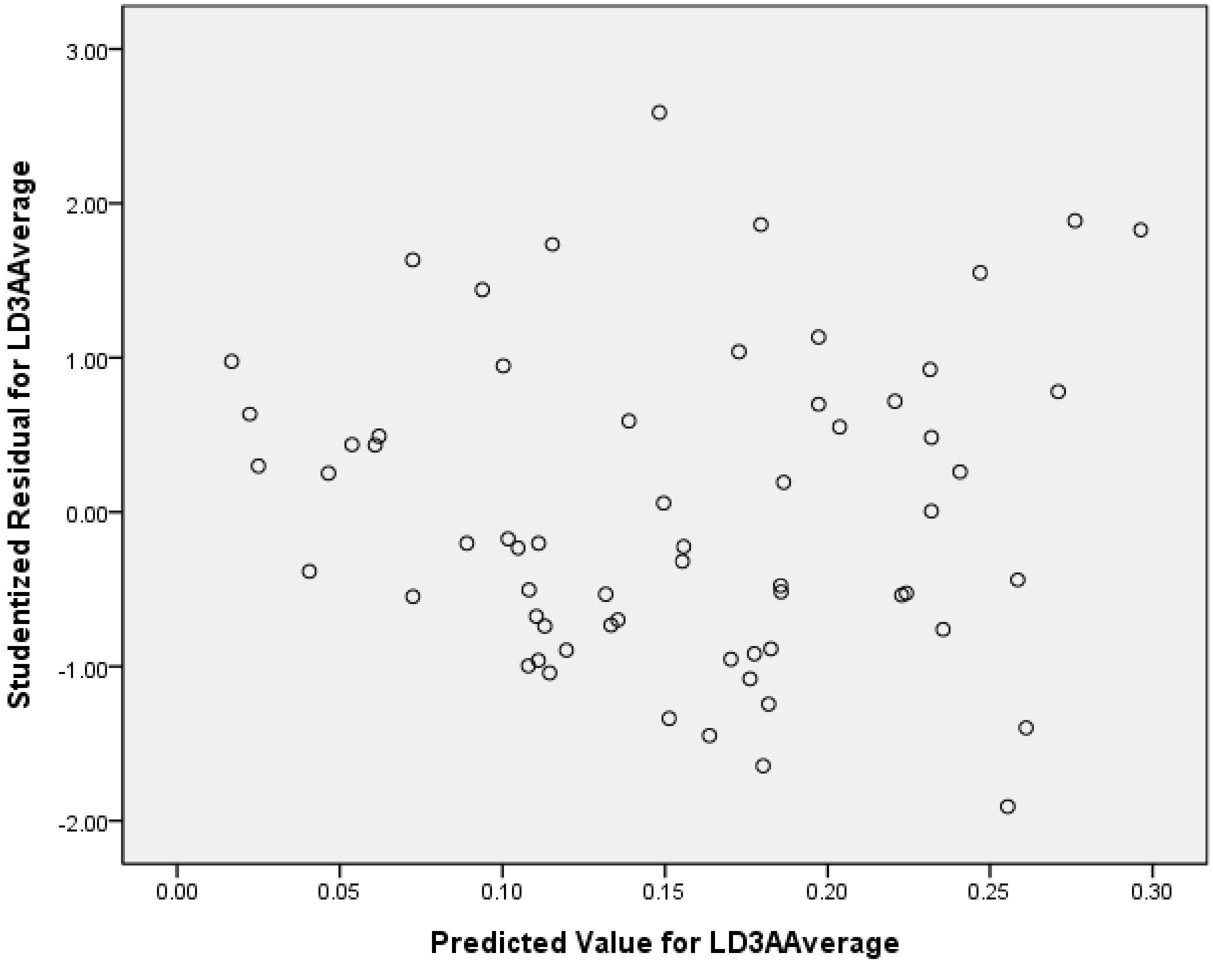

Figure 11 Residuals graph of GLM analysis in Table 7

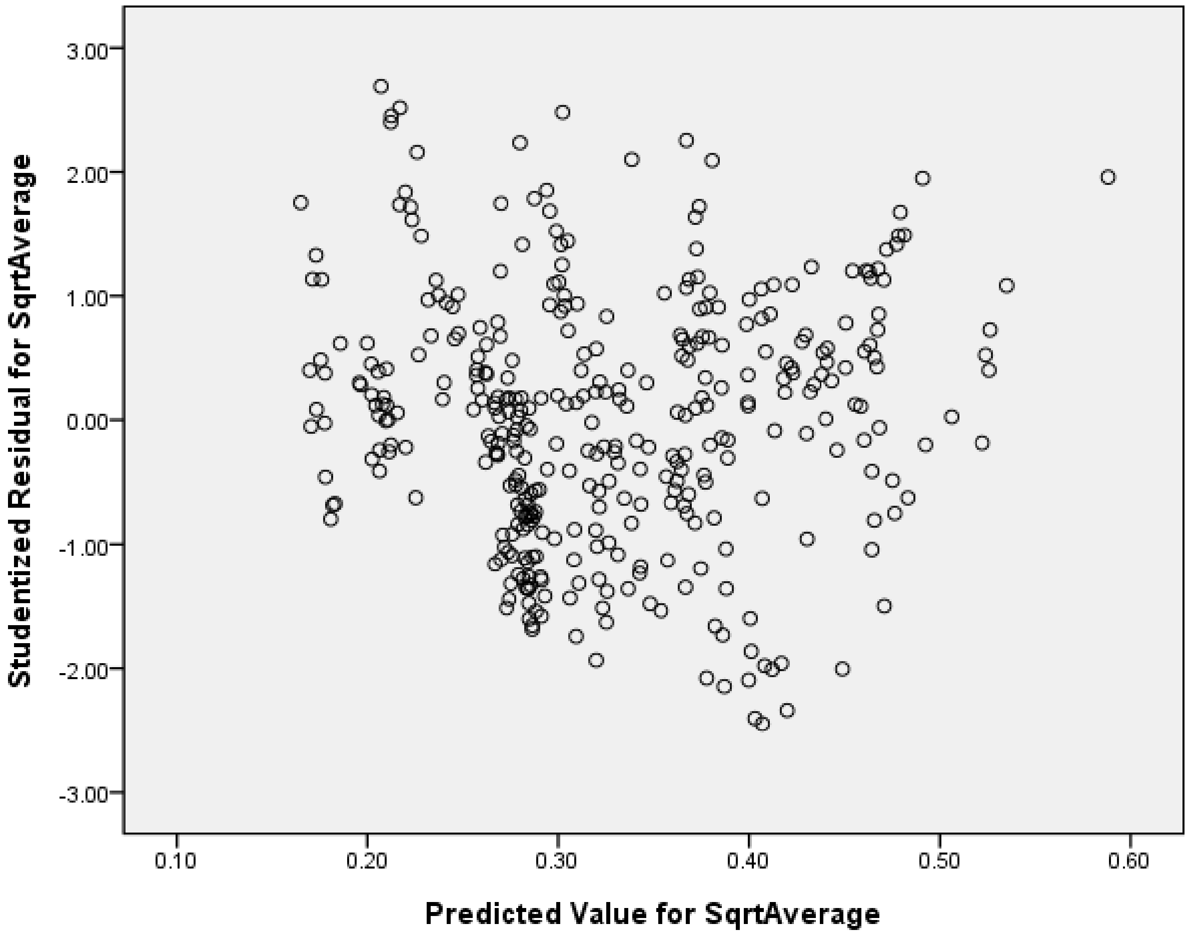

Figure 12 Residuals graph of GLM analysis in Table 8

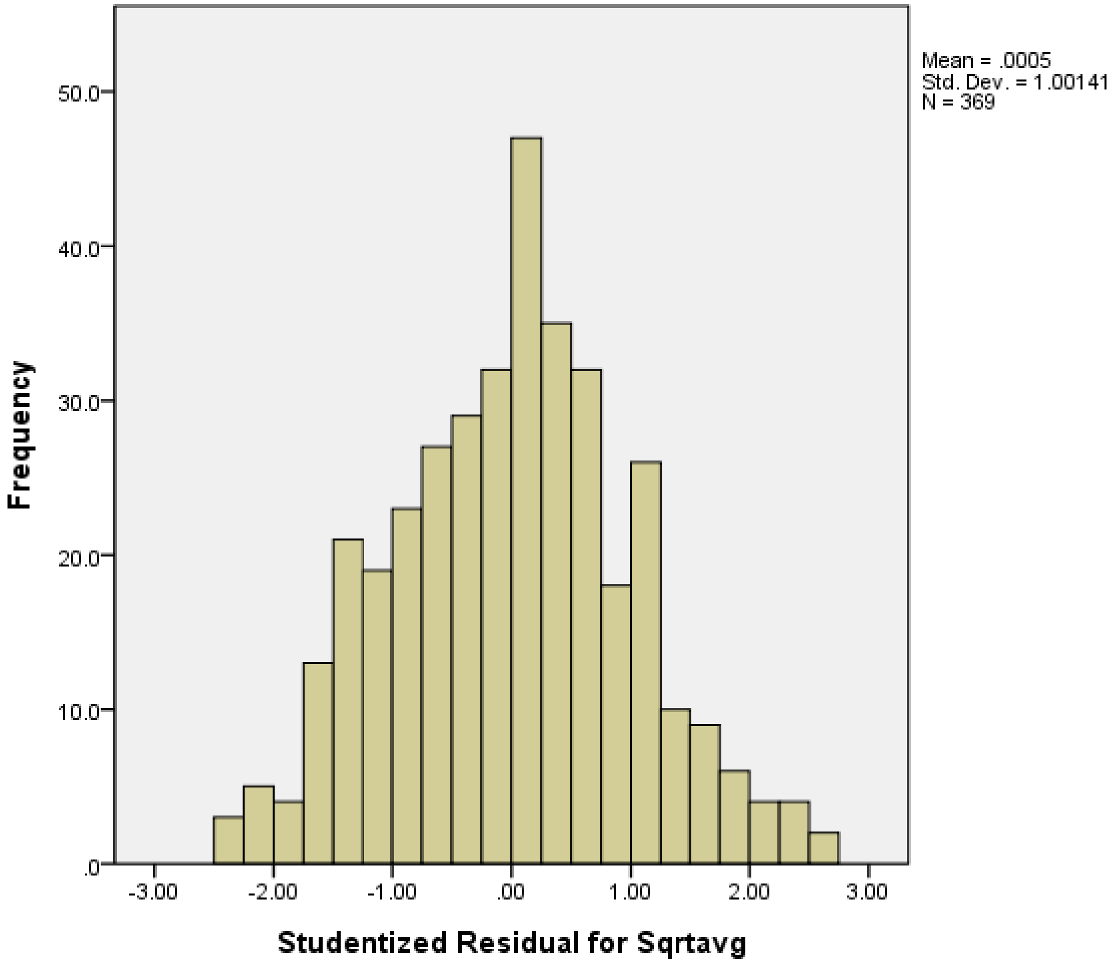

Figure 13 Histogram of the residuals of the GLM analysis in Table 8

Figure 14 A digital photograph of the blackberry-dominated old field habitat south of Pond 2

Figure 15 A digital photograph of the low-lying grassy area east of fence 10

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