VARIATION IN DEMOGRAPHY OF TURTLES IN A SEMI-ARID LANDSCAPE, WITH AN EMPHASIS ON THE ANNUAL SURVIVAL OF YELLOW MUD TURTLE POPULATIONS

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Abstract

Turtle species are declining rapidly across the world because of factors such as habitat degradation, overharvest, and climate change. These declines coupled with a lack of knowledge on many species' life history and factors that directly affect their populations has led to sparse backing for management and conservation. The use of annuli as an aging technique can lead to quick, targeted research in order to fill these gaps within the literature for species of concern. However, there is disagreement within the scientific community of the use of this technique because some researchers have questioned its validity. Therefore, I examined the variation in annual survival, growth rates, and sex ratios of yellow mud turtle (*Kinosternon flavescens*) populations at 9 sites within the species' range in Texas. I also compared survival estimates from age data using annuli to commonly used estimates within the scientific community on both these populations and a population of ornate box turtles (*Terrapene ornata*) to examine the techniques utility. From 2003 to 2016, a total of 2,428 captures of 1,629 individual yellow mud turtles were obtained, and 930 captures of 637 individual ornate box turtles were collected. The annual survival estimates (62.2%-85.6%) and sex ratios varied amongst the different sites, but the growth rates were not significantly different among all of the sites for yellow mud turtles. For the ornate box turtle population, annual survival was calculated to be 72.3% using a Jolley-Seber capture-mark-recapture estimate, 79.4%

with age-structured regression, and 80.75% using a Kaplan-Meier procedure for radio telemetry data. Rainfall, climate, and nutrition in relation to growth rates did not display to have a direct influence on the varying annual survival in the different study sites for yellow mud turtles. Biased sex ratios had a slight relationship with the annual survival rates with sites estimated to have lower survival displaying these biases, but this could be influenced by weaker data sets of some of the study site. Predator abundance and permanence of aquatic habitat are hypothesized to be possible causal factors for annual survival rate differences, but must be researched in the future. The biased sex ratios at the given sites are hypothesized to be influenced by the differential mortality amongst sexes at these sights. Compared to other literature, the Matador WMA ornate box turtle population displayed a lower than average survival rate causing them to be a population of particular concern. When comparing survival estimation of annuli analysis to accepted methods within the scientific community, CMR data and annuli data estimate differences were less than 1.1% in yellow mud turtles. The ornate box turtle annual survival estimate differences were less than 1.35% between data from CMR, radio telemetry, and annuli analysis. These similarities amongst estimates argue for the utility of annuli as a good estimate of age structure for turtle species and provide significant backing for the use of annuli analysis as an estimate of survival.

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INTRODUCTION

Herpetofaunal species are arguably the most imperiled taxa in the present day and age. Over the last couple of decades, amphibians have garnered noteworthy attention amongst the scientific community over their worldwide declines (Houlahan et al. 2000, McCallum 2007). Gibbons et al. (2000) make the argument that reptile species are showing equally significant declines without receiving as much attention. Within the reptile group, turtle species have one of the highest percentages of species that are either threatened or endangered. In order to address these concerns, Gibbons et al. (2000) appeal for an increase in monitoring of reptile populations in order to gain an understanding of population statuses and potential causes for decline. The information and knowledge from these data could then be used to establish management implications, standards, and techniques for the betterment of these at risk species and populations (Gibbons et al. 2000).

The collection and estimation of vital rates is a key step to the initial assessment of a given population. An estimate of survival, particularly, can lead to an understanding of a population's status and potential causes of decline. There are numerous, varying methods that can be used to estimate survival (Silvy 2012, Skalski et al. 2005). However, most commonly used methods, such as capture-mark-recapture (CMR) and radio telemetry data estimators, are both labor and time intensive. A potential method that could cut down on labor and time is age structured regression through catch-curve analysis (Chapman and Robson 1960, Skalski et al. 2005, Robson and Chapman 1961). This method calls for the use of a population's age data to estimate survival which can be more viable given the easier availability of said data.

One simplistic way of collecting age data from a population is through the use of annuli that can be found deposited in bones, scutes, and the shells of turtles (Germano and Bury 1998, Zug 1991). Annuli are the growth rings that are laid after a cessation of growth during periods of inactivity. In turtle research, the technique's validity has been controversial. Most of the debate centers on the limited availability of data and studies for the presence of a relationship between annuli and age (Wilson et al. 2003). However, the technique has been validated for a population of Texas tortoises (*Gopherus berlandieri*) in the Rio Grande Plains of Texas (Hellgren et al. 2000). Additionally, Iverson (1991) conducted a study on the demography and life history of the yellow mud turtle (*Kinosternon flavescens*) in Nebraska using this technique and found it to be accurate with the species.

The yellow mud turtle is a semi-aquatic species that has the presence of and maintains annuli on its plastral scutes. The geographic range of the species stretches from northern Nebraska to parts of northern Mexico, and expanses from southeastern Arizona to western Illinois with the largest portion falling in Texas. With this immense range, the habitat and ecological regions vary significantly. The species prefers quiet, soft-bottomed bodies of water, but the body type can range from sloughs to creeks to cattle tanks (Ernst and Lovich 2009). The species is listed as state endangered in Illinois and listing is being considered in Iowa and Missouri (Tuma 2006). Because of a lack of literature, Iverson (1991) called for the collection of life history data on more populations throughout the range of this species to quantify variation in its demography.

Limited research on the variation in yellow mud turtle populations has left a poor understanding of the demography of the species as a whole. Therefore, there are few data to infer what affects the vital rates, specifically survival, of populations of this species and those of similar ecological niches. This ultimately leads to a lack of management implications for threatened or endangered populations. To start the process of filling these missing gaps of knowledge, my objective for this study was to characterize variation of yellow mud turtles populations within their range in Texas with a focus on estimating annual survival. Secondarily, I had the objective of comparing annuli analysis as a survival estimation technique to the more universally accepted method of estimating annual survival using capture-mark-recapture methodology.

METHODS

Study Area and Sites- Nine sites were identified for data sampling located in Texas within the yellow mud turtle's range (Figure I.1). These sites have a north-south gradient of 940 km. Across this gradient, these sites fall within 4 different ecoregions: the High Plains, the Rolling Plains, the Trans-Pecos, and the Rio Grande Plains (Figure I.2). Rita Blanca National Grasslands (NG) and Yoakum Dunes Wildlife Management Area (WMA) are located within the High Plains ecoregions. Gene Howe WMA, Sturgeon Ranch, Cross Bar Management Area (MA), Cal Farley's Boy's Ranch, and Matador WMA fall in the Rolling Plains ecoregion. Black Gap WMA is the only representative found in the Trans-Pecos ecoregion, and Chaparral WMA represented the Rio Grande Plains (Figure I.2). In addition to the habitat varying between the different ecoregions, sites within the same ecoregion exhibited variances in both vegetation and aquatic habitat types.

The High Plains ecoregion consists of approximately 8,093,000 ha comprised of mainly short and mixed-grass prairies (Correll and Johnston 1979). The grass community is primarily composed of blue grama (*Bouteloua gracilis*), black grama (*Bouteloua eriopoda*), little bluestem (*Andropogon scoparius*), side-oats grama (*Bouteloua curtipendula*), Indian grass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*), and western wheatgrass (*Agropyron smithii*). Although most of this region is open grassland, there are areas with significant encroachment of honey mesquite (*Prosopis glandulosa*), yucca (*Yucca* spp.), shinnery oak (*Quercus havardii*), and salt cedar (*Tamarix parviflora*, Correll and Johnston 1979). The vegetation community can differ significantly within the region depending on soil type and/or water availability. The region receives an average annual rainfall of 38 to 53 cm. The aquatic habitat is mainly limited to playa lakes that form after heavy periods of rain and man-made, windmill overflow ponds that come with the region's large holdings of private ranches (Correll and Johnston 1979).

The Rolling Plains ecoregion is about 9,712,000 ha of gently rolling to moderately rough topography characterized by canyons and stream valleys. Aside from the topographic difference, this region is similar to the High Plains region in its' vegetation community with the addition of big bluestem (*Andropogon gerardii*), sand dropseed (*Sporobolus cryptandrus*), and three-awn (*Aristida* spp.) as common grasses. Soils are often sandier than the High Plains, often making sand sagebrush (*Artemisia filifolia*) and shinnery oak more common. This region's average annual rainfall is 56 to 76 cm with highly variable seasonal precipitation. The aquatic habitat is also similar to that of the High Plains region with the addition of ephemeral rivers and small streams found commonly throughout the region (Correll and Johnston 1979).

The Trans-Pecos ecoregion is comprised of nearly 7,689,000 ha of arid, valleys and plateaus with a few areas of montane woodlands. The vegetative community structure varies significantly through different areas of this region. Notable habitats include: desert scrublands of creosote (*Larrea tridentata*) and tarbush (*Flourensia cernua*), grama (*Bouteloua* spp.) grasslands, yucca and juniper (*Juniperus* spp.)

savannahs, and forest of pinon pine (*Pinus* spp.) and oak (*Quercus* spp.). Precipitaion is generally less than 30 cm annually for the region, the Pecos and Rio Grande Rivers are the only notable large, aquatic habitats, and soils tend to be shallow and rocky (Correll and Johnston 1979). Man-made ponds for livestock are scattered throughout the region, but they can be ephemeral. These factors contribute to the region's sparse available aquatic habitat with vast distances between individual sites.

The Rio Grande Plains ecoregion consists of about 8,093,000 ha of level to gentle rolling thornscrub. The vegetation that dominates this scrubland includes honey mesquite, various *Acacia* species, granjeno (*Celtis pallida*), and various cacti. Soils tend to be deep red sands. The region receives 41 to 76 cm of precipitation annually, and the aquatic habitat is mainly represented by streams, natural depressions, and windmill overflow ponds or cattle tanks (Correll and Johnston 1979).

Rita Blanca NG- Rita Blanca NG contains 41,682 ha in Texas and is managed by the US Forest Service in Dallam County, Texas. It is located 43 km northwest of Dalhart, Texas, and falls within the northern part of the High Plains ecoregion. The property is composed of short-grass prairie and has sparse woody vegetation. Dominant vegetation includes blue grama, side-oats grama, burrograss (*Scleropogon brevifolius*), and buffalograss (*Buchloe dactyloides*). The property is primarily managed for the leasing of portions of the area for livestock grazing (McGee et al. 2006). The aquatic habitat of the property consists of playa lakes and windmill overflow ponds. The average annual rainfall (2010-2016) for this site is approximately 38.46 cm based on a weather station in Texline, Texas, approximately 18 km from the boundary of the property (NOAA 2017h).

Gene Howe WMA- Gene Howe WMA is a 2382 ha property in Hemphill County, Texas, that is managed by the Wildlife Division of Texas Parks and Wildlife Department (TPWD). It can be found 2 km northeast of Canadian, Texas and is located in the Canadian river portion of the Rolling Plains ecoregion. The area is divided into 2 main habitat types: the northern rolling sand hills and the southern moist lowlands. The northern part of the property is characterized by little bluestem, switchgrass, blue gramma, hairy grama (*Bouteloua hirsuta*), and sand dropseed. The lowlands are dominated by cottonwood (*Populus deltoides*), Siberian elm (*Ulmus pumila*), black locust (*Robinia pseudo-acacia*), western soapberry (*Sapindus drummondii*), and Russian olive (*Elaeagnus angustifolia*). The area is primarily managed as a research and demonstration site. The aquatic habitat is made up of windmill overflow ponds in the sand hills portion, and sloughs and natural catchments in the lowlands (Lange 2011). The average annual rainfall (2010-2016) for this site is approximately 56.95 cm based on a weather station approximately 5.5 km east of Canadian, Texas (NOAA 2017c).

Sturgeon Ranch- The Sturgeon ranch is a 3,800 ha property in Hemphill County, Texas that is privately owned and managed by Brit and Beth Sturgeon. The area is 21 km southeast of Canadian, Texas, and is similar in habitat to that of Gene Howe WMA. The exception being that the moist lowlands of the Canadian river drainage are replaced with the upper reaches of the Washita River drainage. The area is only 14 km south of Gene

Howe WMA. The property is primarily managed for livestock grazing. The aquatic habitat found on the area includes windmill overflow ponds, natural depressions, and river headwaters (personal observation). The average annual rainfall (2010-2016) for this site is approximately 56.95 cm based on a weather station approximately 21 km southeast of Canadian, Texas (NOAA 2017c).

Cross Bar MA- The Cross Bar MA is a 4,789 ha property in Potter County, Texas, that is managed by the Bureau of Land Management. The MA can be found 16 km north of Amarillo, Texas and is located in the Canadian River portion of the Rolling Plains ecoregion. The area is characterized by gently rolling, short-grass prairie dominated by blue grama, side-oats grama, and sand dropseed, and dense thickets of honey mesquite and cholla (*Cylindropuntia* spp.). The property's management focus is restoration towards to its historic state of more open, savanna-like habitats. The aquatic habitat consists of catchment ponds and West Amarillo Creek that flows north through the property into the Canadian river (Walker 2009). The average annual rainfall (2010-2016) for this site is approximately 48.06 cm based on a weather station approximately 21 km North-northwest of Amarillo, Texas (NOAA 2017a).

Cal Farley's Boy's Ranch- Cal Farley's Boy's Ranch is a 417 ha property in Oldham County, Texas that is privately owned and run by the Farley family. The area is located 15 km south-east of Channing, Texas and is similar in habitat to that of the Cross Bar MA. The exception being that the property does not have a creek present, and some catchment ponds are available on the property from a small center pivot agricultural operation. The property is run as a community for children with troubled backgrounds. The aquatic habitat is made up of stock ponds, natural depressions, and farmland runoff pools (personal observation). The average annual rainfall (2010-2016) for this site is approximately 42.57 cm based on a weather station located on the property (NOAA 2017b).

Matador WMA- Matador WMA is a 11,410 ha property in Cottle County, Texas, that is managed by the Wildlife Division of TPWD. The WMA is located 10 km north of Paducah, Texas and is found in the middle of the Rolling Plains ecoregion. The area is comprised of grass savannas and riparian corridors. The savannas contain bluestem and grama species, honey mesquite, and sand sagebrush primarily. Cottonwood, western soapberry, and hackberry (*Celtis occidentalis*) dominate the riparian areas (Spears et al. 2002). The property is managed primarily as a research and demonstration site, and secondarily for recreational hunting. That aquatic habitat of the area includes windmill overflow ponds, the Middle Pease River, stock ponds, natural depressions, and ditches. The average annual rainfall (2010-2016) for this site is approximately 54.43 cm based on a weather station approximately 11 km North-northwest of Paducah, Texas (NOAA 2017g).

Yoakum Dunes WMA- Yoakum Dunes WMA is a 13,800 ha property in Cochran County, Texas, that is managed by the Wildlife Division of TPWD. The area can be found 16 km west-southeast of Sundown, Texas and is located in the southern portion of the High Plains ecoregion. The WMA is mainly made up of sandy, rolling hills dominated by honey mesquite, sand sagebrush, and shinnery oak. The management practices focuses on improving habitat for the threatened lesser prairie chicken (*Tympanuchus pallidicinctus*). The aquatic habitat is limited to only windmill overflow ponds on small patches of clayey soils. The average annual rainfall (2010-2016) for this site is approximately 45.97 cm based on a weather station approximately 35 km southwest of Levelland, Texas (NOAA 2017e).

Black Gap WMA- Black Gap WMA is an about 41,600 ha property in Brewster County, Texas, managed by the Wildlife Division of TPWD. The WMA is located 82 km southeast of Marathon, Texas in the southern portion of the Trans-Pecos ecoregion. The area consists of arid roughlands and considerable vertical relief with sparse, diverse vegetation. The property is managed for research and demonstration purposes. The aquatic habitat is limited to large catchments referred to as tanks with long distances between each individual body of water (Axtell 1959). The average annual rainfall (2010-2016) for this site is approximately 41.00 cm based on a weather station approximately 62 km south-southeast of Marathon, Texas (NOAA 2017f).

Chaparral WMA- Chaparral WMA is 6,150 ha property in Dimmitt and La Salle County, Texas, that is managed by the Wildlife Division of TPWD. The WMA is 16 km southwest of Cotulla, Texas, and is found in the middle of the Rio Grande Plains. The property consists of honey mesquite dominated woodlands and parklands with prickly pear cactus (*Opuntia engelmannii*), tasajiilo (*Opuntia leptocaulis*), brasil (*Condalia hookeri*), spiny hackberry (*Celtis pallida*), *Acacia* spp., hogplum (*Colubrina texensis*), and Texas persimmon (*Diospyros texana*) as other common woody species. The property is managed primarily as a research and demonstration site and secondarily for recreational hunting (Burrow 2001). The aquatic habitat varies greatly within this site, but is characterized by natural catchments and windmill overflow ponds. The average annual rainfall (2010-2016) for this site is approximately 54.23 cm based on a weather station approximately 16 km southwest of Cotulla, Texas (NOAA 2017d).

Capture Gear and Protocol- In order to collect data that would be able to address the question asked of this study, my main focus in trapping efforts was to maximize captures of yellow mud turtles across all age classes. Varying types of aquatic habitat were sampled at each site using multiple traps and capture techniques in order to both maximize capture success and collection of a good representation of the property's age structure. These aquatic habitats included but were not limited to: windmill overflow ponds, playa lakes, ephemeral ponds, ditches, natural depression, and sloughs. Active and passive trapping techniques were utilized to maximize capture success as well. Sampling occurred throughout the active season of the species, which was typically May-July for most sites from 2003-2016. However, at Black Gap WMA, sampling occurred mostly in September.

All passive trap gear was set during the morning to midafternoon, left overnight, and checked within 24 hours of placement. Each trap was set with an air space left in the trap to reduce trap mortality. Rebar stakes were used to fix the trap at each location, hold each trap upright, and prevent drift into deeper water. All traps were baited with the bait being suspended in the cod end, placed loose in the trap, or positioned in the appropriate bait bag when available. Baits used throughout the course of the study included: sardines in oil, catfood, raw chicken, and raw chicken coated in buffalo sauce.

Large hoop nets- The large hoop nets (Sterling Net and Twine, Montclair, New Jersey, USA) were made up of 3 rings in a series that support 1 funnel in the middle. Each individual ring has an 88 cm diameter and the total length of the trap is 245 cm. The front of the trap has a mouth, or opening, that is 31 cm across. The square mesh size is 25 mm. These traps are the traditional "turtle trap".

Small hoop nets- The small hoop nets (Memphis Net and Twine, Memphis, Tennessee, USA) were made up of 4 rings in a series that support 2 funnels within. Each individual ring has a 47 cm diameter and the total length of the trap is 155 cm. The front of the trap has a mouth, or opening, that is 27 cm across. The square mesh size is 25 mm. These traps are specifically designed for catfish sampling.

Mini hoop nets- The mini hoop nets (Promar: Gardena, CA, USA) were made up of 2 outward facing funnels supported by a coiled, spring frame. The trap can be collapsed for storage by compressing the spring frame. The total length of the trap is 59 cm and each end ring has a diameter of 30 cm. The square mesh size is 10 mm. These traps are traditionally designed for crayfish sampling

D hoop nets- The D hoop nets (Privately made) were made up of 3 semi-circular rings with a flat bottom, making the shape of a D, in a series that support 1 slit funnel in the middle. Each individual ring has a 78 cm diameter. The trap when set is 130 cm in total length and 52 cm in height. The slit funnel opening at the front of the trap has a 54 cm length. The square mesh size is 35 mm.

Large domed collapsible traps- The large domed collapsible traps (Promar: Gardena, CA, USA) were comprised of a series of supporting arches that can collapse down from a fixed point in the center for storage purposes. When the trap is assembled there are two mouths at each of the trap that measure 15 cm across. The trap has a base of 96 cm in length and 64 cm in width. The assembled trap has a height of 61 cm. The square mesh size is 25 mm. These traps are a traditional designed to sample sea bass.

Small domed collapsible traps- The small domed collapsible traps (Promar: Gardena, CA, USA) were identical to the larger version in overall design. The mouths of the trap measure 12 cm in diameter. The trap has a base of 79 cm in length and 48 cm in width. The assembled trap has a height of 35 cm. The square mesh size is 10 mm.

Large box traps- The large box traps (Promar: Gardena, CA, USA) were similar are similar in design to the dome collapsible traps except they have square frames and horizontal slit funnels equal to the width of the trap. The base of the trap is 60 cm in width and 80 cm in length. The total height of the trap, when erect, is 28 cm. The square mesh size is 10 mm. This is a design for flounder sampling

Small box traps- The small box traps (Memphis Net and Twine, Memphis, Tennessee, USA) were identical to the larger version is overall design. The base of the trap is 43 cm in width and 59 cm in length. The total height of the trap, when erect, is 22 cm. The square mesh size is 12 mm.

Large modified fyke nets- The large fyke nets (Christiansen's Nets, Duluth, Minnesota, USA) were made up of a net fence that is supported by weighted bottom and a series of floats leading to the trap. The trap consists of 2 rectangle frames that have a slit funnel within followed by a series of 5 rings containing 3 regular funnels. The net fence is 14.5 m long and 88 cm in height. The total length of the trap section is 4.5 m. The rectangular frames are 88 cm in height and 120 cm in width. The square mesh size is 10 mm.

Small modified fyke nets- The small fyke nets (Christiansen's Nets, Duluth, Minnesota, USA) had the same design as the larger version with the only difference being that they have 4 rings and 2 regular funnels. The net fence is 7.4 m long and 67 cm in height. The total length of the trap section is 3.3 m. The rectangular frames are 67 cm in height and 95 cm in width. The square mesh size is 10 mm.

Active trapping was utilized whenever the opportunity presented itself, therefor time of day varied. A bag seine (Sterling Net and Twine, Montclair, New Jersey, USA) was used in areas where passive traps could not be set, there was little obstruction, and/or the water was shallow. The bag seine used was 9.1 m with a square mesh size of 2.5 mm. Fortuitous encounters often occurred when either setting or checking trap gear, and the individuals were caught by hand. In some instances where high densities of individuals were observed, sifting through mud/unclear water with one's hands and feet was utilized. Spotlights were also used in order to locate and capture individual by hand during the night in areas where the water was clear. In some instances, individual mortalities resulting from depredation were found and available data were collected.

Marking and Measuring- Each individual that was captured was marked, measured, weighed, and sexed. Either a passive integrated transponder (PIT) tag accompanied by a 2R (second marginal scute on the right side of the shell) notch cohort mark, or an individual notch code on the marginal scutes was used to mark each turtle (Cagle 1939). PIT tags were typically used on populations that will be sampled in the foreseeable future and notch codes were used on more temporary study populations because of the duration of each marking technique. The PIT tags were inserted by a syringe through the rear leg opening into the abdomen and the incision point was sealed with super glue. Individual notch codes were administered by using a Dremel tool (Robert Bosch Tool Corporation, Mount Prospect, Illinois, USA) with a cutoff wheel. The straight-line carapace length (SCL) was measured with a set of dial calipers and an electronic balance was used to collect mass in grams (g). Sex was determined by the presence of longer tails in males once sexual maturity is reached (Ernst and Lovich 2009).

Annuli Analysis- In order to collect age data from each individual, I used plastral scute annuli to age each turtle captured. An impression was made of each individual's plastron in order to estimate age while providing a permanent data source (Galbraith and Brooks 1987). This process began by the cleaning of each turtle's plastron with a sponge to clear the surface from any substance that could alter the mold. Dental alginate

(Matech Inc., Sylmar, California, USA) was then mixed and applied to the plastron of the individual. Once dry, the alginate negative mold was wrapped in a wet paper towel and stored in an individually labeled zip lock bag to keep the impression from distorting by drying out. At a later date, these impressions were removed from their bags and all excess alginate is cut away from the plastron. The alginate mold is then placed up on a tray and a basin is formed around it using molding clay. The mold is then filled with a dental stone (GC America, Alsip, Illinois, USA) and left to dry creating a permanent, positive impression of the turtle's plastron. The impressions were analyzed under light and magnification in a blind fashion to reduce bias when counting the annuli (Ewing, 1939, Galbraith and Brooks 1987, Germano and Bury 1998). All 8 plastral scutes were analyzed and the mode of all counts were taken to estimate the age for each given individual (Zug 1991). Field scute analysis was utilized in a few instances when dental alginate was not available.

Analysis- *Annual Survival*- Both age structured regression and capture-mark-recapture were used to estimate annual survival when possible for both sites. The age structured regression technique begins by compiling age data into an age structure histogram for each site by plotting number of annuli versus frequency. When more than one impression existed for each individual, I randomized which impression was included in the analysis so that each individual was only represented once. The frequency of each histogram is then natural logged, and a linear regression was used to calculate the slope of the line. The slope of the line was then anti-logged to derive the annual survival estimate. Age classes that were poorly represented were removed from this analysis in order to reduce bias (Chapman and Robson 1960, Kazmaier et al. 2001, Skalski et al. 2005, Robson and Chapman 1961). For sites that were sampled over 3 or more years, I used a Jolley-Seber open population capture-mark-recapture model to estimate survival in Program Mark (White and Burnham 1999). For this analysis, I computed all possible survival models for each site and chose the model with lowest AIC as the best fit.

Yearly and Daily Growth Rate- Yearly growth rate was calculated from sites where individuals were recaptured in more than one year. The SCL and mass between initial and recapture measurements were divided by years between captures to calculate this rate. Daily growth rate was calculated from sites where individuals were recaptured within the same year. The SCL and mass between initial and recapture were divided by the days between captures to calculate this rate. When individuals were recaptured more than once, only the first and last captures were used to maintain independence and maximize the growth period for analysis. Both daily and annual growth rates were compared between sites and sexes using an analysis of variance.

Adult Sex Ratio- Total numbers of all sexable individuals, excluding recaptures, for each sex at each site were totaled. These totals were then used in chi-square analyses to determine if their adult sex ratios differed for 1:1. In order to reduce bias from juveniles classified incorrectly, a questionable status was given to any individual for which sex was not 100% certain. After all data had been collected, the smallest individual that had a 100% certainty of being identified as a male was used as the breakpoint from juvenile to adult, and any individual smaller than the breakpoint was considered unsexable (Kazmaier et al. 2001).

RESULTS

Yellow mud turtle populations differed in variation amongst different sites beyond measured variables. The active seasons for each population varied between each location. A majority of the sites had active seasons from late April to July with capture success dropping significantly in the later months. Yoakum Dunes WMA and Black Gap WMA, however, displaying actives seasons much later than the others. Yoakum Dunes WMA still had high capture success into the month of August, and Black Gap WMA still had significant capture success well into the month of September. Data were collected from a total of 2,428 captures of 1,629 individual turtles throughout this study. Collection occurred, depending on site, from 2003 to 2016 (Table I.1).

Survival- For estimating annual survival through age structured regression, 8 of the study sites had appropriate annuli data for analysis, excluding the Chaparral WMA. Ages were estimated for 1,561 individuals. I was able to assign ages 0-14 to the individuals captured. The age 0 assignment was given to the individuals that exhibited no annuli which was a result of said individual having hatched after the latest winter. The age range for which I was able to use for survival estimation varied from site to site depending on the structure of the age classes (Figures I.3-I.10). Annual survival varied across the different study sites , range from 62.2% to 85.6% (Table I.2). Estimations were 65.2% (r^2 = 0.919) for Rita Blanca NG (Figure I.11), 79.5% (r^2 = 0.981) for Gene

Howe WMA (Figure I.12), 69.6% (r^2 = 0.859) for Sturgeon Ranch (Figure I.13), 78.3% (r^2 = 0.645) for Boy's Ranch (Figure I.14), 63.4% (r^2 = 0..811) for Cross Bar MA (Figure I.15), 75.5% (r^2 = 0.858) for Matador WMA (Figure I.16), 62.2% (r^2 = 0.818) for Yoakum Dunes WMA (Figure I.17), and 85.6% (r^2 = 0.839) for Black Gap WMA (Figure I.18).

At 3 of the study sites, capture data were appropriate for a survival estimate through capture-mark-recapture analysis. In all cases, the model with the lowest AIC was a model with constant survival over the sampling period. At Gene Howe WMA, the annual survival was estimated to be 79.7% (SE = 0.031). The population at Matador WMA was estimated to have a 75.1% (SE = 0.034) annual survival rate. The Black Gap WMA population was estimated to have an annual survival rate of 86.7% (SE = 0.034, Table I.4).

Yearly and Daily Growth Rates- For annual growth rate estimates, only Gene Howe WMA, Matador WMA, and Black Gap WMA had the appropriate data for analysis. These 3 sites with the addition of Rita Blanca NG and Sturgeon Ranch had data appropriate for estimation of daily growth rates. Daily growth rates based on both carapace length (F_4 = 1.508, P = 0.200) and mass (F_4 = 0.690, P = 0.599) did not differ amongst the sites tested. Annual growth rate based on mass did not differ amongst sites (F_2 = 0.448, P = 0.639), but annual growth rate based on carapace length was lower at Black Gap WMA than the other two sites (F_2 = 8.136, P = <0.001; Table I.5). Similarly, daily growth rates based on both carapace length (F_4 = 1.414 , P = 0.229) and mass (F_4 =

0.237, $P = 0.917$) did not differ amongst the different sexes at each site. Annual growth rate based on mass did not differ amongst sexes through the sites ($F_2 = 1.846$, $P = 0.160$), but annual growth rate based on carapace length was lower in Black Gap WMA males than males of the other two sites ($F_2 = 3.974$, $P = 0.020$). The females amongst the sites did not differ from one another (Table I.6).

Adult Sex Ratios- Sex ratios were able to be calculated for all 9 of the sites from the study. The sex ratios from Sturgeon Ranch ($X^2_1 = 0.08$), Black Gap WMA ($X^2_1 = 0.11$), Chaparral WMA ($X^2_1 = 1.20$), and Gene Howe WMA ($X^2_1 = 0.47$) did not differ significantly from a 1:1 (F:M) adult sex ratios ($p > 0.273$). The sex ratios were calculated to be significantly different from 1:1 at Cross Bar MA ($X^2_1 = 11.26$) and Boy's Ranch ($X^2_1 = 8.32$) which were male biased, and Rita Blanca ($X^2_1 = 13.59$) and Yoakum Dunes WMA ($X^2_1 = 6.95$, $p < 0.008$) which were female biased. The sex ratio for Matador WMA is approaching a significant difference from 1:1 ($X^2_1 = 2.74$, $p = 0.098$, Table I.6).

DISCUSSION

These results help begin to shed light on the variation and similarities amongst different yellow mud turtles populations, even those of close proximity. They also present data that could be used as supporting evidence for the use of annuli analysis and age-structured regression as an annual survival estimation technique.

Survival- In my study, the annual survival rates from age data varied in an unpredictable pattern across the gradient of my study sites. In the case of Sturgeon Ranch and Gene Howe WMA, even sites within close proximity of each other had significantly varying annual survival rates. This leads me to believe that this species does not have a uniform annual survival rate, but instead, survival is dependent upon local factors. Other studies' data hint towards these conclusions with populations having varying survival rates (Iverson 1991, Lange 2011). These varying survival rates did not follow a gradient indicating to an absence of a clinal climate effect. When compared to the average annual rainfall for each site, the survival rates do not follow that gradient either. Even with the removal of sites with less trapping effort, the results still reflect the same outcome. This leads me to the assumption that neither clinal trends in climate nor average annual rainfall have a significant effect of the annual survival of yellow mud turtle populations.

A comparison to the calculated sex ratios insinuates a possible, slight relationship with survival. The sites with the 3 lowest survival rates (Cross Bar MA, Rita Blanca NG, and Yoakum Dunes WMA) have bias sex ratios, and the sites with the two highest survival rates (Black Gap WMA and Gene Howe) display a ratio that does not differ from 1:1 (Table I.6). This could be attributed to the increased movement of individuals within a population without an even sex ratio in order to increase reproduction odds. It is not uncommon for yellow mud turtles to travel a large distance, and Mahmoud (1969) found yellow mud turtles travel further distances than species of similar ecology.

This movement could potentially lead to increased mortality because of exposure to the elements and predation events which could contribute toward lower annual survival. Unequal sex ratios also reduce the effective population size, because the rarer gender's genes are disproportionally contributed to the next generation (Silvy 2012, Wright 1931). With the removal of problem sites, the relationship between sex ratio and annual survival lessens yet still remains with less convincing results.

The relationship between growth rates comparisons and nutrition are complex because of the variation in both individuals and populations (Dunham and Gibbons 1990). However, I hypothesize that nutrition does not have a significant effect on annual survival, because of the similar growth rates amongst sites and the varying survival estimates. Even though my study did not indicate them, more factors that directly affect the survival rates of this species' survival rate may exist.

Further research must be carried out in order to determine the causal factors that leads to these varying annual survival rates amongst populations of yellow mud turtles if their presence holds. Through observations in my research, I have 2 hypotheses that I believe may be the causal factors for this variation and should be researched going forward. First, I would consider attempting to develop an estimate for predator abundance. After examining the different annual survival estimates for the sites I noticed that sites with low survival were those that displayed high predation and vice versa for those with high survival. For example, numerous predated individuals were found at Yoakum Dunes WMA (annual survival = 62.2%) while turtle predators are likely present in fewer numbers because of aridity at Black Gap WMA (annual survival = 85.6%). The development and collection of a predator abundance index on populations of known predators of yellow mud turtles could provide some insight on a causal factor to these varying survival rates. Secondly, I believe that permanence of bodies of water that the populations in habitat could be influencing these annual survival rates. The aquatic habitat of the populations with high survival rate, such as Black Gap WMA, was observed to retain water for a longer period while other sites that were estimated to have lower survival rates displayed more ephemeral aquatic habitat. Therefore, I believe that the creation of a measurable estimate of aquatic habitat permanence or hydroperiod may also lead to an understanding of the causal factors behind these varying survival rates.

The fact that CMR is such a widely accepted estimator for survival and the standard errors of these estimates all being less than 0.034 give backing to the validity of this technique (Table I.4). Following the assumption that the technique requires a true representation of the population of interest, these close similarities between age structured regression and CMR lead me to believe that annuli can be a good measurement of age (Chapman and Robson 1960, Skalski et al. 2005, Robson and Chapman 1961, Hellgren et al. 2000). If nothing else, these similarities provide a strong backing for the use of annuli and age structured regression for annual survival estimation purposes.

With the results of this study, it is important to address the inherent issues that can possible come from the data collected that may introduce bias. The age structure regression technique hinges on the assumption that the data collected is a true representation of the natural population (Chapman and Robson 1960). Therefore, some of the study sites from this data set that have few years of sampling could have differing estimates from true survival in the wild (Table I.1). The data sets collected also has some underrepresentation of younger age classes is some of the sites. This problem is commonly found in research because of the difficulty in trapping juveniles, but could be attributed to limited trapping efforts, or time of trapping effort (Skalski et al. 2005). The presence of a peak in the older age classes of the age structures of Black Gap WMA and somewhat in Cross Bar MA are also present. These peaks can be explained by the fact that as turtles begin to slow growth as they age laying annuli closer together causing them to increase in aging difficulty (Zug 1991). These 2 factors restrict our ability to estimate survival for these underrepresented age classes and has the capability of possible skewing our estimates. These issues will be considered with our interpretation of the study's data. This can be addressed in the future continuation of this study with more trapping efforts and focused trapping.

Growth Rates- There is little literature on the growth rates on yellow mud turtles that exists. The estimates from this research suggest a uniform growth rate for the species across the wide range of habitat in the areas within this study. The only statistically significant difference amongst the different calculations of growth rate was found in the annual growth based on carapace length for both site and sex (Table I.5, Table I.6). The annual growth rate based on carapace length for Black Gap WMA is statically different than that of the other site addressed in these estimations. Looking at the site's age distribution histogram, the population displays the presence of a large proportion of older individuals within the population as opposed to that of the other sites (Figure I.10). This observation combined with the fact that turtles significantly decrease growth as they increase in age leads me to believe that Black Gap WMA's annual growth rate based on carapace length in skewed lower than its actual rate (Ernst and Lovich 2009, Zug 1991). Therefore, I believe the true growth rates of the different populations within this study do not differ from one another. This differs from much of the literature which describes turtle species in the south of having higher growth rates than that of northern populations especially considering the 940 km north-south gradient of this study (Ernst and Lovich 2009). This notion also leads me to believe that the nutritional status of these populations is similar amongst sites.

Sex Ratios- In my study, roughly half of my sites had sex ratios that displayed a difference from 1:1 (Table I.6). This differs from the literature which supports that yellow mud turtle populations maintain a near 1:1 adult sex ratio (Lange 2011, Mahmoud 1969). It is important to consider factors that may influence the perception of a turtle population's sex ratios as opposed to its actual sex ratio. A common factor that can influence the perception of sex ratios within this type of research is sampling bias (Gibbons 1990). I attempted to address this bias through multiple collection methods and different trap types. However, my study did not avoid the possibility of influence by sampling time of year or of season in which we trapped each site (Gibbons 1990). Mitigation of this bias was attempted through multiple collection efforts throughout the active seasons and over multiple years, but was not achieved for all study sites. Sites that displayed this mitigation (Black Gap WMA, Gene Howe WMA, Matador WMA, and Chaparral WMA) all exhibited sex ratios near 1:1 (Table I.1, Table I.6). Therefore, there is a possibility that these unbalanced sex ratios could be influenced by trapping period effort or an unseen collection method bias.

True sex ratios differing from 1:1 can result from skewed primary sex ratios, differential mortality, differential immigration and emigration, and differential age at maturity (Lovich and Gibbons 1990). Shine and Iverson (1995) state that turtles, as a general group, reach maturity when they meet 72% of their maximum body size. Because the growth rates estimated between sexes at each site do not differ, I hypothesize that differential age at maturity is not a likely cause of the skewed sex ratios (Table I.6). When the survival between sexes at each site is compared to the sex ratio of the same site, you can argue that the differential mortality could be a causing factor to the sex ratios differing from 1:1.

In conclusion, I believe the results of this research give us some knowledge that we can use towards management of yellow mud turtles and species of similar ecology. I hypothesize that annual survival will vary from population to population, and further research must be conducted in order to determine the causal factors. However, I believe this variation makes an argument for targeted sampling in order to clarify population statuses. The use of annuli analysis and age-structured regression have demostrated to aide in this short term, targeted sampling possibly assisting in this management. I also hypothesize that managing a yellow mud turtle populations' sex ratio could improve annual survival.

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Table I.1. Total number of captures and individuals of yellow mud turtles sampled from 9 sites in Texas with the years each site was sampled.

			Years Sampled				
Site	Captures	Individuals	2003	2004	2006	2007	2008
Gene Howe WMA	767	328				X	X
Black Gap WMA	496	405				X	
Matador WMA	356	224			X	X	X
Rita Blanca NG	291	211					
Yoakum Dunes WMA	155	140					
Sturgeon Ranch	146	133					
Chaparral WMA	80	68	X	X			
C.F. Boys Ranch	74	63					
Cross Bar MA	63	57				X	
Total	2,401	1,629					

Table I.1. Cont. Total number of captures and individuals (in parentheses) of yellow mud turtles sampled from 9 sites in Texas with the years each site was sampled.

	Years Sampled							
Site	2009	2010	2011	2012	2013	2014	2015	2016
Gene Howe WMA	X	X	X	X	X	X		X
Black Gap WMA		X					X	X
Matador WMA	X	X		X	X	X	X	X
Rita Blanca NG								X
Yoakum Dunes WMA							X	X
Sturgeon Ranch								X
Chaparral WMA		X	X	X	X	X	X	
C.F. Boys Ranch							X	X
Cross Bar MA							X	X

Table I.2. Age range, r^2 of regression lines, and annual survival derived from age structured regression for yellow mud turtles from 8 study sites in Texas from 2003-2016.

Site	Age Range	r^2	Annual Survival
Rita Blanca NG	6-14	0.919	65.2%
Gene Howe WMA	4-12	0.981	79.5%
Sturgeon Ranch	4-10	0.859	69.6%
Boy's Ranch	4-11	0.645	78.3%
Cross Bar MA	5-10	0.811	63.4%
Matador WMA	4-11	0.858	75.5%
Yoakum Dunes WMA	6-13	0.818	62.2%
Black Gap WMA	4-9	0.839	85.6%

Table I.3. Survival comparison between age structure regression and CMR methods for yellow mud turtles from 2003 - 2016.

Site	Age Structured Regression Survival	Jolley-Seber CMR	
		Survival	SE
Gene Howe WMA	79.5%	79.7%	0.031
Black Gap WMA	85.6%	86.7%	0.034
Matador WMA	75.5%	75.1%	0.034

Table I.4. Annual and daily growth rates of yellow mud turtles across 5 sites in Texas, within a row means followed by the same letter were not significantly different (α = 0.05).

Factor	Period	Matador WMA	Gene Howe WMA	Black Gap WMA	Rita Blanca NG	Sturgeon Ranch	P Value
Carapace Length (mm)	Annual	4.322 A	4.703 A	2.251 A	-	-	< 0.001
	Daily	0.066 A	0.066 A	0.044 A	-0.009 A	0.012 A	0.200
Mass (g)	Annual	17.805 A	17.097 A	15.001 A	-	-	0.639
	Daily	0.228 A	0.468 A	0.504 A	0.262 A	-0.455 A	0.599

Table I.5. Annual and daily growth rates for each sex at 5 sites in Texas, within a row means followed by the same letter were not significantly different ($\alpha = 0.05$).

		Matador WMA		Gene Howe WMA	
		Male	Female	Male	Female
Carapace Length (mm)	Annual	5.507 AB	3.137 BC	5.711 A	3.694 ABC
	Daily	0.097 A	0.034 A	0.073 A	0.060 A
Mass (g)	Annual	21.490 A	14.199 A	21.278 A	12.917 A
	Daily	0.304 A	0.153 A	0.605 A	0.331 A

Table I.5. Cont. Annual and daily growth rates for each sex at 5 sites in Texas, within a row means followed by the same letter were not significantly different ($\alpha = 0.05$).

Black Gap WMA		Rita Blanca NG		Sturgeon Ramch		P Value
Male	Female	Male	Female	Male	Female	
1.669 C	2.832 BC	-	-	-	-	0.020
0.073 A	0.015 A	-0.066 A	0.049 A	0.023 A	0.001 A	0.229
14.207 A	15.795 A	-	-	-	-	0.160
0.209 A	0.799 A	0.212 A	0.313 A	-0.407 A	-0.504 A	0.917

Table I.6. Sex ratios of yellow muds turtles from 9 sites in Texas, 2003 – 2016.

Site	Female	Male	F:M	Chi-square	P
Sturgeon Ranch	55	58	1:1.05	0.08	0.778
Black Gap WMA	156	162	1:1.04	0.11	0.737
Cross Bar MA	12	35	1:2.92	11.26	0.001
Boy's Ranch	16	37	1:2.31	8.32	0.004
Chaparral WMA	12	18	1:1.50	1.20	0.273
Gene Howe WMA	135	124	1:0.92	0.47	0.494
Matador WMA	91	70	1:0.77	2.74	0.098
Rita Blanca NG	97	52	1:0.53	13.59	<0.001
Yoakum Dunes WMA	75	46	1:0.61	6.95	0.008

Figure I.1. Study sites used to explore demography in yellow mud turtle populations (2003 – 2016) and the yellow mud turtles geographic range in Texas (shaded area): Rita Blanca NG(1), Gene Howe WMA (2), Sturgeon Ranch (3), Boy's Ranch (4), Cross Bar MA (5), Matador WMA (6), Yoakum Dunes WMA (7), Black Gap WMA (8), Chaparral WMA (9).

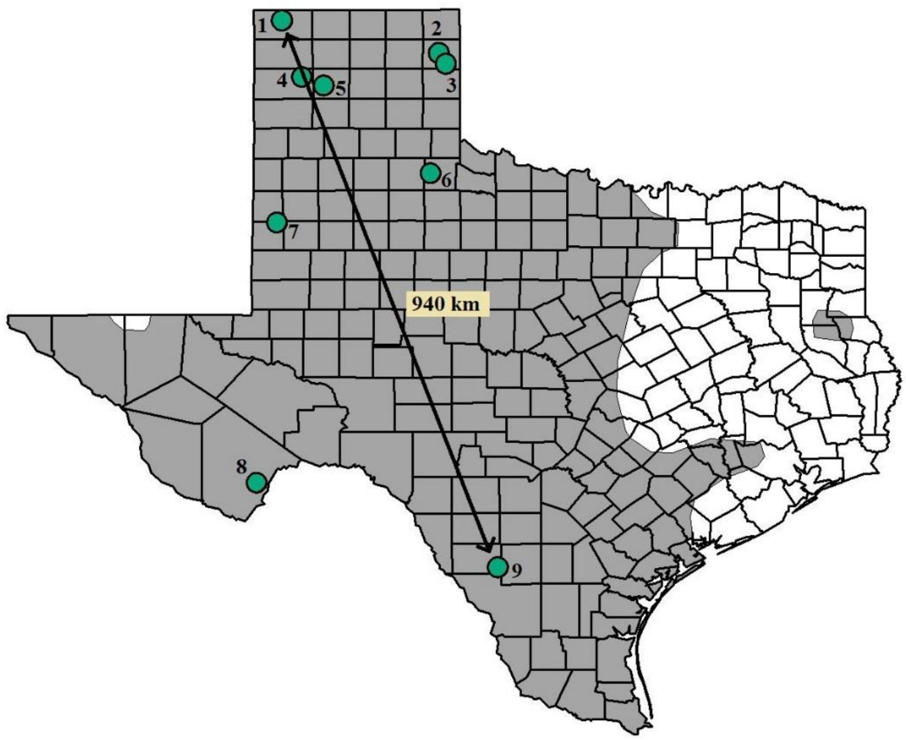


Figure I.2. Study sites used to explore demography in yellow mud turtle populations and the ecoregions of Texas: Rita Blanca NG (1), Gene Howe WMA (2), Sturgeon Ranch (3), Boy's Ranch (4), Cross Bar MA (5), Matador WMA (6), Yoakum Dunes WMA (7), Black Gap WMA (8), Chaparral WMA (9).

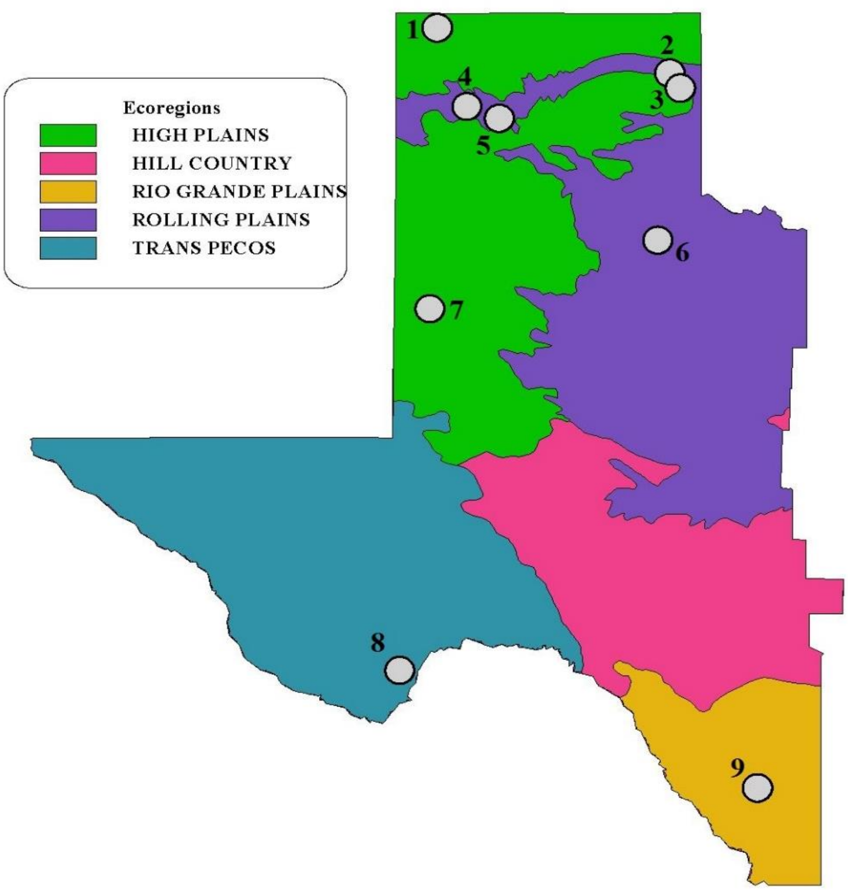


Figure I.3. Age distribution of yellow mud turtles at Rita Blanca National Grasslands in Dallam County, Texas, in 2016 (n = 211).

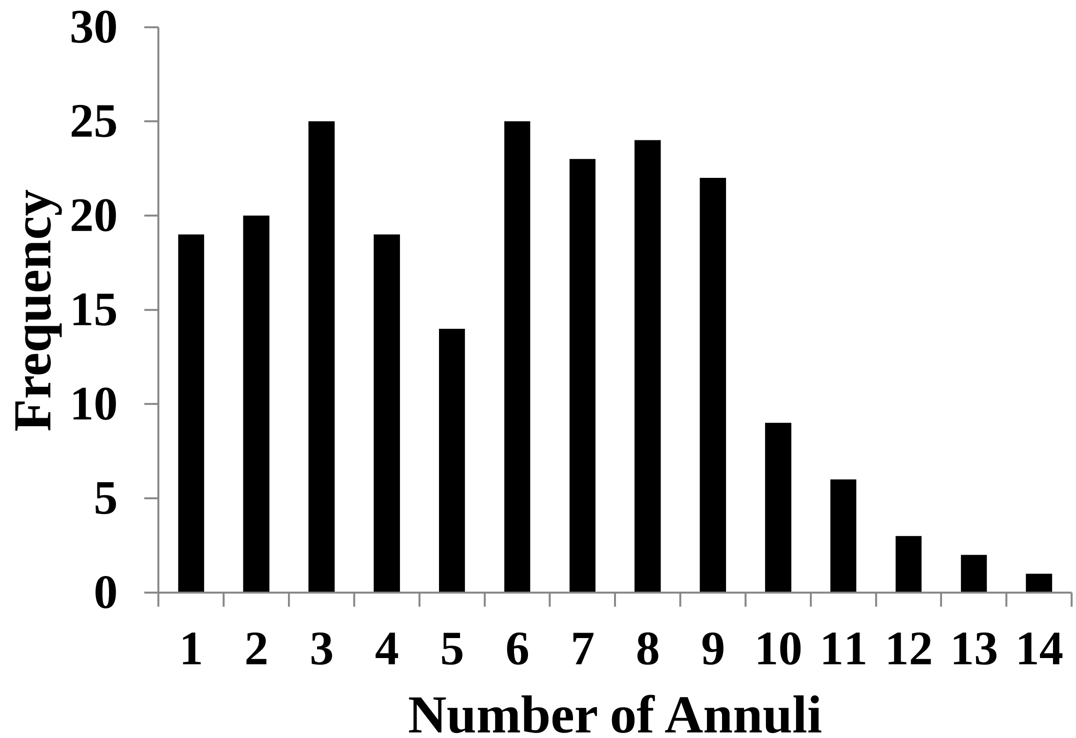


Figure I.4. Age distribution of yellow mud turtles at Gene Howe Wildlife Management Area in Hemphill County, Texas, from 2007-2016 (n = 328).

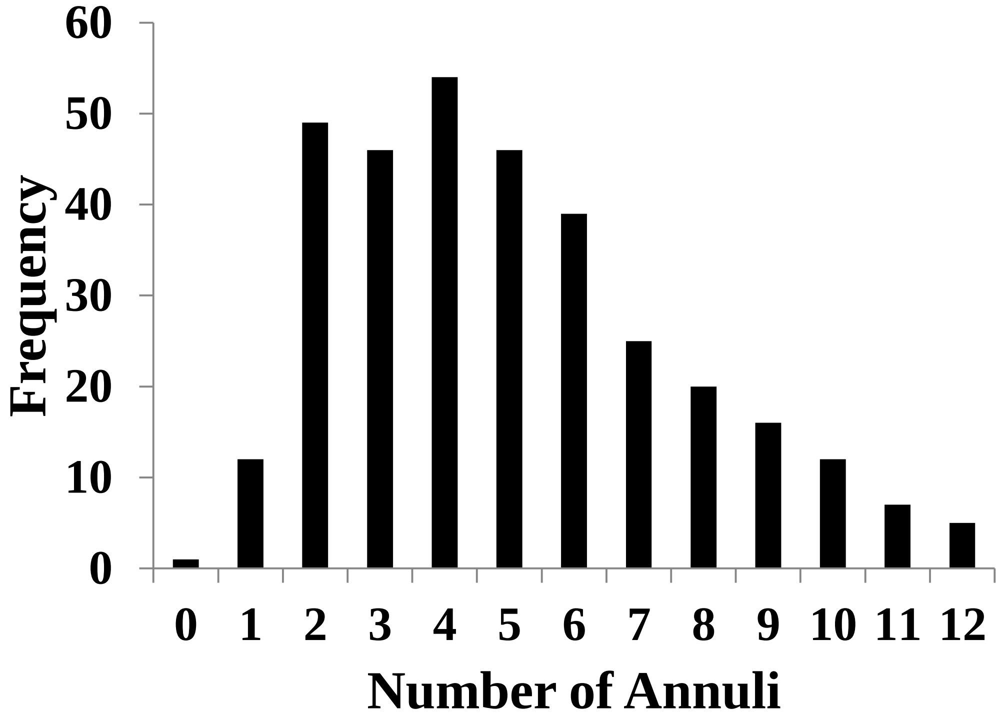


Figure I.5. Age distribution of yellow mud turtles at Sturgeon Ranch in Hemphill County, Texas, in 2016 (n = 133).

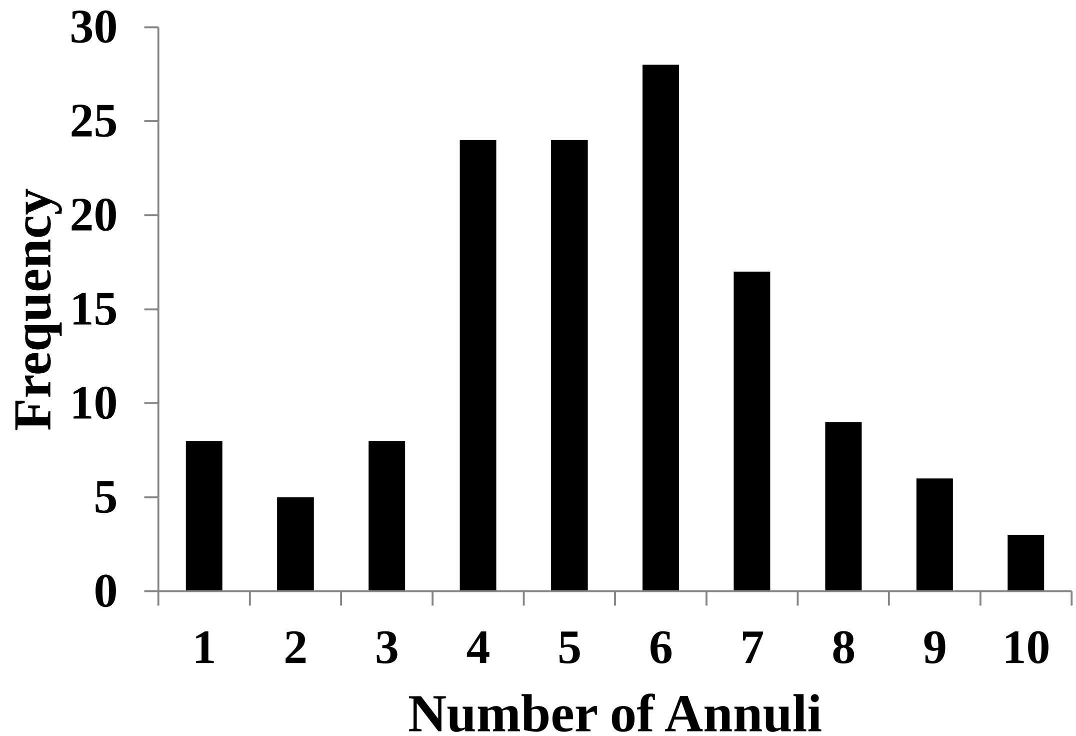


Figure I.6. Age distribution of yellow mud turtles at Cal Farley's Boy's Ranch in Oldham County, Texas, from 2015-2016 (n = 63).

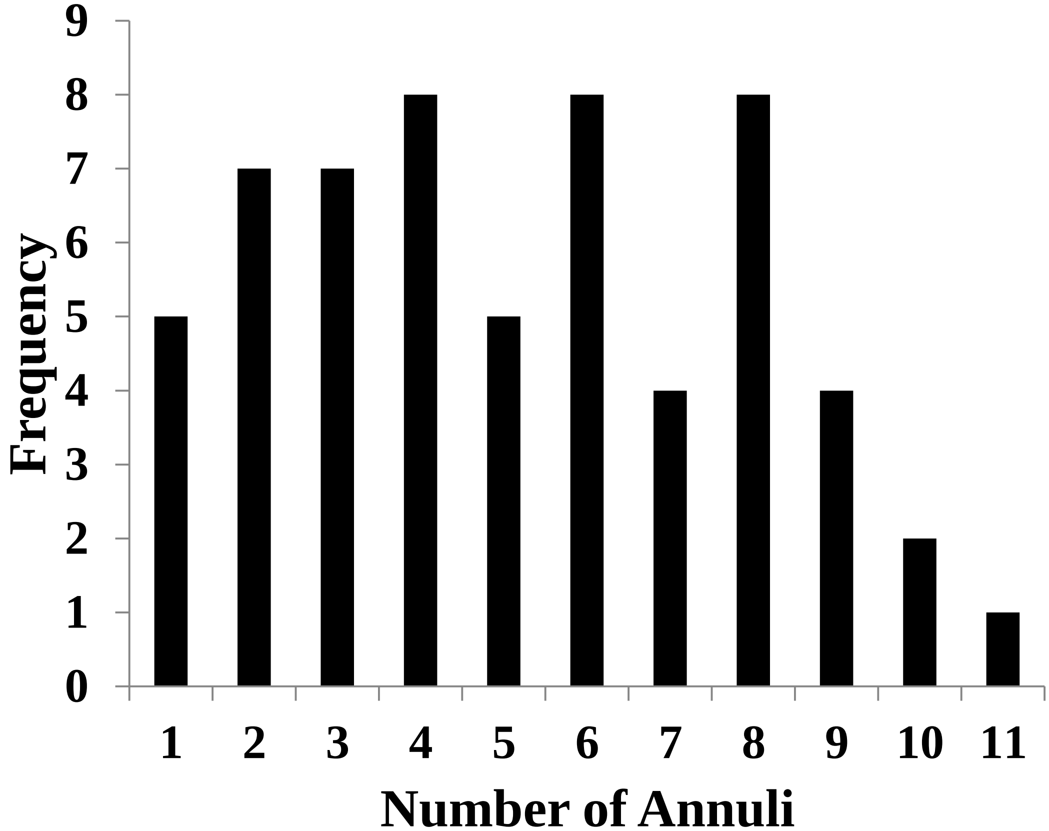


Figure I.7. Age distribution of yellow mud turtles at Cross Bar Management Area in Potter County, Texas, in 2007, 2015, and 2016 (n= 57).

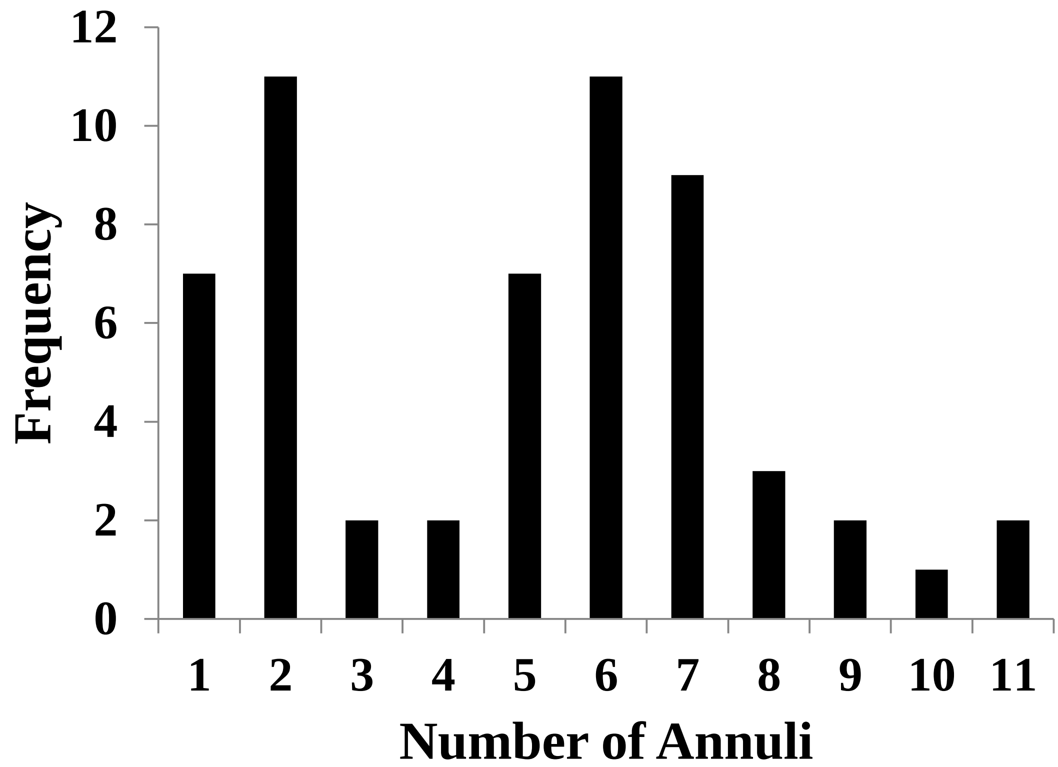


Figure I.8. Age distribution of yellow mud turtles at Matador Wildlife Management Area in Cottle County, Texas, in 2006-2016 (n = 224).

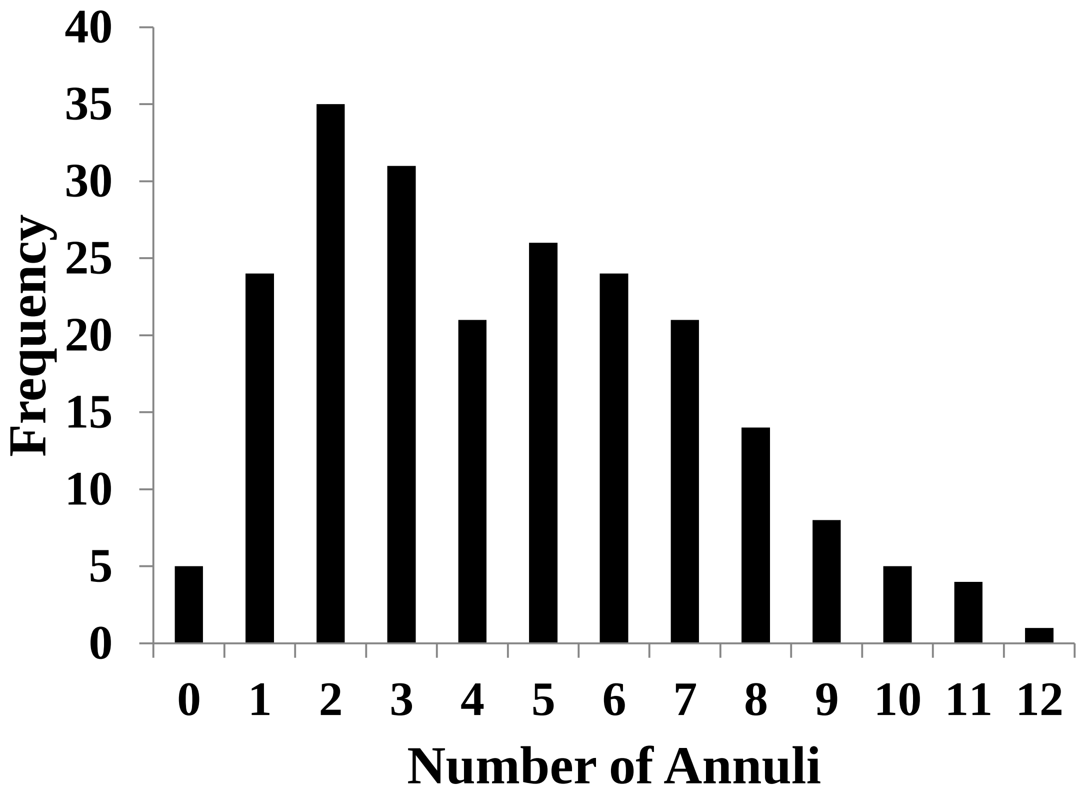


Figure I.9. Age distribution of yellow mud turtles at Yoakum Dunes Wildlife Management Area in Cochran Coutny, Texa, in 2015 and 2016 (n = 140).

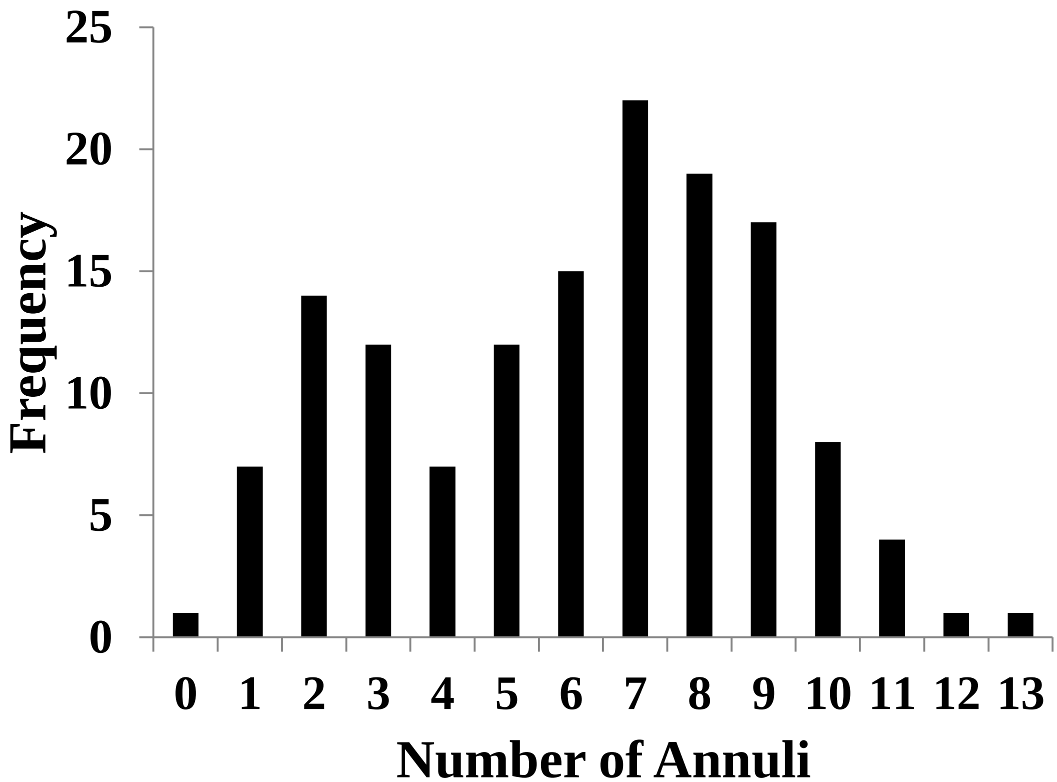


Figure I.10. Age distribution of yellow mud turtles at Black Gap Wildlife Management Area in Brewster County, Texas, in 2007, 2010, 2015, and 2016 (n = 405).

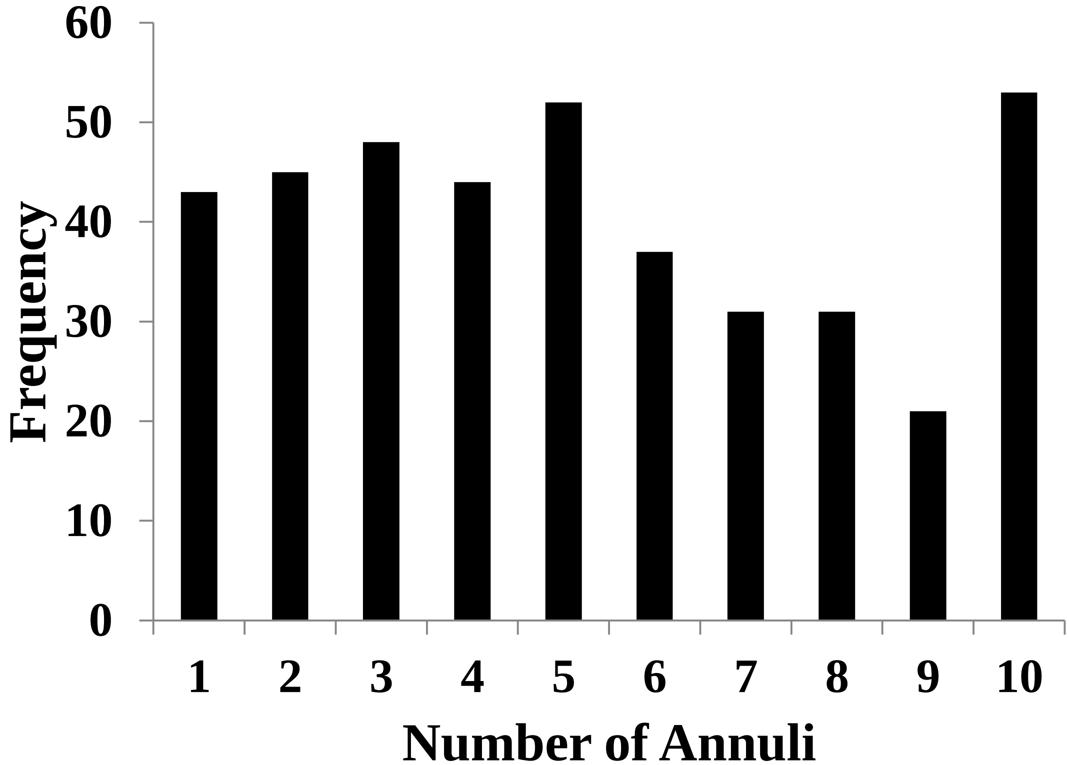


Figure I.11. Age structured regression Age of yellow mud turtles at Rita Blanca National Grasslands in Dallam County, Texas, in 2016 (n = 211).

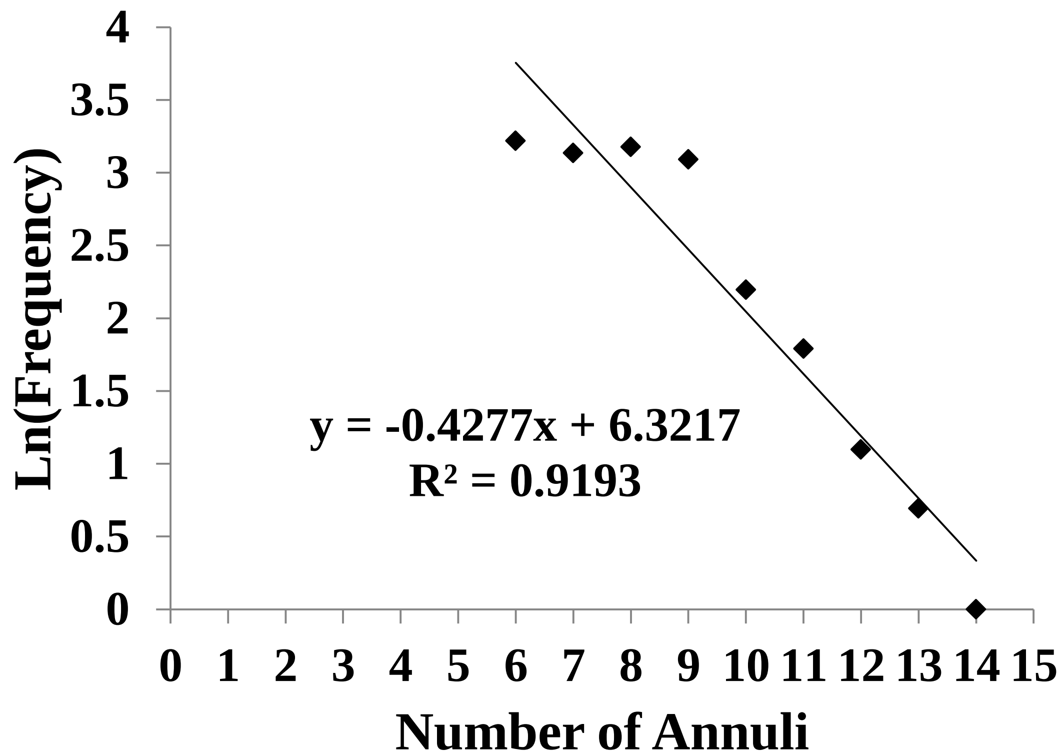


Figure I.12. Age structured regression of yellow mud turtles at Gene Howe Wildlife Management Area in Hemphill County, Texas, from 2007-2016 (n = 328).

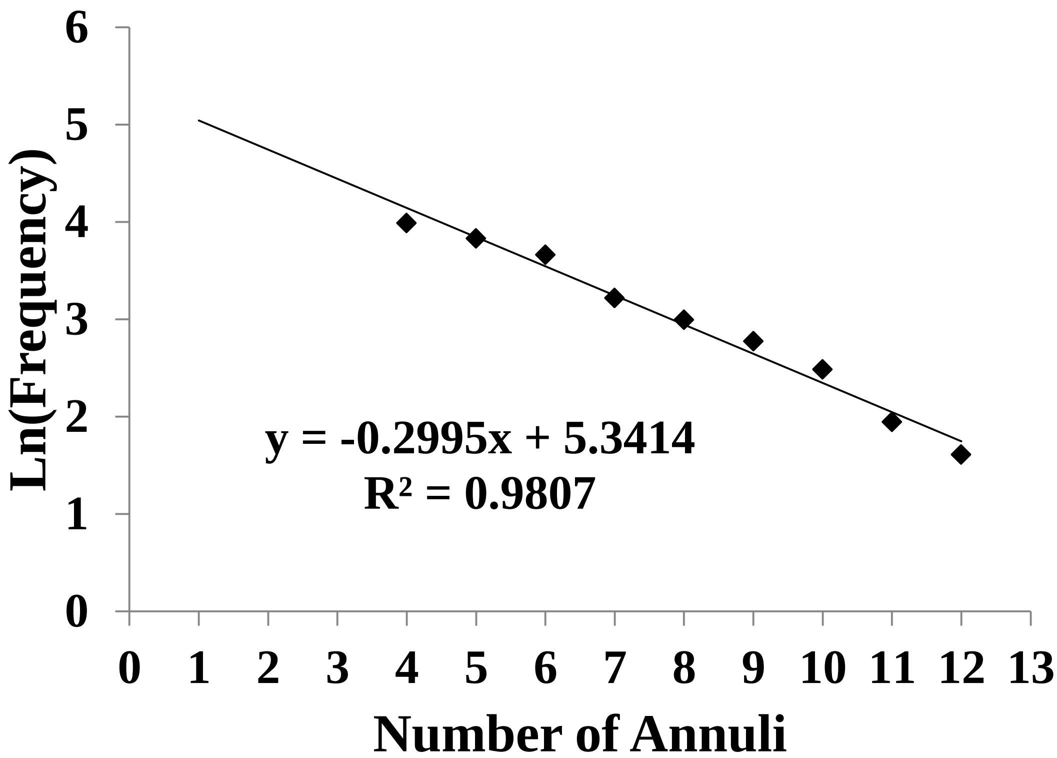


Figure I.13. Age structured regression of yellow mud turtles at Sturgeon Ranch in Hemphill County, Texas, in 2016 (n = 133).

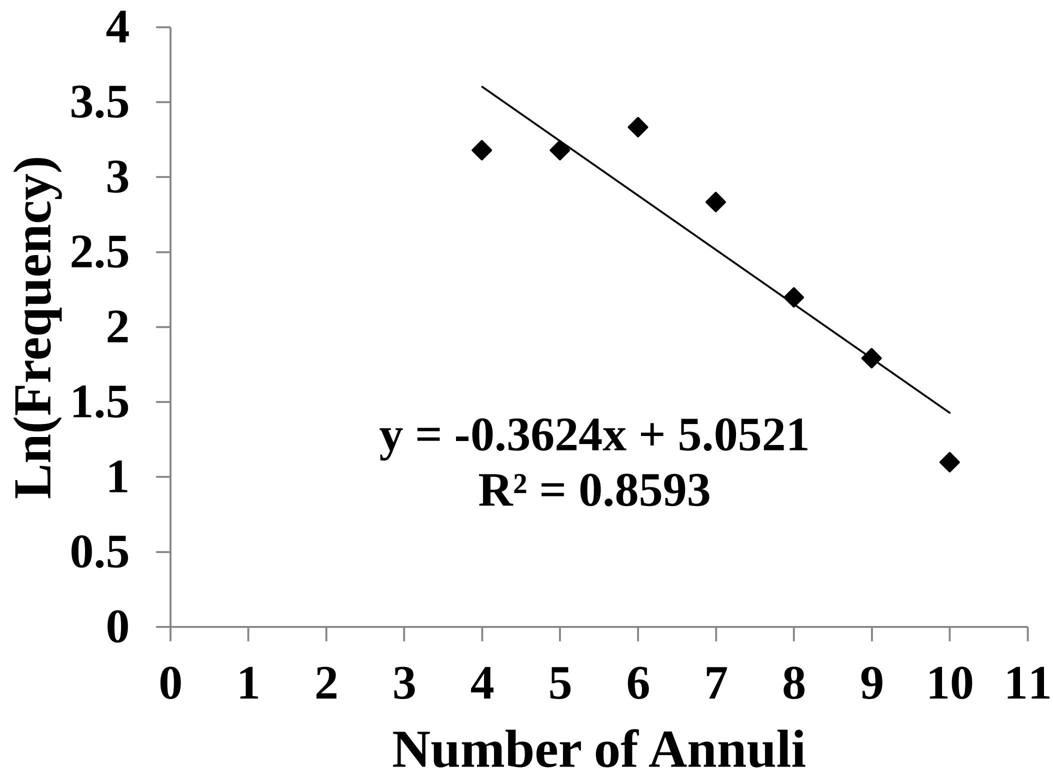


Figure I.14. Age structured regression of yellow mud turtles at Cal Farley's Boy's Ranch in Oldham County, Texas, from 2015-2016 (n = 63).

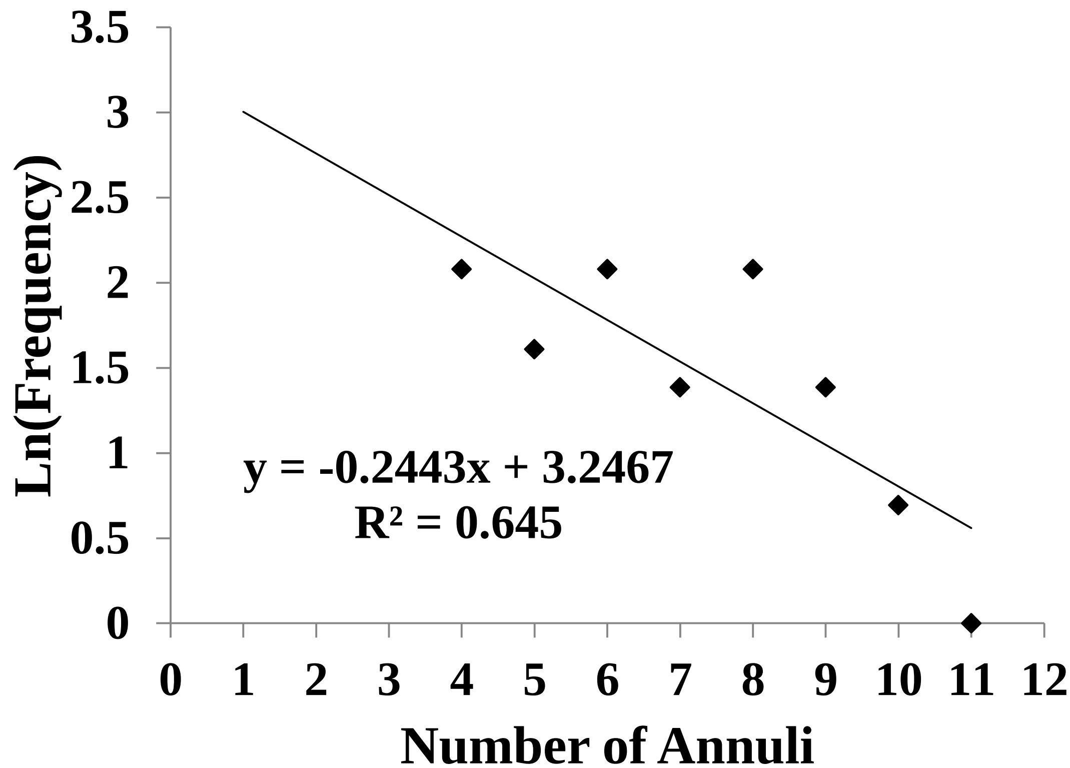


Figure I.15. Age structured regression of yellow mud turtles at Cross Bar Management Area in Potter County, Texas, in 2007, 2015, and 2016 (n= 57).

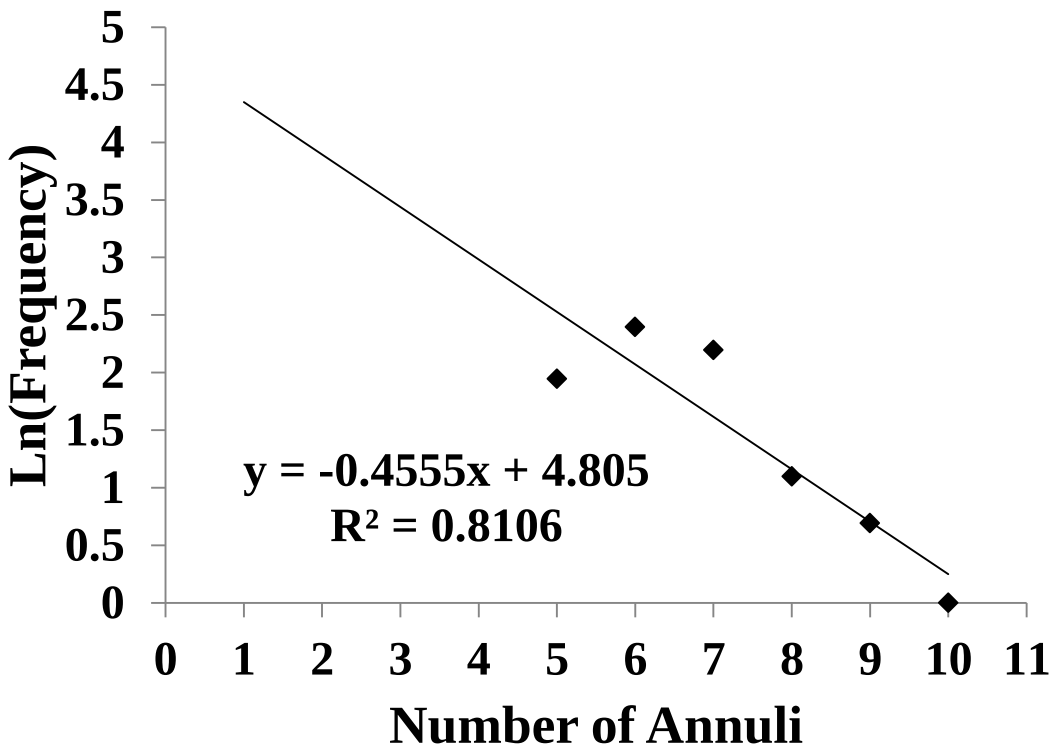


Figure I.16. Age structured regression of yellow mud turtles at Matador Wildlife Management Area in Cottle County, Texas, in 2006,-2016 (n = 224).

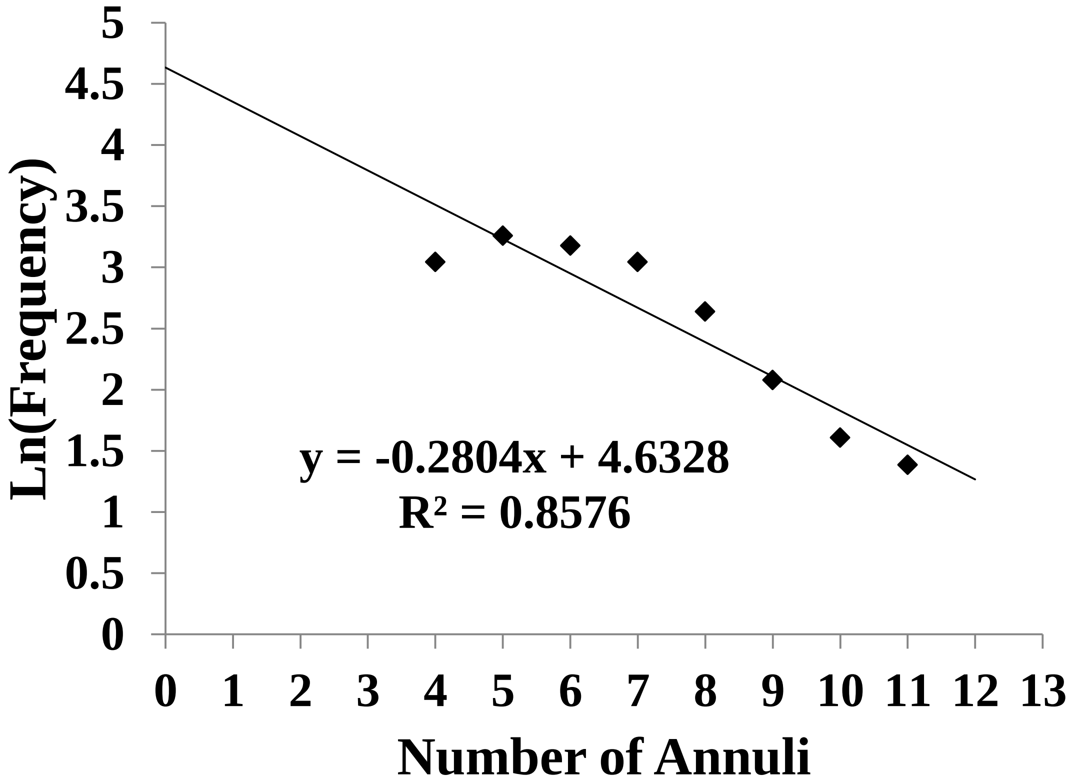


Figure I.17. Age structured regression of yellow mud turtles at Yoakum Dunes Wildlife Management Area in Cochran Coutny, Texa, in 2015 and 2016 (n = 140).

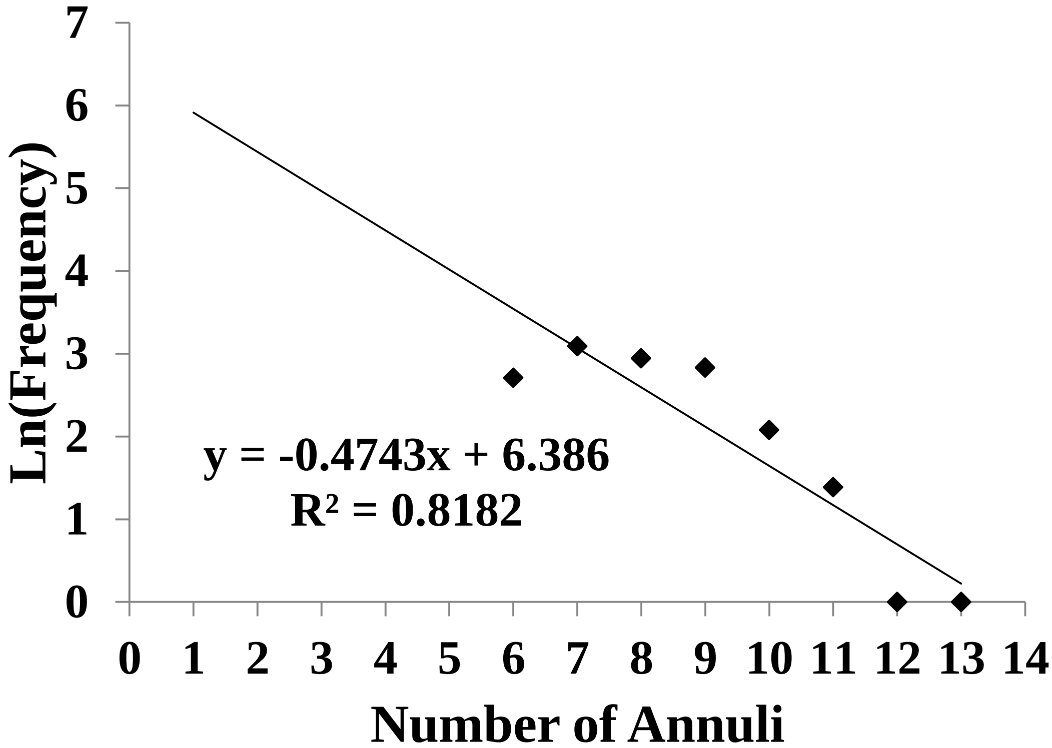
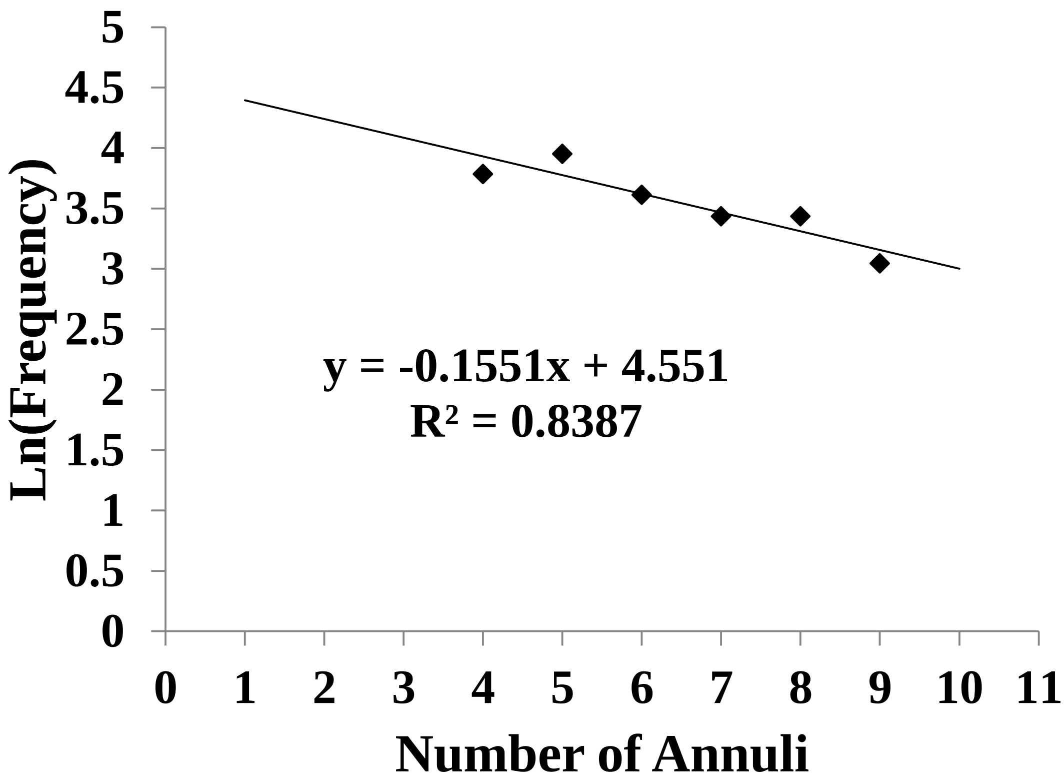


Figure I.18. Age structured regression of yellow mud turtles at Black Gap Wildlife Management Area in Brewster County, Texas, in 2007, 2010, 2015, and 2016 (n = 405).



Chapter II

A Comparison of Survival Estimation Methods for an Ornate Box Turtle population in Texas

INTRODUCTION

The use of annuli on the scutes of turtles to determine an individual's age began with Agassiz's (1857) descriptive studies on painted turtles (*Chrysemys picta*) well over a century ago (Germano and Bury 1998). Aside from the exception of species with leathery, uncornified epidermal shell covering, turtles develop a new scute during every major growth season. Each new scute is formed underneath the previous growth cycle's scute and usually extending past the margin of the previous scute. In many species, scutes are shed constantly, but in others the scutes stack each growing season leaving a countable set of scute growth layers, also known as annuli. In theory, a researcher can use this morphological attribute along with seasonal periodicity to estimate an individual's age (Legler 1960, Moll and Legler 1971, Zug 1991). This is a commonly used technique within the literature. However, a controversy exists amongst researchers on the accuracy of the technique.

The controversy amongst the use of annuli as an age estimation technique is split between those who believe with careful consideration of certain factors it can be utilized and those who argue that the annuli do not correlate with anything that can produce an age estimation. There have been many studies that aimed to synthesize the available data on the subject and come to conclusions regarding the techniques validity (e.g., Germano and Bury 1998, Wilson et al. 2003, Zug 1991). Most of these review studies acknowledge the potential issues with this technique. Complications with aging adult turtles are recognized by Zug (1991). As turtles grow old and reach maturity, growth can slow or cease which increases the difficulty in recognition of annuli (Zug 1991). Germano and Bury (1998) highlight the problems associated with the presence of false annuli. False annuli are annuli deposited during temporary cessations of growth within the growing season that can be indistinguishable from true annuli for those unaware or unfamiliar with the phenomenon (Germano and Bury 1998). Wilson et al. (2003) even goes as far to say that the technique has no basis for application across turtle species and populations because of the lack of literature they found to support it. Finally, other researchers such as Ashton and Ashton (2008) believe that annuli cannot ever be used to estimate the age of an individual because of numerous factors that can affect the length between and number of annuli.

Controversy aside, the technique of using annuli to estimate the age of individual turtles has the possibility to be an important method within research if its validity holds. The technique could provide the advantages of being non-lethal, low cost, and shorter in time requirements as opposed to other methods seeking the same results, such as skeletochronology (Zug 1991). There are speculations that up to two-thirds of turtle species throughout the world are either threatened or endangered (Bonin et al. 2006, Ernst and Lovich 2009). These species are subjected to numerous factors that contribute to these declines. Loss/degradation of habitat, overharvest for food/pet trade, and climate change are a few of the main factors contributing to turtle declines. Further diminishing these conservation issues is the severe lack of knowledge and understanding of most turtle species. This leads to the inability to understand what factors are directly affecting a given population and the management implications that will appropriately address these issues (Gibbons et al. 2000). With the current concerns over managing declining turtle populations throughout the world, validation of a scute annuli technique would greatly increase our ability to collect age-structured data for some species.

In order to both test the utility of annuli analysis as an aging technique and a survival estimator, my objective for this study was to compare this technique to other commonly accepted and supported methods of estimating annual survival (open population and known fate capture-mark-recapture methods). Secondarily, I will also aim to evaluate the status of an ornate box turtle (*Terrapene ornate*) population in a Rolling Plains ecosystem within the state of Texas.

METHODS

Study Area and Site- The Rolling Plains ecoregion is located in the northern part of the state of Texas with a majority of the area falling in the “Panhandle”. This region along with the High Plains ecoregion makes up the southern extent of the Great Plains region of the central United States. This ecoregion is comprised of about 9,700,000 ha of gently to moderately rolling topography and a section of the area cuts through the High Plains ecoregion following the Canadian river (Correll and Johnston 1979).. This region has a highly variable seasonal precipitation with average annual rainfall falling within 56 to 76 cm. Soils found in this region vary from coarse sands near streams to tight clays and shales. The original vegetative community of the Rolling Plains was tall to mid-grass prairies containing common species such as blue grama (*Bouteloua gracilis*), sand bluestem (*Andropogon hallii*), big bluestem (*Andropogon gerardii*), sand dropseed (*Chloris cucullata*), three-awn (*Aristida* spp.), little bluestem (*Andropogon scoparius*), side-oats grama (*Bouteloua curtipendula*), Indian grass (*Sorghastrum nutans*), switchgrass (*Panicum virgatum*), and western wheatgrass (*Agropyron smithii*). Common invaders include honey mesquite (*Prosopis glandulosa*) through most of the region, and shinnery oak (*Quercus havardii*) and sand sage (*Artemisia filifolia*) in sandy soils. Two thirds of the area remains in range land with the primary class of livestock being cattle that are grazed on large ranches (Correll and Johnston 1979).

The Matador Wildlife Management Area (WMA) was the selected study site for this project. The WMA is an 11,410 ha property found in the northern portion of the Rolling Plains ecoregion and is managed by the Wildlife Division of Texas Parks and Wildlife Department (TPWD). It falls within Cottle County and is located 10 km north of the town of Paducah, Texas (Figure II.1). The area is characterized by grasslands dominated by bluestems (*Andropogon* spp.) and gramas (*Bouteloua* spp.) with scattered or clumped woody species (Spears et al. 2002). Sand sage, honey mesquite, and shinnery oak dominate the vegetative cover (Becker et al. 2009). The aquatic habitat varies substantially and includes windmill overflow ponds, natural depression, sloughs, stock ponds, the Middle Pease River, and ditches. The property receives 562 mm of annual rainfall on average with most precipitation accumulated in the months of May and June. The primary management practices that TPWD uses on the area include prescribed burning, grazing, mechanical treatments, and herbicide application (Becker et al. 2009).

CMR- TPWD initiated a capture-mark-recapture (CMR) program for the ornate box turtle in 2004. In this program, individual ornate box turtles are collected within the property through fortuitous encounters and road cruising. Every new individual is given a notch code on the marginal scutes for identification using a Dremel tool (Robert Bosch Tool Corporation, Mount Prospect, Illinois, USA; Cagle 1939). These CMR data were then used in an open population model (=Pradel) within Program MARK in order to estimate annual survival (White and Burnham 1999). The Pradel model also calculates Lambda, which will allow me to evaluate the status of the ornate box turtle population through population growth rate.

Annuli Analysis- In 2007, the TPWD CMR program was supplemented with the collection of age data. An impression of the plastron was taken from each captured individual (Galbraith and Brooks 1987). The impressions were collected by mixing a batch of dental alginate (Matech Inc., Sylmar, California, USA) and applying it to the plastron of the turtle. It was left on the individual's plastron until the mixture had set completely then removed carefully. These alginate impressions were then stored in zip lock bags with a wet paper towel in order to retain moisture. These impressions were then transferred to West Texas A&M University to produce the permanent impressions. At the university, alginate molds were trimmed and surrounded by a basin made of modeling clay. A mixture of dental stone (GC America, Alsip, Illinois, USA) was then poured atop the alginate impression and left to set overnight. Each final impression was analyzed under illuminated magnification in order to obtain annuli counts in a blind fashion to avoid bias (Ewing, 1939, Galbraith and Brooks 1987, Germano and Bury 1998). The annuli were counted on all usable plastral scutes and the mode of these counts was used to represent age of each individual (Zug 1991). When individuals were captured multiple times, I randomized which capture was included in the analysis so that each individual was only represented once. These data were then used in an age-structured regression to estimate annual survival (Chapman and Robson 1960, Kazmaier et al. 2001, Robson and Chapman 1961).

Radio Telemetry- The data from a study by Grant (2010) on spatial ecology of Ornate Box turtles at Matador WMA were used for a survival estimate from radio telemetry for comparison. Turtles that were caught through the TPWD CMR program within the prescribed fire matrix between 2007 and 2010 were used. Each individual was outfitted with a radio transmitter (Holohil Systems, Carp, Ontario, Canada) using silicon to attach each to the carapace of the turtle. From June to September 2007, turtles were fixed with 0.95 g, 45 day transmitters, and RI 2-B, 14.3 g, 24 month transmitters were applied for those caught after September 2007. Individuals within the study were then located daily in the active season (May-August) and weekly in the inactive season

(September-April), and a GPS point was recorded for each location. The Kaplan-Meier staggered entry procedure was used to estimate an annual survival rate for these data (Pollock et al. 1989, Kazmaier et al. 2001). In this analysis, both the assumptions that all censored individuals were considered dead and all censored individuals remained alive were considered for a minimum and maximum survival estimate (Grant 2010). I then averaged these estimates to develop an overall survival estimate.

RESULTS

CMR Data- A total of 930 captures or 637 individuals were logged from 2004 to 2014 on the property. The annual survival was estimated at 72.3% with a lower 95% confidence interval of 0.680 and an upper of 0.762 (SE = 0.021). Lambda was estimated to be 0.930 with a lower confidence interval of 0.891 and an upper of 0.970 (SE = 0.020).

Age Data- A total of 360 individual turtles were aged from 2007 to 2014 were used from the CMR data set. The estimated ages ranged from 0 to 18 years with the most frequent age class being 9 years (Figure II.2). The annual survival rate for ages 8-17 was estimated to be 79.4% (r^2=0.834, Figure II.3).

Radio Telemetry Data- A total of 40 ornate box turtles (17 female, 13 male) were radiotracked for 15,600 radio-days (one radiotransmitter on one ornate box turtle is 1 radio-day) from 2007-2010. Seven individual (4 female, 3 male) were censored (= unknown fate). One female died in a prescribed fire and two males presumably died from avian predation events. The annual survival rates were estimated to be 86.7% maximum and 74.8% minimum with the differences approaching significance (p = 0.066,

Figure II.4). These curve shapes did not differ between the two estimates ($X^2 = 2.641$, $p = 0.104$, Grant 2010). The average annual survival between the 2 methods was 80.75%.

DISCUSSION

When comparing the results between the different methods of estimating annual survival within my study, there is a difference of less than 8.45% between the annual survival estimates of all techniques. Between the Kaplan-Meier and age structured regression methods, there was only a 1.35% difference (Table II.1). Given that both radiotelemetry and CMR techniques are commonly used and recommended within the scientific community for estimating annual survival because of their robustness (Silvy 2012), the similarity of these results gives backing to the validity and use of age structured regression with the use of annuli as an annual survival estimate.

In order to properly function, age structured regression as a technique has assumptions that must be met. If the assumptions are not met, the annual survival estimate could be different from true annual survival. One of these assumption with the technique is that all of the ages are accurately recorded (Chapman and Robson 1960, Skalski et al. 2005, Robson and Chapman 1961). Because of the fact that the annual survival estimate of age structured regression using annuli was calculated to be so close to that of commonly used methods, I come to the conclusion that annuli can be an accurate way to record the age of a turtle in this population. Considering these 2 backing results, I find this technique to be a useful method of aging this population of ornate box turtles. However, I believe that before using the technique within the field that one should be aware of the issues that can arise with the technique and practice the method thoroughly before using data collected to come to conclusions.

The first issue I believe that users of this technique must address or be aware of is the presence of false annuli. These false annuli can often be confused for true annuli and ultimately have the ability to drastically effecting both age estimation and survival calculation. There are two observations that can help the age estimator distinguish false from true annuli. First, false annuli do not form completely around the entirety of the scute and true annuli do (Germano and Bury 1998, Landers et al. 1982, Legler 1960). If you follow each annuli consistently around to both margins of the scute they encounter, you can distinguish between the two annuli types. Second, false annuli form shallower indentations on the scute than true annuli (Germano and Bury 1998, Legler 1960). This way a differentiating between the two annuli types can be more difficult to observe and is not recommended to those newer to the technique.

All factors considered, I recommend thorough practice of this technique's use before applying it in the field. The technique has a learning curve that must be passed before becoming proficient with its use. I recommend practicing use of the technique under supervision of an experienced user, on known-age individuals, and/or on a teaching collection of molds such as those produced in this study. The discernment between the two types of annuli can also be increased by examining all or multiple scutes of the species of turtle in question (Gemano and Bury 1998). Through this study, there were many instances where a false annuli seemed extremely prominent on one scute of an individual, but was easily recognized upon inspection of the other scutes of the individual. I also recommend observation under an illuminated magnifier to help clarify robustness and presence of annuli, particularly at the junction of scutes in older individuals.

A second issue that can arise from data collected with this technique is underrepresentation of age classes in portions of the population of focus. As previously stated, older individuals become difficult to age as growth slows which leads to an underrepresentation of older age classes (Zug 1991). This along with the common difficulty of sampling juveniles within most turtle species leads to issues that may influence annual survival estimates using age structured regression. By simply removing these underrepresented age classes to follow an assumed constant decline of a natural population, these issues are mitigated.

The validity of using annuli as an aging technique allows for many different advantages in research in both turtle species and the scientific community as a whole. One of these advantages is the amount of time the techniques has the ability to save. Radiotelemetry data requires the collect of information daily and weekly respective of the period of the research. This can prove to be both time and labor intensive. CMR data collection often requires multiple successive occasions in order to get the appropriate information to run statistical tests (Silvy 2012). With annuli and age structured regression, a quick surgical strike of sampling can provide appropriate data for an annual survival estimate given the availability of appropriate data. This allows for a quick evaluation of the status of a species and can prove significantly useful for species of high risk that have little scientific literature backing. At the least, this technique's convenience allows it to be administered alongside CMR data collection in order to provide more insight and further evidence support the resulting estimates.

Another advantage that comes with the use of this technique is the lower financial costs of carrying out a study using it. It is logical to consider that with time carrying out a technique comes money needed in order to perform the study. Therefore, the time saving aspect of the use of annuli and age structured regression comes with less costs in order to achieve the same goal. Techniques such as radiotelemetry also required gear that can prove to be accumulate large overall costs for a study. This aging technique requires little costs compared to that of radio telemetry and capture-mark-recapture techniques.

With an average of 78.3% annual survival between the three estimation techniques of this study, the Matador WMA ornate box turtle population has a relatively low survival compared to that of other estimates in the literature. In a CMR study done in Illinois, an ornate box turtle population was estimated to have an annual survival of 97% (SE = 0.06) using a Pradel model (Bowen et al. 2004). A radiotelemetry study on Eastern Box Turtles, *Terrapene carolina*, in Indiana estimated populations to have an annual survival of 97% (SE = 0.03, Currylow 2010). Although this is a different species, their similar ecologies allow for a coarse comparison of the Kaplan-Meier estimates. After observing this low survival estimate, I looked toward the Lambda or population growth rate to further look into this population's status. Because the upper confidence interval is less than 1, it suggests this population is in slow decline.

In conclusion, I find that annuli and age-structured regression is a viable technique of the estimation of annual survival for this population of ornate box turtles. I hypothesize that this technique could be applicable to other populations and species, but should be appropriately tested and researchers require a familiarity with the technique before in can be used to base management decisions upon. If proven valid for a given population, this technique could save both labor and money compared to the techniques of radio telemetry and capture-mark-recapture for survival estimation. Further research and management is needed, to determine the causal factors of decline for the Matador WMA ornate box turtle population and appropriate addressment.

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Figure II.1. The location of the Rolling Plains ecoregion within Texas (upper left), Cottle county within the Texas Panhandle and Rolling Plains (lower left), and Matador WMA location within Cottle county in relation to the town of Paducah, Texas (right).

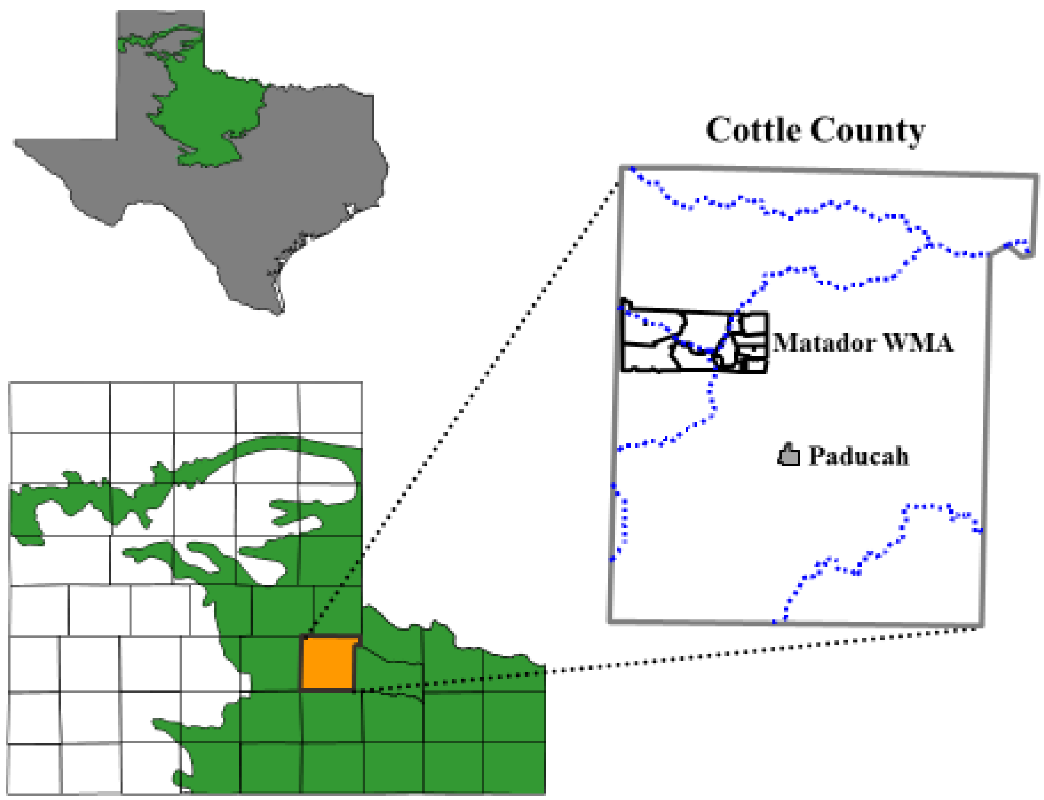

Figure II.2. Age distribution of ornate box turtles at Matador Wildlife Management Area in Cottle County, Texas, from 2007-2014 (n = 360).

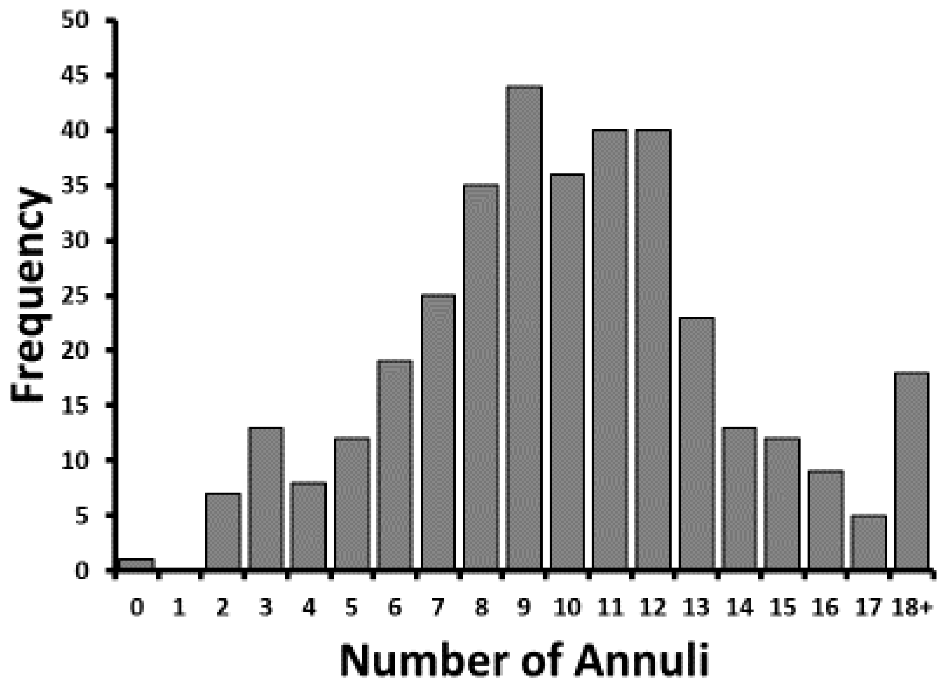

Figure II.3. Age structured regression of ornate box turtles at Matador Wildlife Management Area in Cottle County, Texas, from 2007-2014 (n = 360).

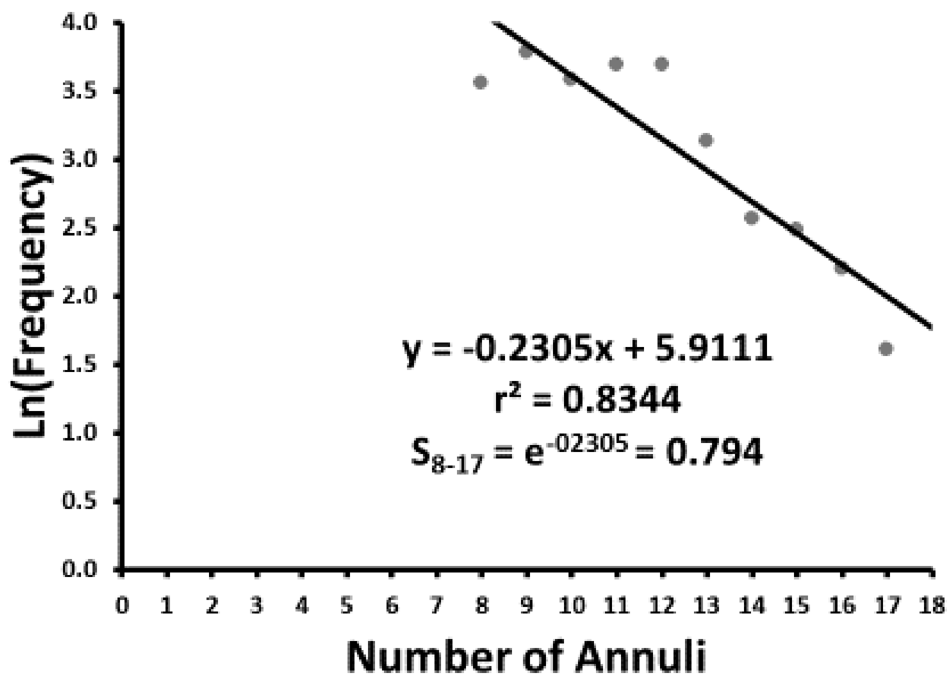

Figure II.4. Annual Kaplan-Meier survival estimates assuming censored individuals are either live or dead for ornate box turtles at Matador Wildlife Management Area, Cottle County, Texas, from 21 June 2007 – 22 April 2010.