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Population Structure And Habitat Association Of Aquatic Testudines In Quivira National Wildlife Refuge

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POPULATION STRUCTURE AND HABITAT ASSOCIATION OF AQUATIC TESTUDINES IN QUIVIRA NATIONAL WILDLIFE REFUGE

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ABSTRACT

I researched aquatic turtle population structure and habitat association of the turtles of Quivira National Wildlife Refuge (QNWR), in central Kansas. This group of organisms was focused on because of the lack of baseline knowledge and understanding of species presences, population sizes, structure, or habitat use on the refuge.

I selected wetland units for sampling based on water permanence and quality of habitat. I then deployed baited hoop traps on these wetland units from April 24 to July 4, 2015. Captures were identified to species, measured, marked, and then release. Schnabel Multiple Census Method and Combined Percent Estimates were used to estimate population sizes. The results from these estimates were then extrapolated to the other wetland units on QNWR and a refuge wide population estimate was calculated. Adult sex ratios and age classes were generated for each species based on measurements taken during sampling.

Abundant population sizes were observed on QNWR, with a male-biased sex ratio for hard-shelled turtles, and a female-biased sex ratio for soft-shelled turtles. This biased sex ratio could be the result of sampling technique, close proximity to roadways, Temperature Sex Determinate species, or due to high mortality rates of breeding and nesting individuals.

Wetland unit variables were measured for sampled wetland units, and their influence on aquatic turtle abundance was investigated with a One-way Analysis of Variance. Significant results was obtained for *Apalone spinifera* and *Chelydra serpentina*, providing support for their known life histories and habitat preferences.

Insights into seasonal turtle activity was recorded, and early season intensive monitoring protocols were suggested for continued research and management for the aquatic turtle populations of QNWR.

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PREFACE

This thesis follows the style for the Transactions of the Kansas Academy of Science. Animals captured in this research project were manipulated in accordance with IACUC protocol 14-0012.

INTRODUCTION

Dynamic environmental conditions and manipulations of habitat by management agencies make it challenging to assess changes in occurrence or species relative abundance, and if change is detected, which of the influences might be causal. Encroachment of trees, introduction of invasive plant species, and continued agricultural development by humans are some of the greatest threats to our native prairies in the Midwest (Larson, Anderson, and Newton 2001; Ratajczak, Nippert, and Collins 2012). Virgin prairies and native grasslands are some of the most rapidly declining ecosystems in North America (Coppedge et. al. 2001). Unfortunately, these habitats are also often overlooked when the conservation of habitats and ecosystems is considered. Additionally, a changing climate, drought cycles, and other conditions result in significant challenges to the mission of wildlife refuges today (Meretsky et. al. 2006). To address the challenges of restoring native habitats, combating threats to biodiversity, and manage ecosystems in flux, there is a need for a greater understanding of how organisms are using the habitats we are attempting to conserve (Meretsky et. al. 2006). These difficulties, along with political and public considerations, make scientific research increasingly complicated to conduct and the resulting management recommendations difficult to execute.

Kansas is an area of interest, as the climate continues to change and ecosystems deviate from historical norms. Average global atmospheric temperatures (IPCC 2007), drought frequency, and severity (IPCC 2001) are expected to increase in the next few decades. These abiotic conditions are some of the most influences on Kansas's major ecosystems. Within the state are well defined ecoregions based on vegetation types that evolved under specific soil and climate conditions and among these is the Arkansas River Lowlands (Lauver et al. 1999), which is the area of interest for this study.

Some vegetation types are widespread (e.g. the Tallgrass and Shortgrass Prairies) and others are restricted to small areas (e.g. riparian habitats and wetlands). Wetland habitats of note are the natural grassland marshes of the prairies (Schaffner 1898). Though restricted in size, these grassland marshes are critical to the life cycle of many species. Amphibians use them for reproduction, growth, and as habitat throughout their lives (Bragg 1967). Some snake species have specialized diets comprised of wetland-dependent organisms (Weatherhead and Prior 1992). Wading birds and waterfowl both feed and nest in these habitats (Bolen, Smith and Schramm 1989, Custer and Galli 2002). Wetlands also function as early successional habitat for small mammal species (Francl, Castleberry and Ford 2004). Their importance is highlighted as these habitats become fractured, degraded, and destroyed (Gibbs 2000; Trenham et al. 2003).

Quivira National Wildlife Refuge (QNWR) is an 8,957 hectare refuge unique to Kansas. QNWR was established in 1955 with funds from the Migratory Bird Commission, but the most recent acquisition was in 1998. QNWR is dominated by marshes, sand prairie grasslands, and riparian and upland prairie complexes (U.S. Fish and Wildlife Service 2013a). These salt marshes are characterized as inland salt marsh, meaning subterranean salt deposits affect the water in the wetland units on the refuge. These salt deposits affect the salinity, or the amount of salt dissolved in the water, of the wetlands on QNWR. The result is a wide range of salinity variation due to precipitation, runoff, and water depth (Lindberg and Harriss 1973; Hackney and Cruz 1978) among the standing water bodies on the refuge (1 to 39 ppt).

This marshland is a critical stopping point for many migratory bird species as they move between breeding and overwintering ranges (Skagen and Knopf 1994). As such, QNWR has traditionally been managed to focus on the needs of migratory bird species, with limited focus on resident species of QWNR. More recently, the National Refuge System has been tasked to take a more holistic approach to management of resources held in the public trust (Meretsky et. al. 2006). Therefore, the challenge of coming to understand how all species use the refuge and respond to current management practices has been undertaken by QWNR.

The challenges of this expanded mission were addressed by the drafting and implementing a Comprehensive Conservation Plan (CCP). The CCP is a document outlining QNWR's goals and strategies for refuge management over the next 10 to 15 years. Their goal is to consider all species in management decisions, and to return as much of the refuge to native grassland conditions as possible (U.S. Fish and Wildlife Service 2013b). To achieve these goals and to monitor their relative success, QNWR needed a record of organism occurrence and habitat association for the refuge, and to develop a set of monitoring protocols. Such monitoring protocols will provide staff with a means of evaluating effective management practices as they continue implementation of their CCP.

Aquatic turtles are a charismatic group of species associated with the presence of water or aquatic habitats (Bennett, Gibbons, and Franson 1970), therefore sampling protocols are distinctive from predominantly nonavian terrestrial species. Turtles are some of the longest lived organisms in North America (Gibbon and Semlitsch 1982; Gibbon 1987), meaning they have a long life cycle, long time to maturity, and it can be nearly a decade before a population recovers from a significant mortality event (Heppell, Crowder, and Crouse 1996). Accordingly, particular care must be taken when habitat manipulations are considered that will affect this group.

Routine management of wetland units typically focused on migratory birds can have great impact on the survivorship of aquatic turtles. The decision to drain a wetland unit influences turtles and other organisms (bird, mammal, snake, lizard, etc.) by forcing them to find a new water source. For the aquatic turtle community, this action leads to the destruction, or at least seasonal loss, of an entire habitat. In addition, direct mortality of juveniles and adults occur as they attempt to relocate to new aquatic habitats (Janzen 1993). A greater understanding of how aquatic turtles use habitats and water control structures within QNWR will help the refuge make informed management decisions as they continue implementing their CCP.

My project attempted to address a small part of the overall implementation of the CCP on QNWR. I estimated population characteristics of aquatic turtles inhabiting the refuge and provided insights on the effectiveness of sampling protocols for this group. Specifically, the focus of my research was on the aquatic turtle species inhabiting 4 ponds and 2 large salt marshes in QNWR. My objectives were to 1) sample the representative habitats on the refuge to document aquatic turtle species, 2) to investigate the population structure of these species, 3) to document broad-scale habitat associations between these turtles and the wetlands they inhabit, and 4) develop sampling protocols that will allow effective long-term monitoring of these species. I hypothesize the wetland units with greater area will follow the Species-Area Relationship, and contain higher species richness with more individuals of those species than the wetland units of lesser area.

METHODS

Study Area

Quivira National Wildlife Refuge is located in central Kansas, and consists of 8,957 hectares, the majority of which is in Stafford County. QNWR is managed by the U.S. Fish and Wildlife service and has been listed as a wetland of international importance for the migration of waterfowl and shorebirds in the Central Flyway (U.S. Fish and Wildlife Service 2015).

Surrounding these wetland units is a large sand-prairie complex comprised of vegetated sand dunes (Eberle, Welker, and Welker 1996). This vegetation community is a mix of eastern tallgrass prairie and western shortgrass prairie species. Both ecoregions are strongly represented side by side, typical of the mixed grass prairie. Patches of grass including big bluestem, *Andropogon gerardii,* can be observed interspersed with sand dropseed, *Sporobolus cryptandrus,* and several *Bouteloua* sp. of grass. This overlap in species' occurrence is observed in the birds, mammals, reptiles, amphibians, and other taxonomic groups using QNWR, resulting in a mixture of eastern and western species inhabiting the refuge (U.S. Fish and Wildlife Service 2014). For example, Eastern Meadow Lark, *Sturnella magna,* and Western Meadow Lark, *Sturnella neglecta,* can be heard calling simultaneously on the refuge. Graham's Crayfish Snake, *Regina grahamii,* is found at the most western edge of its range. The Glossy Snake, *Arizona elegans,* can be observed at the eastern edge of its range (Ernst and Ernst 2003). Aquatic turtles also exhibit an unexpected pattern of overlap with the Pond Slider, *Trachemys scripta,* a southeastern species, and Yellow Mud Turtle, *Kinosternon flavescens,* a south central species occurring in the same habitat (Ernst and Lovich 2009). These and other atypical co-occurences can be observed in this unique wetland complex.

Sampling and Data Collection

I used hoop traps, baited with Louisiana Hot Sauce Sardines, in sampling complexes of 2 traps each to capture aquatic turtles. The traps were purchased from Miller Net Company (Memphis, TN), and consisted of 3 galvanized rings, a single throat, and 2.54-cm^2 pre-tarred mesh netting (Figure 1). The traps measured 0.9 m in diameter, and were 2.1 m in length. Attached to the mouth of each trap were a pair of 3.0 m by 0.9 m leads of the same pre-tarred mesh netting (Figure 2). The purpose of these leads was to funnel the turtles into the mouth of the trap, increasing the likelihood of capture. The distal end of each lead and the cod end of each trap were staked to the bottom to ensure the trap was stable while deployed. (Figure 2).

I selected 6 of a possible 42 wetland units based on the size, location on the refuge relative to roadways, and quality of turtle habitats (Figure 3, Table 1). The relative importance and interest in certain wetland units to QNWR management also was taken into consideration. Habitat quality was judged, in part, on the perceived permanence of water, and a minimum depth of 1.0 m. This was to ensure adequate water depth for overwintering turtles and the proper function of the turtle traps, such that turtles had access to both the trap opening and the water surface.

Sampling was conducted from April 24 to July 4 2015, and divided into 10 sampling periods (Table 2). Turtle traps were deployed on a wetland unit for 3 consecutive nights (1 sampling period). Traps were checked once every 24 hours.

Eighteen turtle traps were used in each sample period and deployed in pairs, to equal 9 trapping complexes or locations per night (Figures 4-8).

Trapping complexes were rotated between wetland units throughout the sampling periods because of limitations in equipment and logistics of checking traps. Large wetland units such as the Little Salt Marsh required a larger trapping effort to assess the turtle populations due to high capture rates and large amounts of suitable habitat. A full rotation through all sampled wetland units was completed between sampling periods 1-6. A second full rotation through all sampled wetland units was completed between sampling periods 7-10.

Each trap was baited the day they were deployed, and rebaited upon each visit to check the traps. Traps were checked daily, and turtles were identified to species. Sex, curved plastron length, and curved carapace lengths were recorded. Age classes were then assigned based on these measurements (Appendix 1). Individuals were marked on a posterior marginal scute (Figure 9), to uniquely identify the wetland of capture for each individual prior to release at the point of capture. The notch was a “filed” mark on the ‘hard-shelled’ species of turtles (Figure 10), and a small clipping of the carapace (Plummer 2008) on the ‘soft-shelled’ species of turtles (Figure 11).

Opportunistic encounters were recorded, but no systematic vehicle surveys were conducted. When a turtle was encountered within approximately 0.8 km of a sampled wetland unit, it was measured and included in the sampling effort for the current sampling period. These road encounters were then released, orientated in the same direction they were traveling before sampling. The road encounters were only used in the population estimations if active sampling was underway on the wetland unit within 0.8

km of capture. Otherwise, these data were used to estimate the overall population of turtles for QNWR and to assess inter-pond movements on the refuge.

I attempted to catch turtles by hand in smaller ponds. Captures were then placed in a tub and measured at the conclusion of the sampling effort to insure individuals were not accidently resampled. These efforts were later abandoned due to low water levels, but the captures were included in the population estimates for the refuge.

Habitat assessments were conducted on each wetland unit, at each individual trapping location. Assessment of the dominant vegetation types of both the shore and aquatic vegetation were conducted. Vegetation classes were broken down into grasses, shrubs, forbs, tall emergent, submerged, and floating vegetation types. I rated each vegetation class at each trap location on a scale of 0 to 3 of relative cover; 0 equaled no vegetation present, 3 equaled complete cover. These rankings were combined and divided by the number of trapping points to yield the estimated cover of each vegetation type at each trapping location. Water samples were collected and salinity and conductivity were measured in the lab with a ___. Soil types were identified from the United States Department of Agriculture National Cooperative Soil Survey (SSURGO) Database, provided by QNWR's staff, and categories of terrestrial vegetation were determined from the high resolution data sets also provided by QNWR.

Data Manipulation

A Schnabel Multiple Census Method (Nelson 2015) was used to estimate population size of all species in each of the 5 wetland units. When there were sufficient numbers of captures and recaptures, 90% confidence intervals were calculated for these population estimates. For species with insufficient recaptures, a population estimate was calculated by a Combined Percent Estimate. The combined wetland unit population was estimate by collapsing all sampled turtles into groups of captured or recaptured individuals. A Schnabel Multiple Censes Model was then conducted for the wetland unit. The population estimate from this model was multiplied by the species percentage observed during the entire sample period. This produced the Combined Percent Estimate for species with few captures. Upper and lower confidence intervals were not estimated.

To estimate the number of aquatic turtles inhabiting QNWR, each wetland unit was categorized by surface area. These wetland units were then divided into 4 categories based on similarities in surface area. Category one was represented by the Park Smith Pond, and included 15 wetland units between 2 to 12 hectares. Category two was represented by the T-Intersection Pond, and consisted of 4 additional wetland units from 14 to 20 hectares. Category three was represented by the Dorrynane Lake Complex, and consisted of 14 wetland units from 20 to 50 hectares. Category four was created by multiplying the Dorrynane Lake Complex results by 7.5 to represent 3 wetland units between 100 to 200 hectares. The Big Salt Marsh population estimates were used for the Wildlife Drive Wetland Unit because of immediate proximity and similarity of habitat features. The Little Salt Marsh was treated separately because of its unique size and habitat characteristics.

Once each wetland unit had an assigned area category, the population results from the wetland units I sampled were applied, and summed to produce an overall estimate of population size for QNWR. In a similar fashion, Lower and Upper confidence intervals (when available) were taken from each sampled wetland unit per species, and then extrapolated to include all wetland units on QNWR.

I used a One-way Analysis of Variance (ANOVA) (Fox and Weisberg 2015) to investigate relationships between turtle abundance and the sampled wetland units for each species. This was accomplished by grouping the sampling periods into 2 rotations (sampling periods 1-6; sampling periods 7-10). Each rotation contained turtle captures from each of the 5 wetland units. Captures from the sampled wetland units were combined for each species within each of 2 rotations. Combining sampling period results was necessary because not all wetland units were sampled during each sampling period because of limitations of equipment and logistics.

I then conducted an ANOVA of wetland units for each individual species. When an ANOVA yielded a significant result, a Tukey's Honest Significant Different (Tukey's HSD) test was conducted to identify differences among the wetland units.

RESULTS

Population Estimates

I captured 6 species, representing 4 families of turtles during the field season. These included the Pond Slider, *Trachemys scripta,* Painted Turtle, *Chrysemys picta* (Emydidae), Yellow Mud Turtle, *Kinosternon flavescens,* (Kinosternidae), the Common Snapping Turtle, *Chelydra serpentina* (Chelydridae), the Smooth Soft Shell, *Apalone mutica,* and the Spiny Soft Shell, *Apalone spinifera* (Trionychidae). *A. mutica* was identified on the refuge for the first time and this observation represents the first county record for the species in Stafford County, KS (Collins, Collins, and Taggart 2010).

I caught 1,024 aquatic turtles (Table 4). *T. scripta* was captured most often and was represented by 474 individuals, followed by *K. flavescens* (372 individuals), *C. serpentina* (103 individuals), *A. spinifera*, (68 individuals), *C. picta* (6 individuals), and *A. mutica* (1 individual).

In the Little Salt Marsh (Figure 4) I captured 6 species (Table 5), and the highest estimated number of turtles (N = 2315 (1510-3066)). *T. scripta* was the most frequently captured (289 individuals) (Table 4), and had the highest number of recaptures (28 individuals). *T. scripta* was estimated to have the largest population (1153 (873-1615) individuals). *A. mutica* was the least captured turtle (1 individual) with no recaptures.

In the Dorrynane Lake Complex (Figure 5) I captured 5 species (Table 5), and the third highest estimated number of turtles (N = 519 (302-1233)). *T.* scripta was captured most often (70 individuals) (Table 4), and had the highest number of recaptures (14 individuals). *T. scripta* was estimated to have the second largest population (114 (80-200) individuals). *K. flavescens* was captured 68 times, with 5 recaptures and had the highest population estimate in the wetland unit (N = 369 (205-923) individuals). *C. picta* was the least captured turtle (1 individual) with no recaptures.

In the Park Smith Pond (Figure 6) I captured 4 species (Table 5), and the second highest estimated number of turtles (N = 584 (402-989)). *K. flavescens* was captured most often (180 individuals) (Table 4) and had the highest number of recaptures (19 individuals). *K. flavescens* was estimated to have the largest population (455 (322-703) individuals). *C. picta* was the least captured turtle (1 individual) with no recaptures.

In the T-Intersection Pond (Figure 7) I captured 3 species (Table 5), and had the second lowest estimated number of turtles (N = 186 (109-429)). *K. flavescens* was captured most often (44 individuals) (Table 4) and had the same number of recaptures as *T. scripta* (7 individuals). *K. flavescens* was estimated to have the largest population (102 (59-237) individuals). *C. serpentina* was the least captured turtle (1 individual) with no recaptures.

In the Big Salt Marsh (Figure 8) I captured 3 species (Table 5), and had the smallest estimated number of turtles (N = 135 (67-306)). *K. flavescens* was captured most often (27 individuals) (Table 4) and had the same number of recaptures as *T. scripta* (2 individuals). *K. flavescens* was estimated to have the largest population (86 (43-257) individuals). *C. serpentina* was the least captured turtle (2 individuals) with no recaptures.

A calculated 32,321 aquatic turtles were projected to inhabit QNWR (Table 5). *K. flavescens* had the largest extrapolated population (N = 22,451 (13,437-47,211)), and *A. mutica* the smallest (5 individuals).

Population Structure

The Little Salt Marsh (Figure 4) had a higher number of juvenile and sub-adult females among the hard-shelled turtles than juvenile and sub-adult males. Adult males were encountered more than adult females in the hard shelled turtles. This trend was not observed in the soft-shelled *Apalone* spp., which had more females observed among juvenile, sub-adult, and adult turtles (Table 6).

The Dorrynane Lake Complex (Figure 5) had a higher number of juvenile and sub-adult females among the hard shelled turtles than juvenile and sub-adult males. Adult males were more frequently captured than adult females in the hard shelled turtles. This trend was not observed in *Apalone* spp., which had more adult females observed. Juveniles or sub-adults were not observed in all species (Table 6).

The Park Smith Pond (Figure 6) had a higher number of sub-adult and adult females among *T. scripta,* and the opposite was found in *K. flavescens.* No juvenile *K. flavescens* were observed (Table 6).

The T-Intersection Pond (Figure 7) had a higher number of adult individuals observed. Adult male *K. flavescens* were more frequently observed than adult females. Adult female *T. scripta* were more frequently observed than adult males (Table 6).

The Big Salt Marsh (Figure 8) had a higher number of juvenile and sub-adult females than males. Adult males were more frequently captured then adult females. No juvenile or sub-adult males were observed (Table 6).

An average adult male to female ratio for QNWR was calculated for species with high capture rates. A male to female ratio of 2 : 1 was estimated for *K. flavescens* and 1.9 : 1.1 for *T. scripta*. A male to female ratio of 3.1 : 1 was estimated for *C. serpentina.* A male to female ratio of 1 : 2.5 was estimated for *A. spinifera*. The individual sex ratios for each wetland unit can be reviewed on Table 6. Individual sex ratios by sample period indicate some seasonality in occurrence in individual units (Figure 12 – 19).

Wetland Unit Associations

Variances between individual wetland unit variables were not investigated due to multicollinearity among habitat variables. Comparisons of the relative abundance of turtle species among wetland units were evaluated by ANOVAs. The ANOVA for *T. scripta* was not significant (DF = 4, F-value = 3.342, p = 0.109). The ANOVA for *K. flavescens* was not significant (DF = 4, F-value = 0.716, p = 0.616).

The ANOVA for *C. serpentina* was significant (DF = 4, F-value = 17.37, p = 0.004). A Tukey's HSD test indicated that abundance of *C. serpentina* was higher in the Little Salt Marsh compared to the other sampled wetland units (Little Salt Marsh vs. Dorrynane Lake p = 0.019; Little Salt Marsh vs. Park Smith Pond p = 0.006; Little Salt Marsh vs. T-Intersection Pond p = 0.006; Little Salt Marsh vs. Big Salt Marsh p = 0.005). The ANOVA for *A. spinifera* also was significant (DF = 4, F-value = 53.87, p = 0.0003). A Tukey's HSD test indicated that the abundance of *A. spinifera* was higher in the Little Salt Marsh compared to the other sampled wetland units (Little Salt Marsh vs. Dorrynane Lake p = 0.001; Little Salt Marsh vs. Park Smith Pond p = 0.0004; Little Salt Marsh vs. T-Intersection Pond p = 0.0004; Little Salt Marsh vs. Big Salt Marsh p = 0.0004).

DISCUSSION

Population estimates

Compared to other estimates of turtle populations in central Kansas, Quivira National Wildlife Refuge possesses some of the most densely populated concentrations of aquatic turtles in the state (House, Nall, and Thomas 2011). Table 5 lists the calculated density per acre of each species of the turtle sampled per wetland unit. My large sample size provides confidence in the results from the Schnabel estimates for the sampled wetland units. In the cases when a Combined Percent Estimate was required, the results should be viewed with caution (Table 5).

I expect my refuge wide aquatic turtle estimate of 32,321 individuals to be a conservative estimate, if all the wetland units of QNWR experienced several seasons of adequate water levels. Some of the wetland units in a category were smaller than my sampled wetland units; however, the majority of wetland units were 2 or 3 times larger than the sampled units used to estimate the populations in those wetlands. Nonetheless, wetland unit size is not likely to be the only factor influencing availability of water or population size. However, it might not be unreasonable to assume a wetland unit 3 times the size and of a reasonable habitat quality could sustain a similar number of turtles as a smaller wetland unit of better habitat quality. More accurate methods for overall population estimations would be available with multiple years of sampling and an increased sample size of the species currently represented by a low number of captures.

Population Structure

Temperature Sex Determination (TSD) is the process where the average temperature of the nest during embryo development determines the sex of the organism (Vitt and Caldwell 2009). In turtles, a higher average nest temperature results in a female, while lower average nest temperatures result in a male (Vitt and Caldwell 2009). Genetic Sex Determination (GSD) is the process where the genetic composition of an individual embryo determines the sex of the organism regardless of environmental factors. The hard-shelled turtles sampled on QNWR were TSD species (Vogt et al. 1982; Wilhoft, Hoating, and Franks 1983; Ewart and Nelson 1991), whereas the soft-shelled turtles were GSD species (Vogt et al. 1982). The species with GSD normally yield a hatchling male to female ratio near 1:1 (Vogt and Bull 1982). The species with TSD are highly variable in their hatchling sex ratios from nest to nest, and season to season (Dodd, Murdock, and Wibbels 2006). Expected hatchling sex ratios of hard-shelled turtles could not be estimated without a specific nesting study.

Male and female age classes were calculated for each of the sampled wetland units (Tables 6). The majority of wetland units had a larger number of males sampled than females. However, more *A. spinifera* females were observed then males. The Park Smith Pond and the T-Intersection Pond also had more female *T. scripta* observed than males. These were the only exceptions to the observed male-biased sex ratios. Ream and Ream (1966) observed baited hoop traps tended to result in a male biased sampling of aquatic turtles. There are other potential reasons to explain this male biased sex ratio and should be considered to properly interpret a biased sex ratio (Swannack and Rose 2003). Other factors, including close proximity to roads might cause a male biased sex ratios in aquatic turtles (Steen and Gibbs 2004). Higher mortality rates of nesting females is shown to skew aquatic turtle sex ratios in medium to large sized turtle species. (Gibbs and Shriver 2002). The close proximity of wetland units to roadways on QNWR might have resulted in the observed male biased sex ratios. In addition, males typically do not move between ponds in significant numbers (House, Nall, and Thomas 2010). The increased amount of time females spend between ponds might expose them to greater road mortality risks when compared to males.

The calculated number of turtles in each age class across the sampled wetland units show few representatives in the Juvenile and Sub-Adult categories. Sampling during a high precipitation year might have contributed to the lack of captures for young individuals. Hatchling and young turtles use smaller, warmer, and less turbid water with more basking sites (Plummer 1977) to facilitate temperature regulation (Janzen, Paukstis, and Brodie 1992). Larger, more permanent water bodies also expose young turtles to potential predators of larger size and aggressive adult turtles (Bury and Germano 2003). High rainfall on QNWR filled all wetland units, greatly increasing the amount of shallow, ephemeral habitat hatchling and young turtles could use. Higher mortalities in smaller bodied females during nesting due to predation also might contribute to the small estimated number of young individuals (Tucker and Filoramo 1999).

Wetland Unit Association

No wetland unit associations were detected for *T. scripta* or *K. flavescens*. This lack of specificity is not surprising based on the well documented resilient nature of these habitat generalists (Cagle 1950; Iverson 1991). Significant ANOVA results were obtained for *C. serpentina* and *A. spinifera.* A Tukey's HSD identified the Little Salt Marsh as the wetland unit with more captures for these species.

Observations of *A. spinifera* were nearly limited to the Little Salt Marsh. This species is reported to favor habitats with soft substrates, plentiful sandbars, and relies on more permanent water sources (Collins, Collins, and Taggart 2010). *C. serpentina* is also a more well adapted aquatic species and although it occurred in more units than *A. spinifera,* the majority of the observations were in the Little Salt Marsh. The Little Salt Marsh also has extensive concrete diking structures. This type of obstructed habitat is preferred by *C. serpentina* (Froese 1978). This high number of obstructions and permanence of water are likely the largest influence on high occurrence of these two species in the Little Salt Marsh. These observations might be explained by other biotic or abiotic factors, but further investigation of detailed habitat variables is required.

The Little Salt Marsh is the most persistent water source during droughts (M. Oldham 2015, pers. comm.). It is also the main water source for the wetland manipulations conducted on the refuge. A trend was observed across QNWR when comparing species richness and number of individual turtles captured (Table 5). The Little Salt Marsh functions as a major source habitat for QNWR. The Little Salt Marsh has the highest area of suitable turtle habitat, and supported the highest number of species. It also supported the highest number of individuals of those species. In general, I observed a decreases in species richness and abundance of individuals with increasing distance from the Little Salt Marsh. It seems reasonable to view the Little Salt Marsh as the source habitat for the colonization of aquatic turtles for other wetland units on the refuge, particularly those vulnerable to annual dewatering due to either natural drought cycles or management manipulations.

Behavioral Observations

Mating between individuals detained in traps was observed throughout the summer. The frequency of mating in *C. serpentina* appeared to increase noticeably in June. Nesting behavior was observed across QNWR during June in *A. spinifera, K. flavescens,* and *T. scripta*. This period aligns with sampling periods 6 through 8. Sampling period 8 was the second highest in overall capture rates, suggesting nesting was largely completed by this date, and female turtles had returned to the wetland units. Sampling period 9 resulted in a more balanced male to female capture ratios.

A number of recently hatched *K. flavescens* and *T. scripta* were observed emerging from nests and traveling to water sources. This movement was observed on April 25, 2015 on one of the first warm days following a precipitation event. These emergence events are typical for *K. flavescens* (Long 1986), but are more variable in *T. scripta* (Packerd et al. 1997; Tucker and Packard 1998). The majority of emergence events were observed in late April and early May at moderate frequency. No emergence of nestlings for the other turtle species were observed.

The highest number of captures occurred during sampling periods 1 and 2. Due to a relatively cool spring, atmospheric and water temperatures had not reached optimal levels for turtle activity until the last week of April (Ernst 1972). Presumably the majority of turtles were still in the water, feeding, becoming more active, and preparing to mate as environmental temperatures continued to rise. Ongoing monitoring might reveal a local temperature threshold marking the beginning of aquatic turtles' seasonal activity.

A high number of captures in sampling period 1 was observed for *K. flavescens*, with a steady decrease through period 4 (Figure 16-17). Natural reproduction in this species is not well documented (Iverson 1991) but is thought to take place in April to May. There was a steady decline in male *K. flavescens* captured from May 1 to 20. This might be attributed to the male turtles traveling from pond to pond in search of females. In sampling period 5 through 8, I observed relatively high capture numbers of males while captures of females steadily declined. This decline might be attributed to females leaving the water to seek nesting habitats (Christiansen et al. 1985). In sampling period 9, a more equal number of males and females were captured. Accordingly, I suspect by the beginning of June the majority of nesting activity had been concluded for *K. flavescens* on QNWR.

The described trend of increasing activity, mating, and nesting is less clear in the other species. I am confident with an increased sampling effort, or a more focused effort on a single sizable wetland unit, patterns in breeding behavior could be documented.

Monitoring Protocols

After comparing the sex ratios of the turtle species, it is apparent during sampling periods 1 and 2 a higher number of captures for the majority of turtle species occurred. I would recommend monitoring occur between April 24 and May 10, once temperatures have reached ~22.5° C. During this time period the activity of aquatic turtles will be increasing with the warming weather. However, it is unlikely the turtles will have begun terrestrial movements in search of mates or nesting sites. Sampling during this time should provide the most consistent estimates and unbiased sex ratios.

I suggest protocols similar to those used in this investigation. The two trap complexes were effective at dividing the high capture numbers between two holding areas to avoid injury to turtles, damage to the traps, and loss of data. The sardine bait is cheap, long lasting in storage, and logistically easy to manipulate. Traps should be set in the late morning for the first night of sampling, and then not checked until the early afternoon the next day. Attention should be paid during sampling to observe any emergence of hatchlings. For the most complete sample of the aquatic turtle community, all pond edges should be sampled to ensure the maximum number of captures is being obtained. Twelve trapping complexes would be the minimum effort invested if only a short period of sampling was conducted on a yearly basis. If longer sampling periods are conducted then a smaller effort might suffice. Twelve trap complexes allows for all of the east side of the Little Salt Marsh to be sampled at the same time. Maximizing sampled habitat should minimize biased sex ratios and provide sufficient captures for population estimates.

The Little Salt Marsh should be the major focus of this future monitoring due to its source effect on the aquatic turtles. Other wetland units should be monitored to investigate inter-wetland movements by turtles and fluctuations in species richness and abundance. Expanded monitoring will better identify nesting habitats. Investigating predation upon nests and emerging hatchlings would provide insights into inclusive habitat requirements.

Future research

Much research can still be focused on the aquatic turtle populations of QNWR. With multiple years of population sampling, more sophisticated population models might be calculated. Some of these include detectability estimates, survivorship probabilities, fecundity levels, and more accurate estimates of population size and sex ratios (Rodda 2012). Hatchling sex ratios need to be determined to better understand the observed male biased sex ratio on QNWR. An investigation into other traditional sampling techniques should be conducted to compare their sex ratio results with the results of this study.

TSD in aquatic turtles might provide a means of monitoring the effects of climate change on the refuge. If a baseline TSD ratio can be established, male to female ratios can be observed on a year to year basis as a means of assessing local climate change. Sex is temperature dependent in most of QNWR's turtles. If ratios become skewed to increasing female bias, this might indicate a shift in higher average local temperatures. Additional studies focused on breeding times, nesting activities, and emergence of hatchling turtles would provide other valuable insights into the local aquatic turtle community and perhaps larger scale weather patterns.

Conclusion

Quivira National Wildlife Refuge is a highly productive habitat for aquatic turtle diversity and populations in central Kansas. Aquatic turtles are a robust group of organisms, dependent upon water. Because a large number of turtle species in North America possess TSD, they might be a model organism for assessing significant local climate change. A shift to increasing female bias in sex ratio would indicate a significant increase in local environmental temperatures. These data might serve to demonstrate local climate change and its impact on native organisms. Reducing or eliminating secondary sources of bias in sex ratios would be important to clearly interpret these data over time.

Turtle populations are fragile when adult mortalities are considered, and care should be taken to reduce the threats to aquatic turtle activity. Road mortalities in both adults and juveniles should be avoided. Disturbance of known turtle nesting habitat should be avoided whenever possible. Reducing sources of high, unnatural turtle mortalities will improve the results of long term monitoring, especially if unnatural mortality is biased to sex. Continued research into QNWR's aquatic turtle population structure and dynamics will be important to establishing a baseline sex ratio for all species. Continued management and monitoring of QNWR might be important in the evaluation of the effects of climate change at a local scale.

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TABLE 1. Table listing the names of the sampled wetland units of Quivira National Wildlife Refuge and their GPS coordinates for the 2015 sampling season.

Pond	Coordinates (UTM)
Little Salt Marsh	N 38.09054˚ W 98.48995˚
Lake Darrynane Complex	N 38.14123˚ W 98.47611˚
Park Smith Pond	N 38.14434˚ W 98.49027˚
T-Intersection Pond	N 38.20085˚ W 98.49193˚
Big Salt Marsh	N 38.17848˚ W 98.54075˚
Y-Road Complex	N 38.19883˚ W 98.54706˚

TABLE 2. List of dates and the associated sampling period number for the 2015 summer sampling of Quivira National Wildlife Refuge.

Sample Period	Date
1	24 April – 27 April
2	01 May – 4 May
3	11 May – 14 May
4	17 May – 20 May
5	25 May – 28 May
6	01 June – 04 June
7	09 June – 12 June
8	17 June – 20 June
9	25 June – 28 June
10	03 June – 04 June

TABLE 3. Common name, scientific name, number of aquatic turtles captured, recaptured, and overall percent of captures by species for the 2015 sampling season of Quivira National Wildlife Refuge.

Common Name	Scientific Name	Number of Individuals	Number of Individuals Recaptured	Percentage of Total Captures
Smooth Softshell	*Apalone mutica*	1	0	0.1%
Spiny Softshell	*Apalone spinifera*	68	5	6.7%
Snapping Turtle	*Chelydra serpentina*	103	12	9.1%
Yellow Mud Turtle	*Kinosternon flavescens*	372	34	34.0%
Painted Turtle	*Chrysemys picta*	6	0	0.6%
Pond Slider	*Trachemys scripta*	474	58	45.2%

TABLE 4. Number of captures and (recaptures) per species of aquatic turtle by sampled wetland unit. Trap nights for each wetland unit are listed in brackets, and the Catch/Trap Unit for 2015 sampling season of Quivira National Wildlife Refuge.

Species	**Little Salt Marsh** [171]	**Dorrynane Lake** [81]	**Park Smith Pond** [134]	**T-Intersection Pond** [62]	**Big Salt Marsh** [54]
A.mutica	1	0	0	0	0
A.spinifera	60 (3)	8 (2)	0	0	0
C. serpentina	62 (7)	16 (4)	4 (1)	1	2
K. flavescens	53 (1)	68 (5)	180 (19)	44 (7)	27 (2)
C. picta	4	1	1	0	0
T. scripta	289 (28)	70 (14)	61 (7)	38 (7)	15 (2)
Catch/Unit	2.97	2.29	2.04	1.56	0.89

TABLE 5. Population estimates for each aquatic turtle species at each sample location for the 2015 season. In parenthesis are 90% Upper and Lower Confidence Intervals. In brackets are the areas of the wetland units in acres. In "{}" are the number of trap nights. The "*" denotes a population estimated by the Combined Percent Estimates.

Species	**Little Salt Marsh** [376.9 ha]	**Dorrynane Lake** [21.9 ha]	**Park Smith Pond** [5.7 ha]	**T-Intersection Pond** [15.2 ha]	**Big Salt Marsh** [489.5 ha]	**Totals**
Density (turtles/hectare)	6.14	23.7	102.5	12.2	0.28	
T.scripta	1153 (873-1615)	114 (80-200)	118 (69-275)	82 (48-190)	41 (16-n/a)	7,808 (5,249-14,547)
K.flavescencs	652 (217-n/a)	369 (205-923)	455 (322-703)	102 (59-237)	86 (43-257)	22,451 (13,437-48,211)
C.serpentina	216 (126-505)	25 (12-99)	9 (n/a)	2* (n/a)	8* (n/a)	1,323 (746-4,387)
C.picta	18* (n/a)	2* (n/a)	2* (n/a)			125 (n/a)
A.spinifera	271 (n/a)	9 (3-n/a)				608 (112-n/a)
A.mutica	5* (n/a)					5 (n/a)
Totals	2,315 (1,510-3,066)	519 (302-1,233)	584 (402-989)	186 (109-429)	135 (67-306)	

TABLE 6. Age classes and adult male to female sex ratios of captured aquatic turtle by species for each sampled wetland unit of Quivira National Wildlife Refuge for the 2015 sampling season.

Age Class	**Juvenile**	**Sub-Adult**	**Adult**	**Adult Sex Ratio (male : female)**
Little Salt Marsh				
A. spinifera Female	0	6	36	1:2
A. spinifera Male	0	0	18	
C. serpentina Female	0	1	20	1.9:1
C. serpentina Male	2	1	38	
K. flavescens Female	0	8	12	2.4:1
K. flavescens Male	4	0	29	
T. scripta Female	19	40	49	3.5:1
T. scripta Male	5	3	173	
Dorrynane Lake Complex				
A. spinifera Female	0	0	6	1:3
A. spinifera Male	0	0	2	
C. serpentina Female	0	0	3	4.3:1
C. serpentina Male	0	0	13	
K. flavescens Female	0	5	20	1.9:1
K. flavescens Male	1	4	38	
T. scripta Female	1	6	23	1.7:1
T. scripta Male	1	1	38	
Park Smith Pond				
K. flavescens Female	0	16	54	1.4:1
K. flavescens Male	6	1	78	
T. scripta Female	2	8	20	1:1.5
T. scripta Male	3	5	13	
T-Intersection Pond				
K. flavescens Female	0	6	11	2.1:1
K. flavescens Male	1	3	23	
T. scripta Female	1	2	19	1.3:1
T. scripta Male	1	1	15	
Big Salt Marsh				
K. flavescens Female	0	1	7	2.6:1
K. flavescens Male	0	1	18	
T. scripta Female	1	4	2	3.5:1
T. scripta Male	0	0	7	

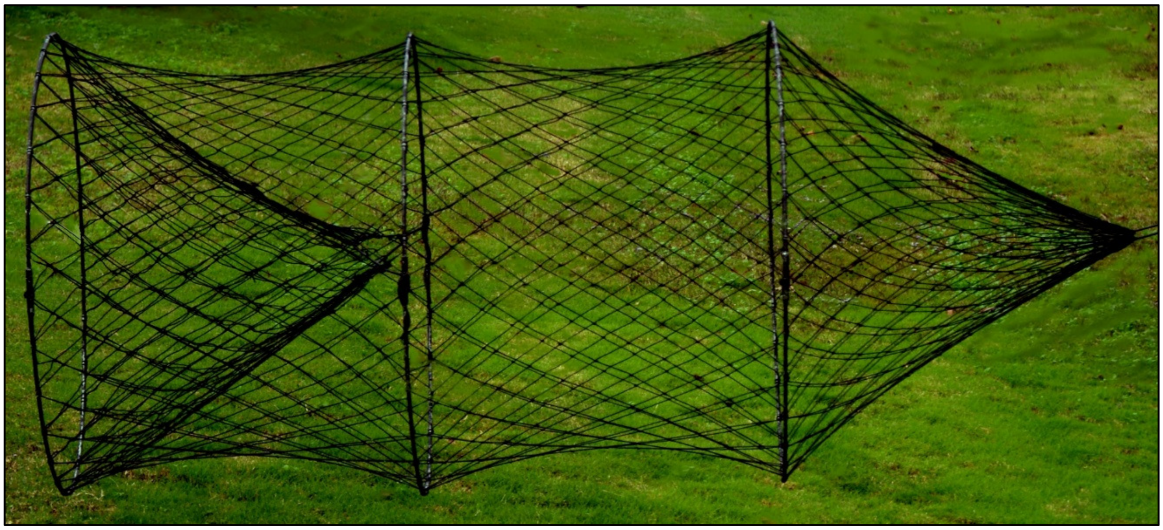

FIGURE 1. Picture of hoop net trap used to sample aquatic turtles during 2015 sampling.

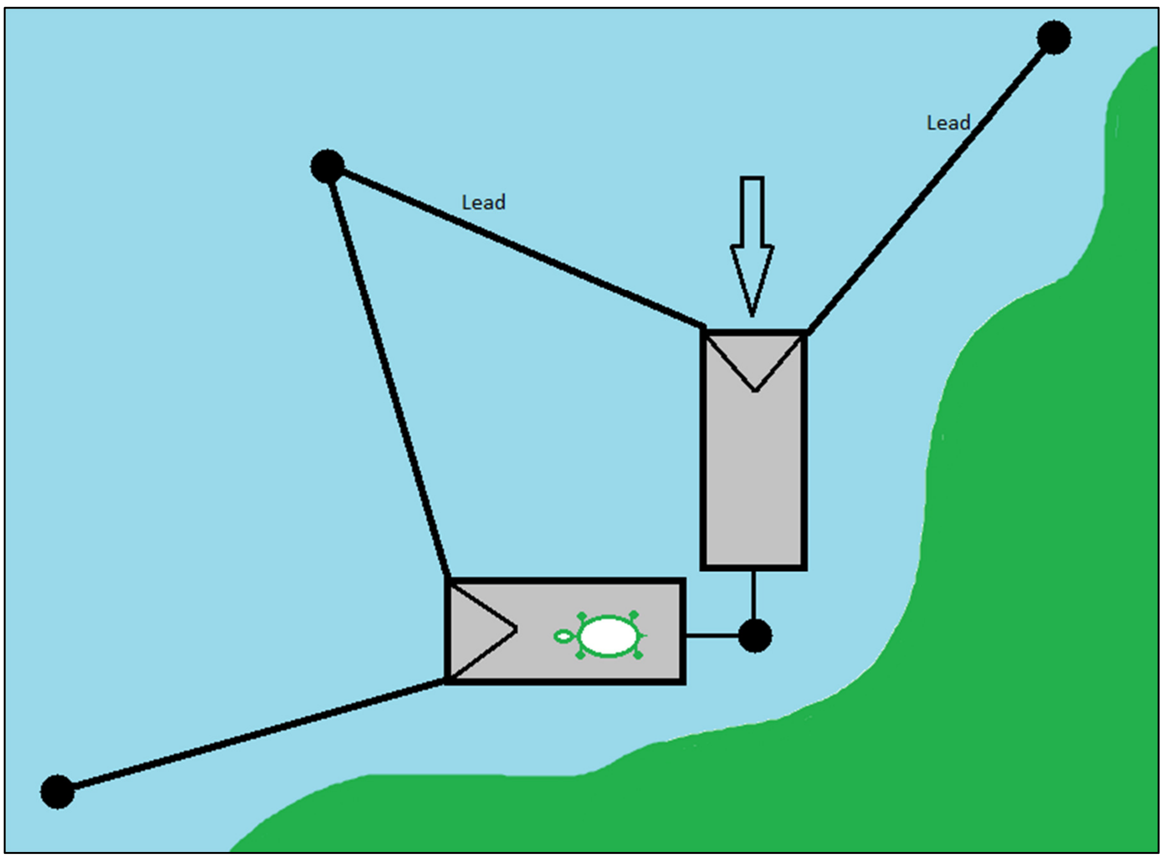

FIGURE 2. Illustration of trap set up for 2015, showing how the leads were attached to the trap and then set towards the middle of the pond, openings facing away from the bank.

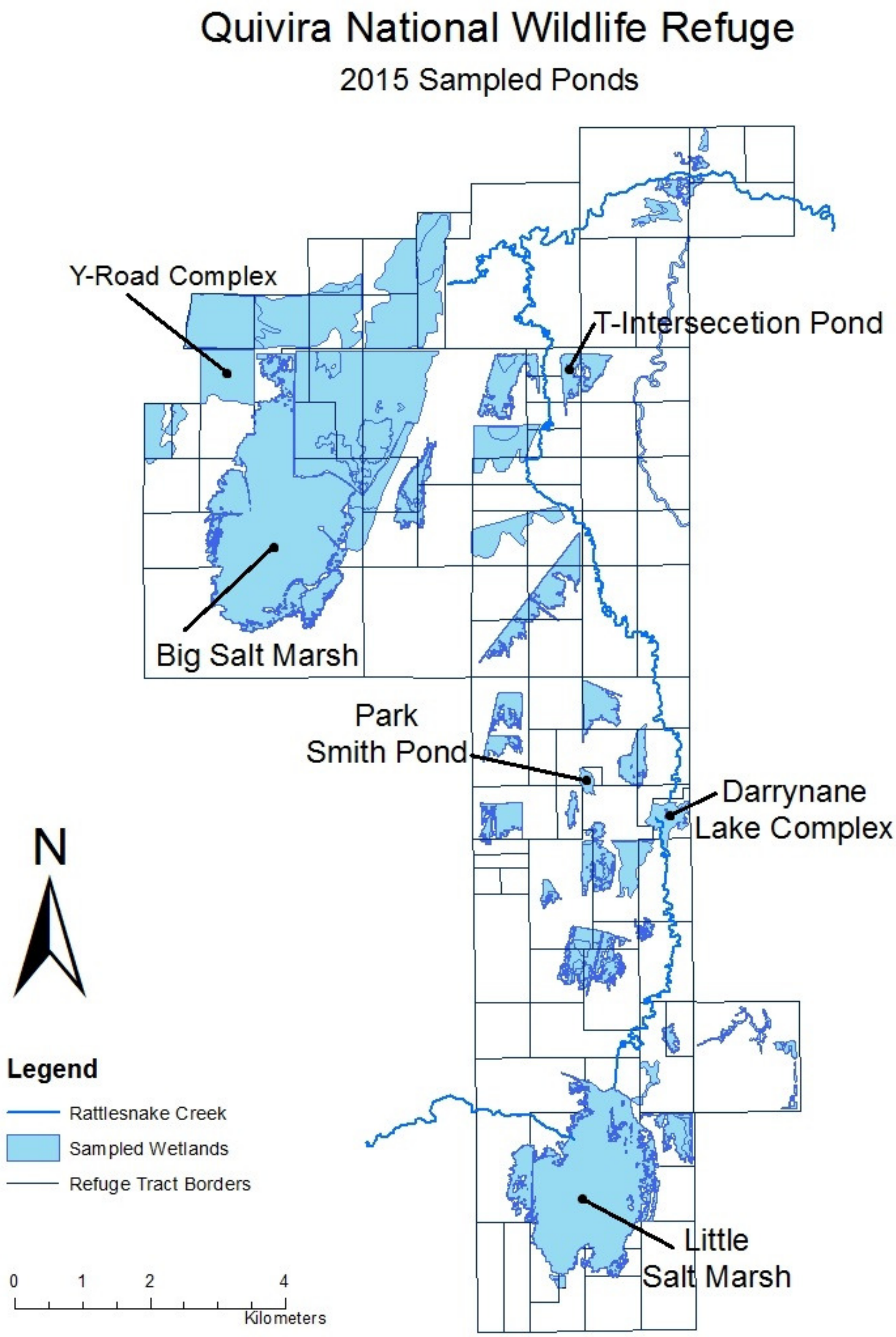

FIGURE 3. Map of the distribution of wetland units and water ways within Quivira National Wildlife Refuge; sampled wetland units for 2015 season are labeled.

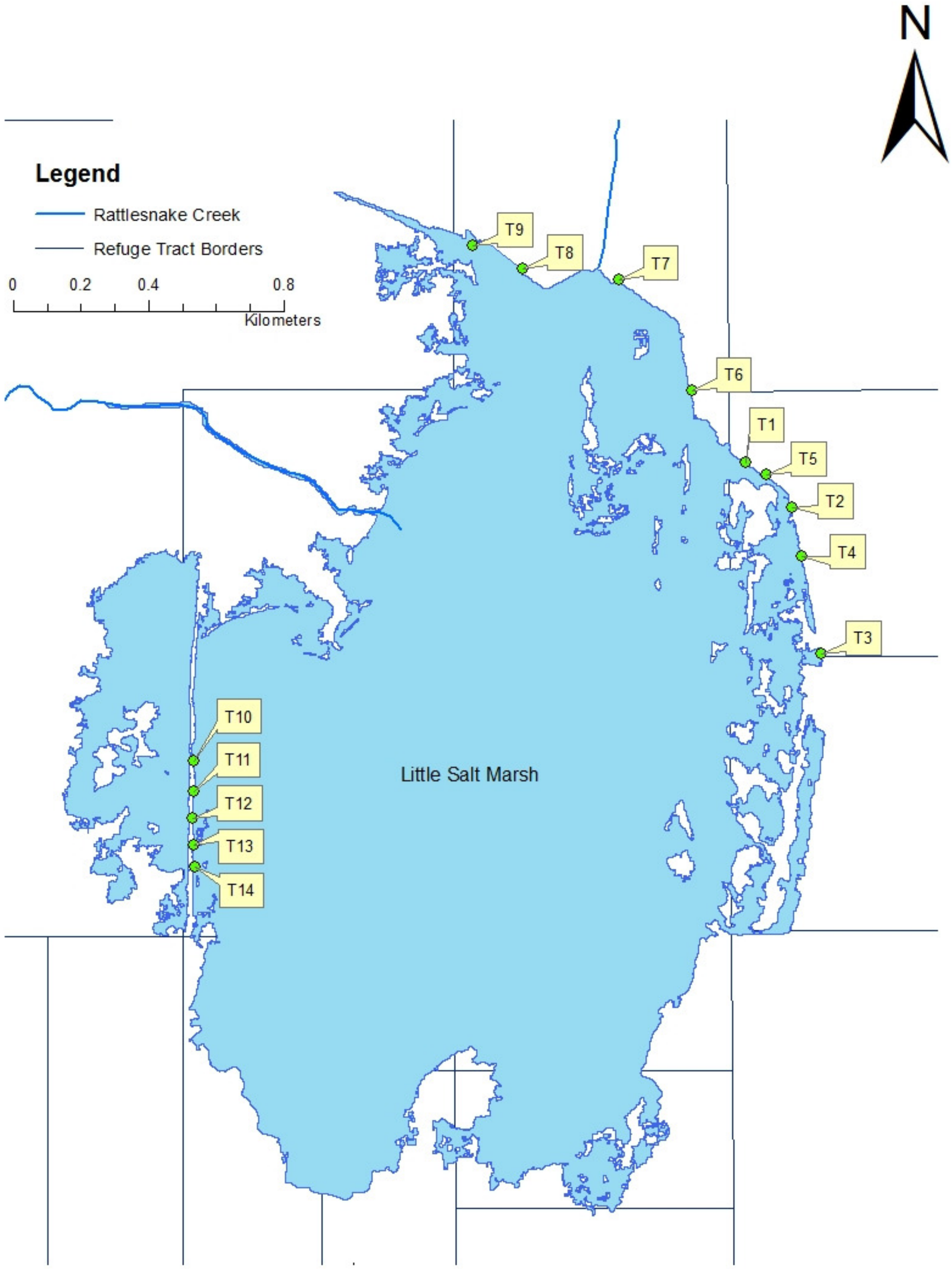

FIGURE 4. Map of the Little Salt Marsh and its individual trapping locations for the 2015 season.

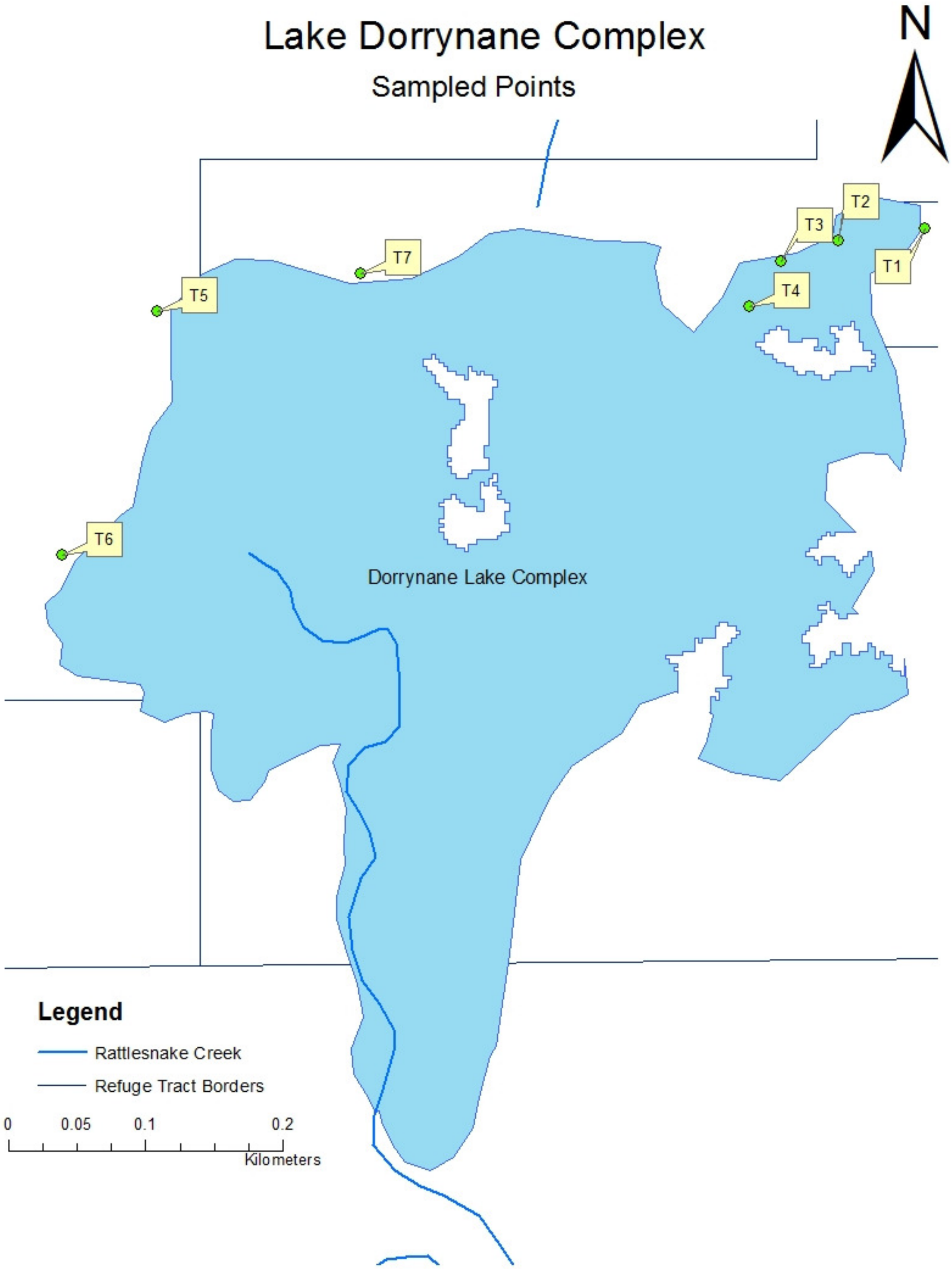

FIGURE 5. Map of the Dorrynane Lake complex and its individual trapping locations for the 2015 season.

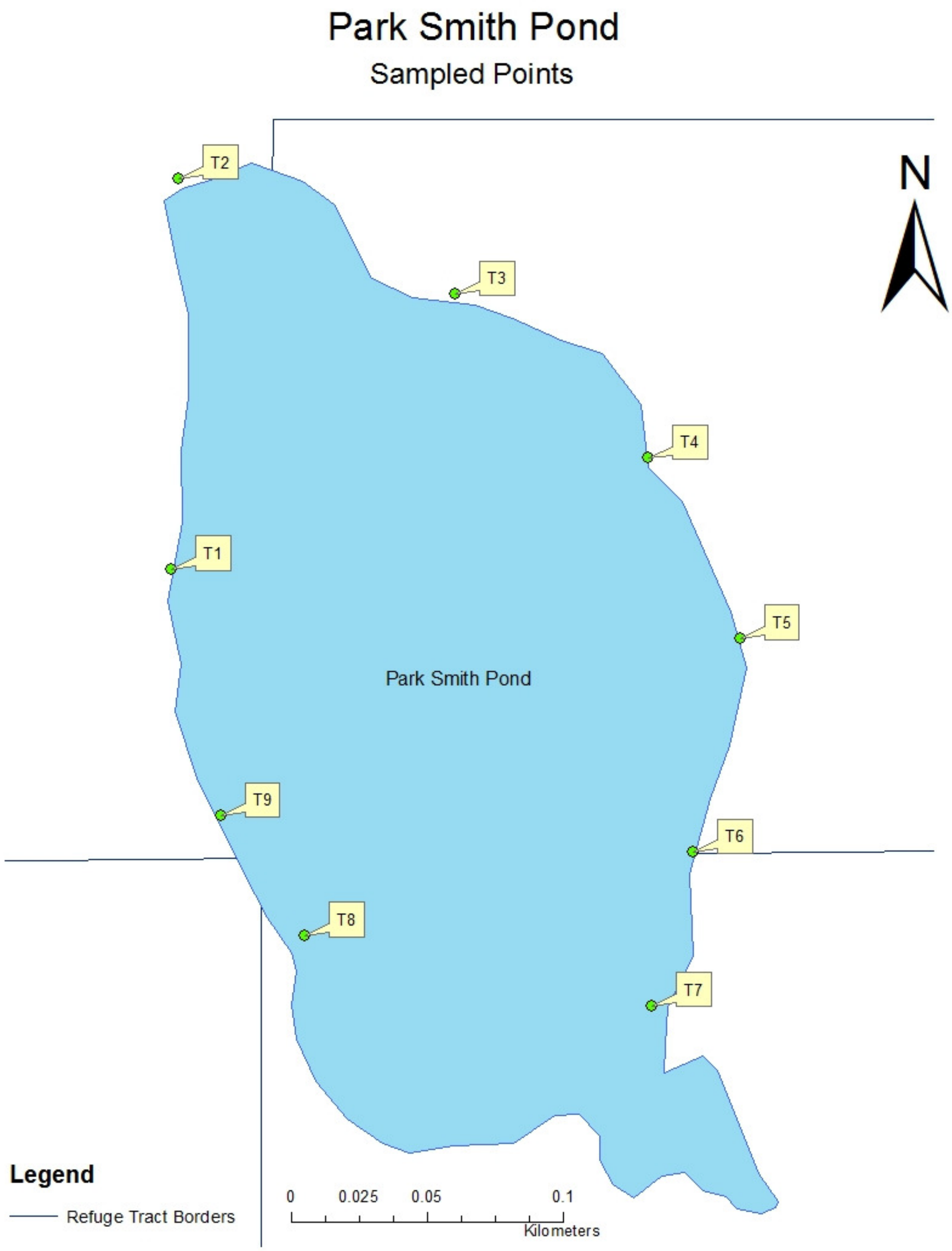

FIGURE 6. Map of the Park Smith Pond and its individual trapping locations for the 2015 season.

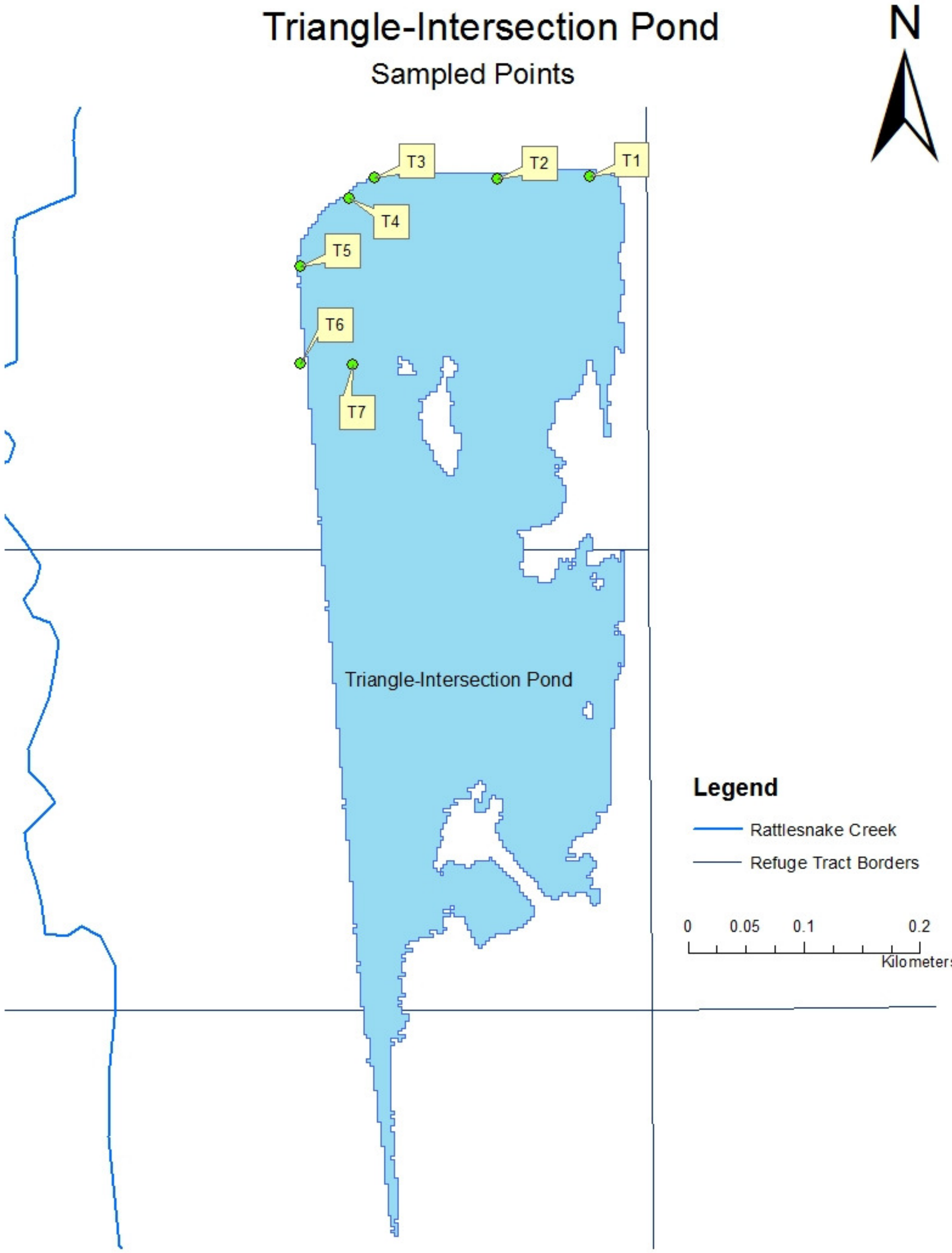

FIGURE 7. Map of the Triangle-Intersection Pond and its individual trapping locations for the 2015 season.

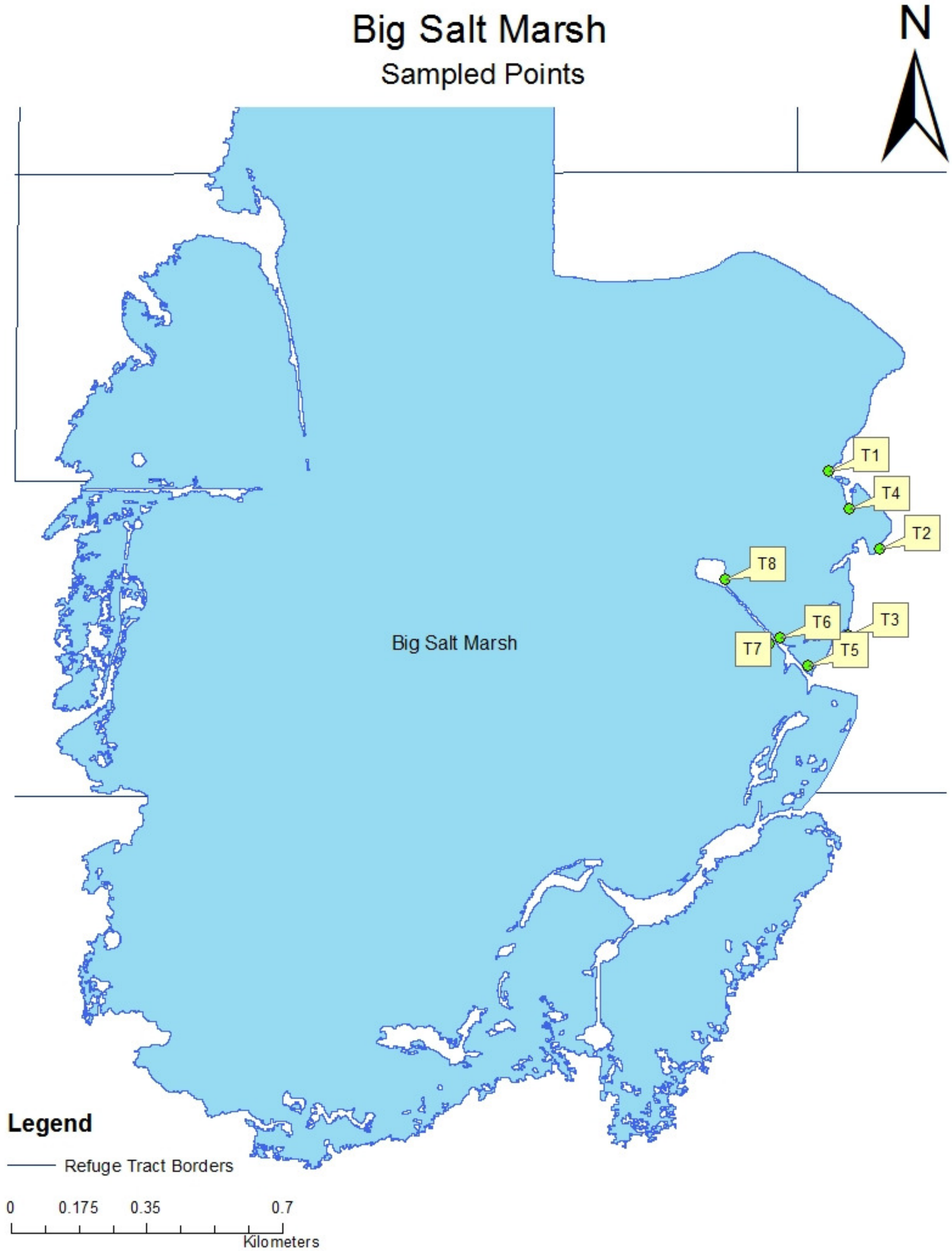

FIGURE 8. Map of the Big Salt Marsh and its individual trapping locations for the 2015 season.

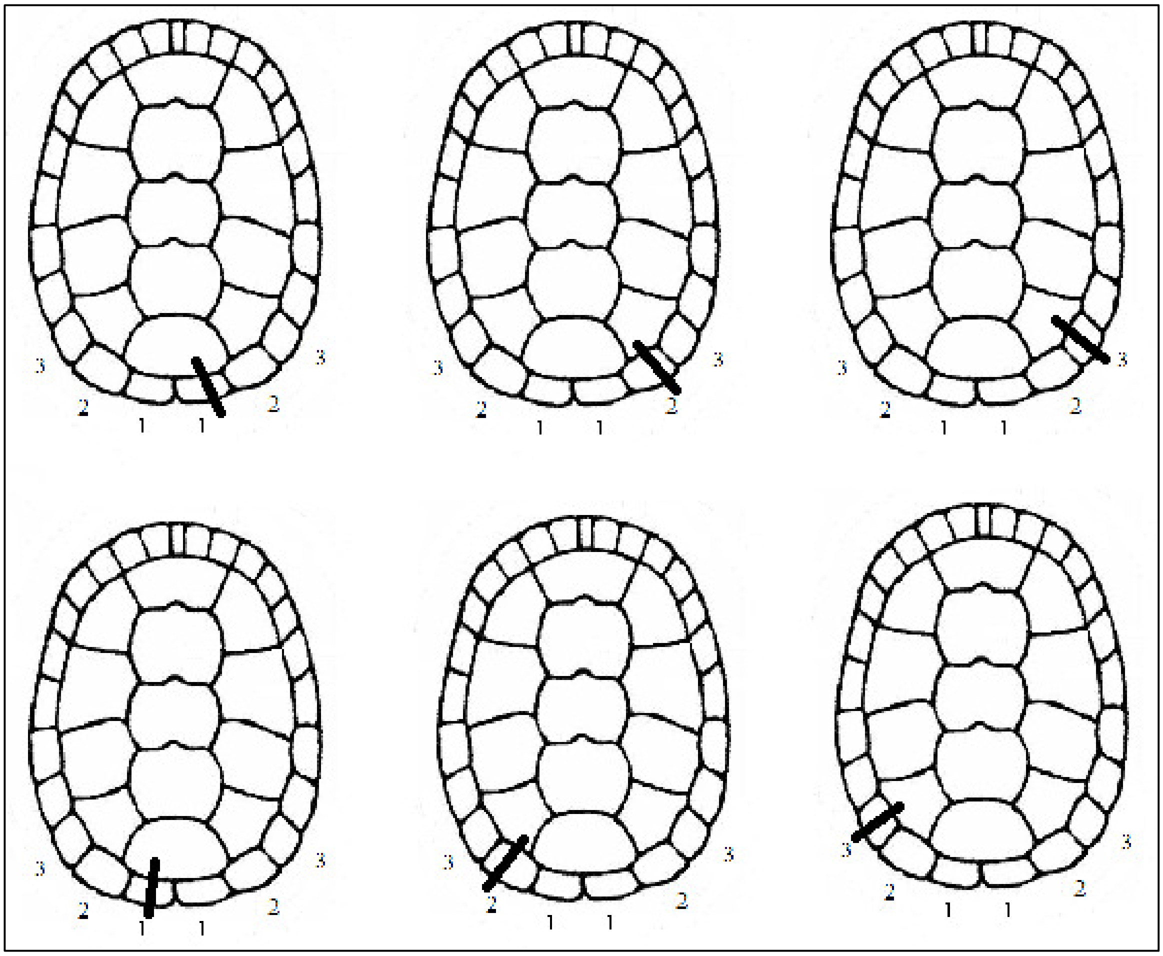

FIGURE 9. Illustration showing the location of the notch made on the marginal scute unique to each pond sampled. Top Left: Little Salt Marsh, Top Center: Park Smith Pond, Top Right: Lake Darrynane Complex, Bottom Left: Big Salt Marsh, Bottom Center: Y-Road Complex, Bottom Right: T-Intersection Pond.

FIGURE 10. Example of carapace file marking on a hard shelled turtle species used for identification of recaptured individuals in the 2015 sampling season.

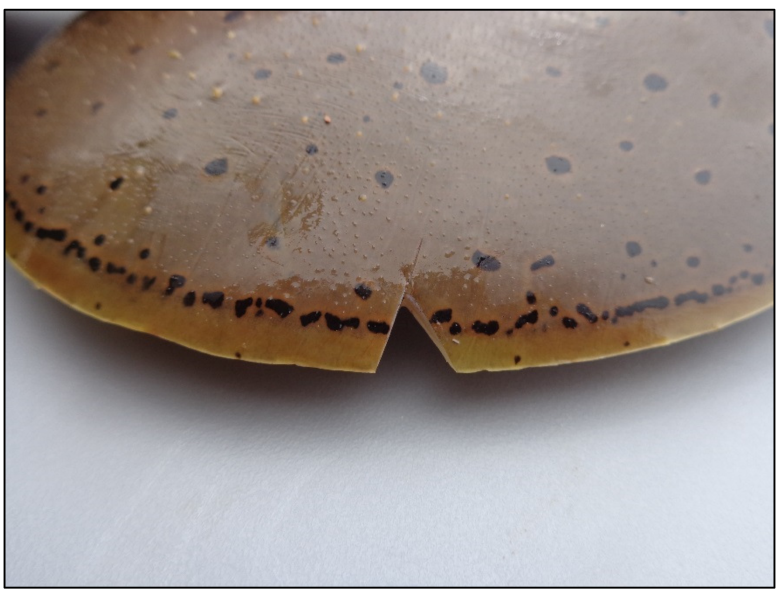

FIGURE 11. Example of carapace clipping mark on a softshell turtle species used for identification of recaptured individuals in the 2015 sampling season.

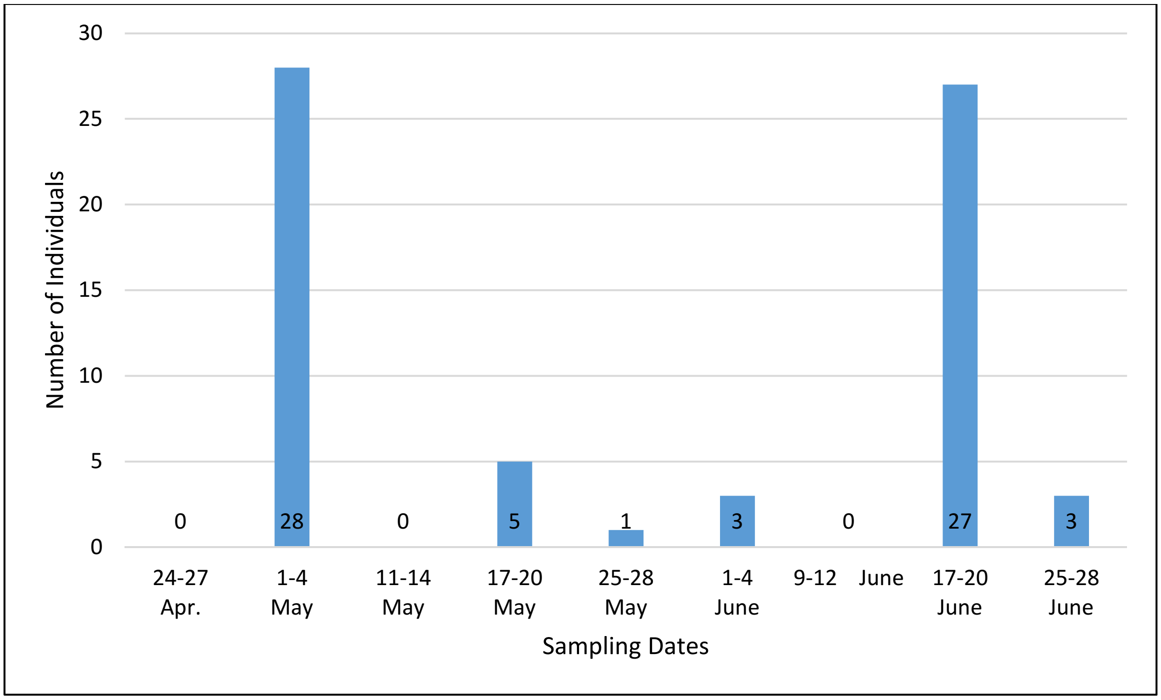

FIGURE 12. Total number of individual captures by rotation of *Apalone spinifera* for the 2015 sampling of Quivira National Wildlife Refuge.

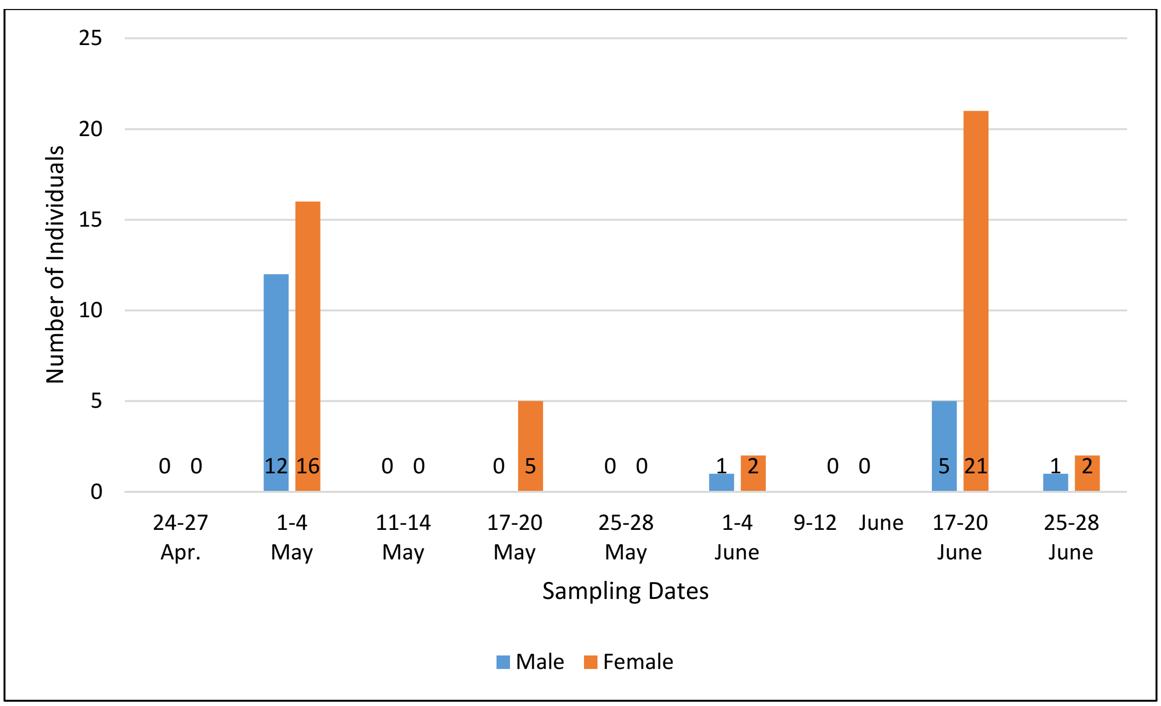

FIGURE 13. Total number of individual male and female captures by rotation of *Apalone spinifera* for the 2015 sampling of Quivira National Wildlife Refuge.

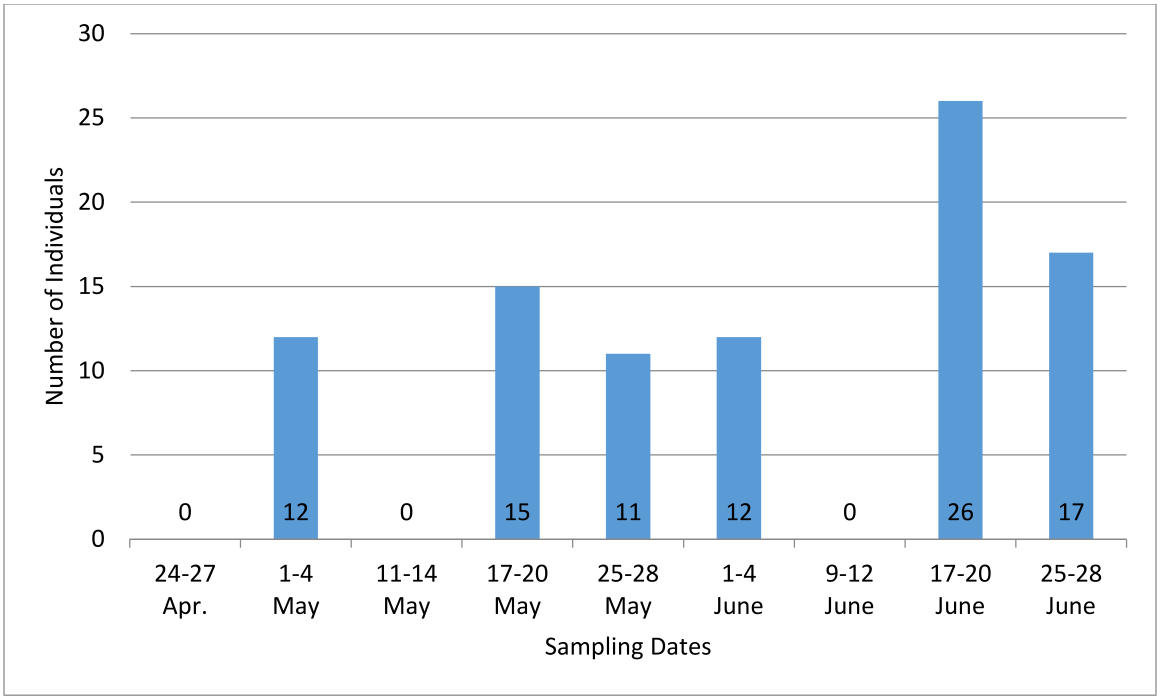

FIGURE 14. Total number of individual captures by rotation of *Chelydra serpentina* for the 2015 sampling of Quivira National Wildlife Refuge.

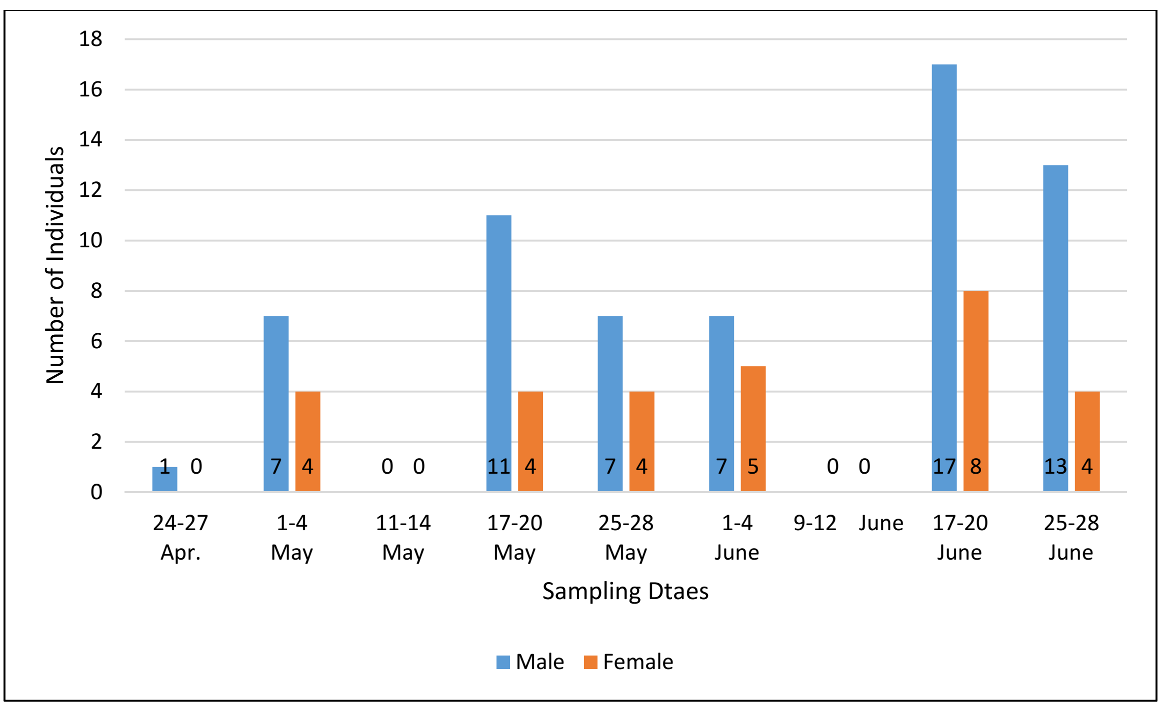

FIGURE 15. Total number of individual male and female captures by rotation of *Chelydra serpentina* for the 2015 sampling of Quivira National Wildlife Refuge.

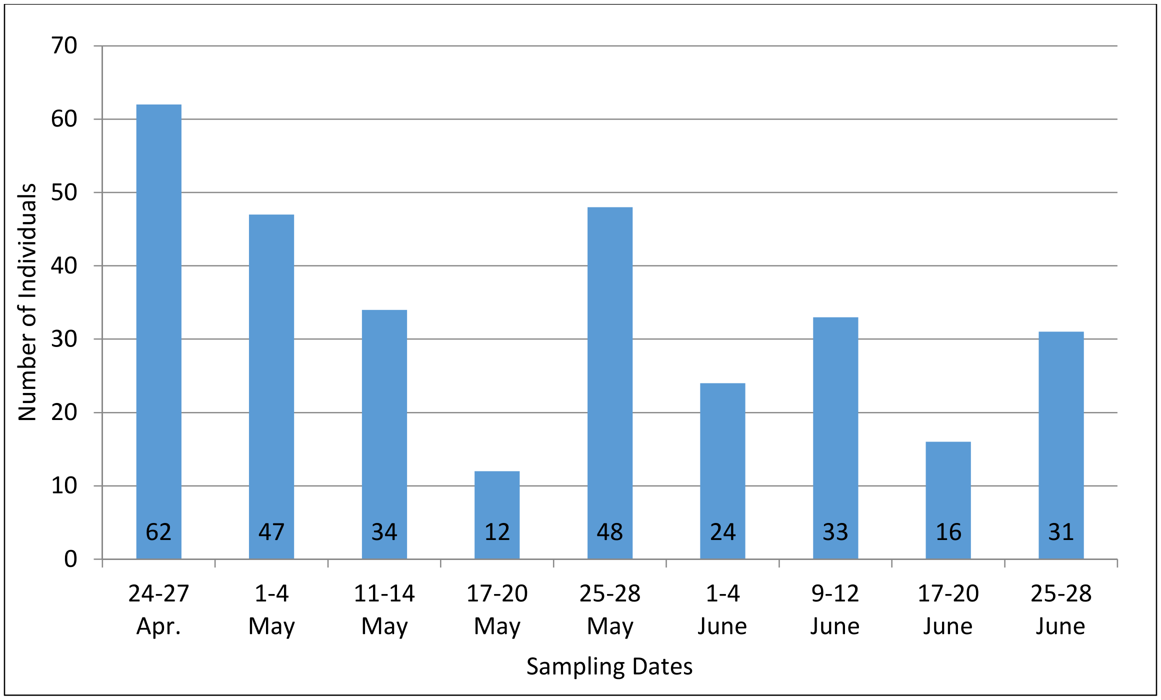

FIGURE 16. Total number of individual captures by rotation of *Kinosternon flavescens* for the 2015 sampling of Quivira National Wildlife Refuge.

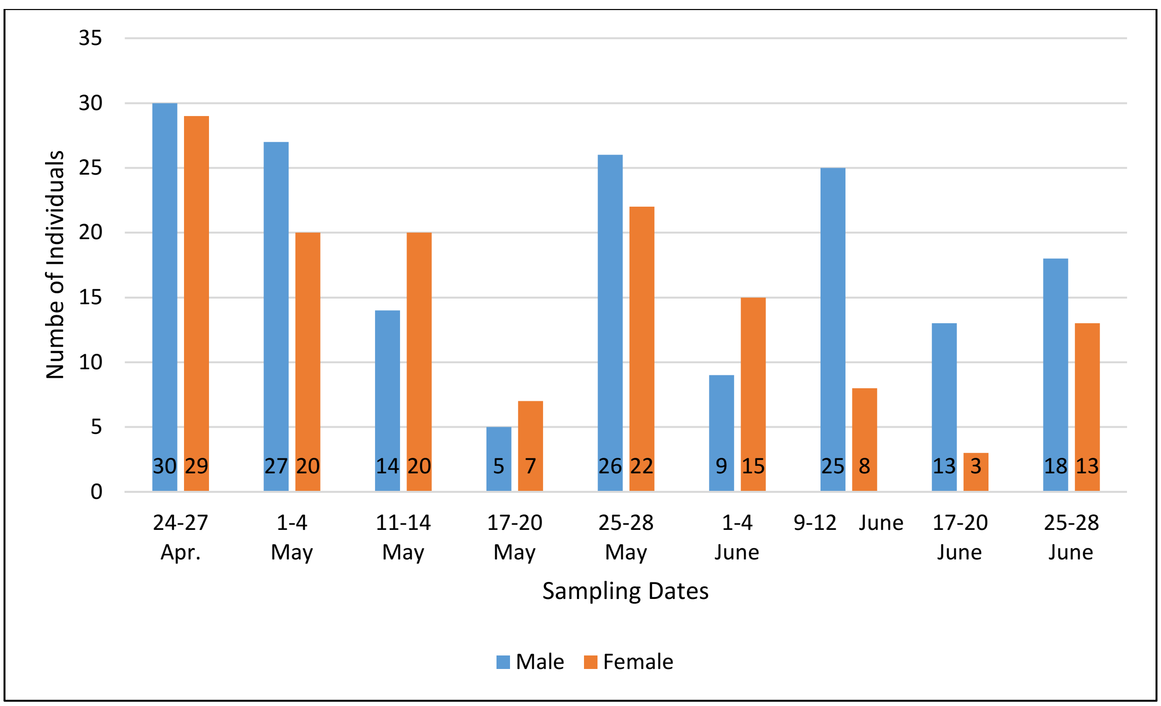

FIGURE 17. Total number of individual male and female captures by rotation of *Kinosternon flavescens* for the 2015 sampling of Quivira National Wildlife Refuge.

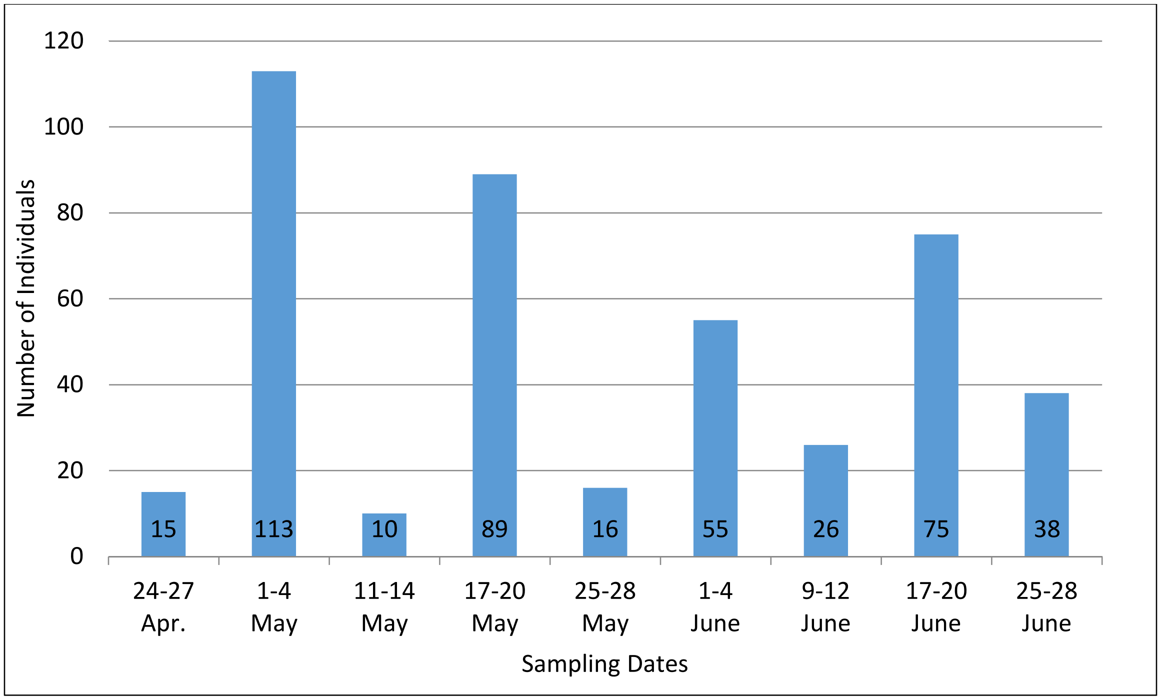

FIGURE 18. Total number of individual captures by rotation of *Trachemys scripta* for the 2015 sampling of Quivira National Wildlife Refuge.

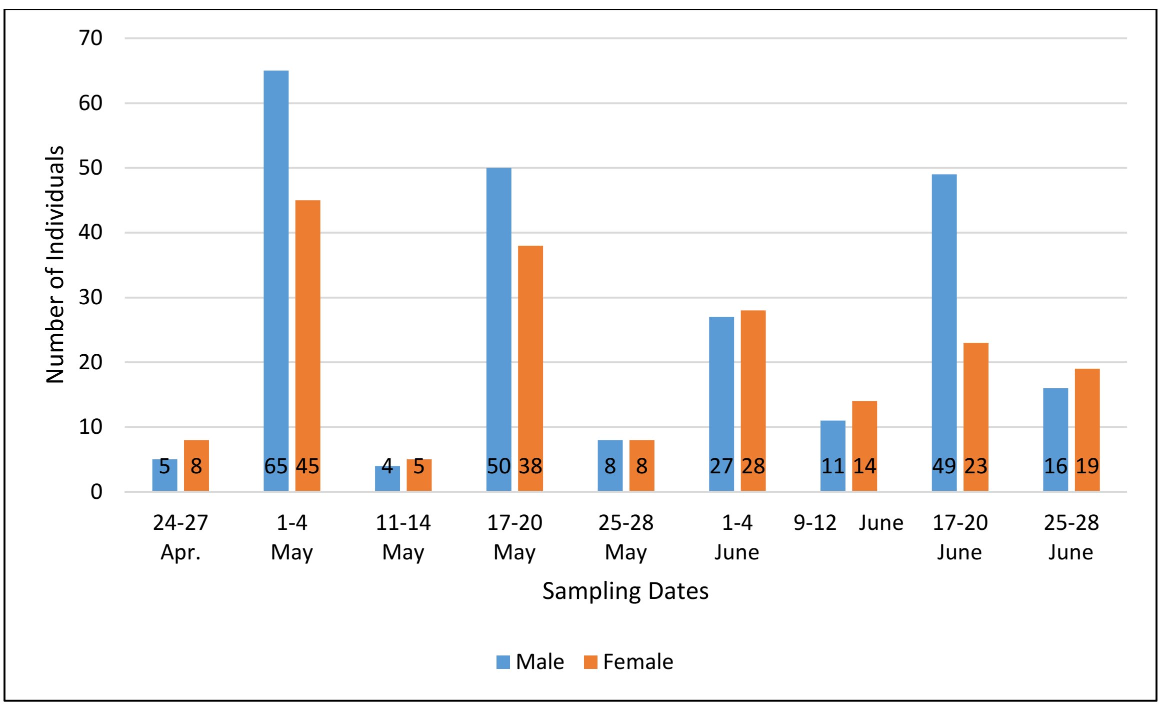

FIGURE 19. Total number of individual male and female captures by rotation of *Trachemys scripta* for the 2015 sampling of Quivira National Wildlife Refuge.

APPENDIX 1. Lengths of carapace and plastron measurements for age class association for each species of turtle sampled on QNWR in 2015. Values used for age classes were obtained from Ernst and Lovich (2009), in which studies conducted closest to Kansas were used as referenced values.

Species Name	**Juvenile Male**	**Sub-adult Male**	**Adult male**	**Juvenile Female**	**Sub-adult Female**	**Adult Female**
A. mutica (plast.)	< 8 cm	8.1-9 cm	9.1+ cm	< 14 cm	14.1 – 15 cm	15.1+ cm
A. spinifera (plast.)	< 8 cm	8.1-9 cm	9.1+ cm	< 18 cm	18.1 – 20 cm	20.1+ cm
C. serpentina (plast.)	< 14 cm	14.1 – 15 cm	15.1+ cm	< 12 cm	12.1 – 17 cm	17.1+ cm
K. flavescens (carap.)	< 8 cm	8.1-9 cm	9.1+ cm	< 8 cm	8.1 – 12 cm	12.1+ cm
C. picta (plast.)	< 7 cm	7.1-9 cm	9.1+ cm	< 9 cm	9.1 – 12 cm	12.1+ cm
T. scripta (plast.)	< 9 cm	9.1 -10 cm	10.1+ cm	< 15 cm	15.1 – 19 cm	19.1+ cm

APPENDIX 2. Geographic Positioning System (GPS) points for the individual trap sites for each wetland unit sampled on Quivira National Wildlife Refuge for the 2015 sampling season.

Pond	Point	Latitude	Longitude
Little Salt Marsh (LSM)	T1	N 38.09818	W 98.48106
	T2	N 38.09697	W 98.47952
	T3	N 38.09305	W 98.47855
	T4	N 38.09568	W 98.47918
	T5	N 38.09784	W 98.48036
	T6	N 38.10010	W 98.48289
	T7	N 38.10309	W 98.48534
	T8	N 38.10339	W 98.48858
	T9	N 38.10400	W 98.49024
	T10	N 38.09029	W 98.49978
	T11	N 38.08947	W 98.49974
	T 12	N 38.08877	W 98.49981
	T13	N 38.08805	W 98.49978
	T14	N 38.08746	W 98.49977
Park Smith Pond (PSP)	T1	N 38.14465	W 98.49146
	T2	N 38.14595	W 98.49142
	T3	N 38.14556	W 98.49026
	T4	N 38.14501	W 98.48945
	T5	N 38.14441	W 98.48907
	T6	N 38.14370	W 98.48927
	T7	N 38.14319	W 98.48945
	T8	N 38.14343	W 98.49091
	T9	N 38.14383	W 98.49126
Dorryanne Lake (DAM)	T1	N 38.14138	W 98.47310
	T2	N 38.14131	W 98.47382
	T3	N 38.14117	W 98.47430
	T4	N 38.14088	W 98.47456
	T5	N 38.14087	W 98.47949
	T6	N 38.13927	W 98.48029
	T7	N 38.14111	W 98.47779
Triangle-Intersection Pond (TIP)	T1	N 38.20121	W 98.4915
	T2	N 38.20119	W 98.4924
	T3	N 38.20121	W 98.49361
	T4	N 38. 20105	W 98.49386

APPENDIX 1. (continued)

	T5	N 38.20052	W 98.49435
	T6	N 38.19976	W 98.49435
	T7	N 38.19975	W 98.49384
Big Salt Marsh (BSM)	T1	N 38.18048	W 98.53146
	T2	N 38.17865	W 98.52997
	T3	N 38.17664	W 98.53091
	T4	N 38.17958	W 98.53086
	T5	N 38.14594	W 98.53211
	T6	N 38.17659	W 98.53294
	T7	N 38.17646	W 98.53327
	T8	N 38.17795	W 98.53454
Y-Road Pond (YRP)	T1	N 38.19806	W 98.54641

APPENDIX 3. Wetland units of Quivira National Wildlife Refuge, area in acres and hectares.

Name	Acres	Hectare
Big Salt Marsh	1209.47	489.46
Little Salt Marsh	931.46	376.95
Wildlife Drive	801.08	324.19
Marsh Road Meadow	493.83	199.85
North Flats/Lake	393.23	159.13
Salt Spring Meadow	252.94	102.36
Unit 61	121.46	49.15
Unit 58	116.37	47.09
Unit 63	103.16	41.75
Unit 50	90.55	36.64
Unit 14A	89.53	36.23
East Lake	88.88	35.97
Unit 49	85.2	34.48
Unit 26	69.11	27.97
Unit 20A	68.89	27.88
Unit 20B	65.83	26.64
Unit 7	61.97	25.09
Unit 28	60.85	24.63
Unit 48	55.18	22.33
DAM/Unit 24	54.1	21.89
Unit 25	53.98	21.84
Unit 37	49.8	20.15
Unit 14B	44.83	18.14
Unit 30	41.57	16.82
TIP/Unit 62	37.52	15.18
Unit 40	36.37	14.72
Unit 11	29.71	12.02
Unit 29	27.32	11.05
No Name 1	26.99	10.92
No Name 2	26.16	10.59
Unit 10A	19.2	7.77
Unit 10B	14.29	5.78
Unit 16	14.24	5.76
Park Smith Pond	14.06	5.69
Migrants Mile	12.05	4.88
Unit 12B	11.47	4.64
Unit 21	11.25	4.55
Unit 91	8.43	3.41

APPENDIX 3. (continued)

No Name 3	7.09	2.87
Unit 90	7.05	2.85
Unit 10C	6.89	2.79
No Name 4	6	2.43

APPENDIX 4. Calculated average of local vegetation community and measured salinity for each sampled wetland unit of Quivira National Wildlife Refuge for 2015.

Wetland Unit	**Little Salt Marsh**	**Dorrynane Lane Complex**	**Park Smith Pond**	**T-Intersection Pond**	**Big Salt Marsh**
Bare Ground	0	0.143	0.111	1.5	0
Grass	0.846	2.714	1.038	1.83	2.5
Forb	1	0.571	1.333	2.167	1.5
Tall Emergent	2.385	2.571	2.44	1	0.833
Submerged	0	0	0	0	2
Floating	0.308	0.429	0	2.33	1.167
Salinity	1.1	1.2	1.9	1.0	2.3