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# **BROOD SURVIVAL OF ATTWATER'S PRAIRIE-CHICKEN (TX)**

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# **INTRODUCTION**

The Attwater's prairie-chicken (APC) (Tympanuchus cupido attwateri) is endemic to prairies along the Texas and Louisiana gulf coast (Bendire 1894). Historically occurring in numbers that may have approached 1 million individuals on 6 million acres of habitat (Lehmann 1968), fewer than 110 have existed in the wild since 1995 despite intensive management intervention (USFWS 2010) (Figure 1). Recovery actions undertaken during the last 20 years have included habitat restoration, captive breeding and release, and a number of research projects focused on identifying factors limiting recovery progress (USFWS 2010). Post-release survival for captive reared birds returned to wild habitats has ranged from 8–43%, and averaged 17% annually. While lower than the approximately 50% annual survival reported for wild prairie-chickens, it is substantially higher than attempts with other pen-reared galliform species (e.g., Toepfer 1988, Pratt 2010, USFWS 2010, Rymesova et al. 2012). Estimates are that >66,000 acres of habitat exist in suitable condition for APC occupation (USFWS 2010). Even given a low carrying capacity estimate of 1 bird/50 acres (Lehmann 1941), this amount of habitat should support far more than the number of APC currently found there. The APC Recovery Plan identified poor survival of chicks in the wild as "...the single-most important factor limiting significant progress toward recovery" (USFWS 2010:40). Observations at the Attwater Prairie Chicken National Wildlife Refuge (APCNWR) in 2003 revealed total loss of 8 broods <12 days post-hatch. Several dead or dying chicks were found with brood hens at night roosts, indicating that predation was not the sole cause of chick mortality (USFWS 2010). Necropsy of these chicks attributed cause of death to inanition and dehydration.

Invertebrates are an important food source for young prairie-chickens (e.g., Lehmann 1941, Jones 1963, Rumble et al. 1988, Savory 1989, Hagen et al. 2005). Savory (1989) noted that invertebrate food comprised >90% of the diet of prairie-chickens <5-8 weeks old. Comparisons of insect abundance in APC brood habitat with that from an increasing Minnesota greater prairie-chicken (GPC) (*T. c. pinnatus*) population found that while total insect biomass did not differ between the areas, APC brood habitat supported <30% of insect numbers (i.e., >70% lower) compared to the GPC brood habitat (P < 0.001) (Pratt et al. 2003). In an attempt to confirm the importance of insect availability as a limiting factor for APC during the first 2 weeks post-hatch, broods (i.e., hen and chicks) were confined on APCNWR at the nest site immediately post-hatch and provided locally collected insects *ad libitum*. From 2004–2012, 83% of 547 chicks treated in this manner survived the critical 2-week period, and were subsequently released back to wild habitats. A number of these chicks survived to adulthood (APCNWR, unpublished data).

Red imported fire ants (RIFA) (*Solenopsis invicta*) were accidentally introduced to the United States at the port of Mobile, Alabama (Allen et al. 1995) circa 1930. Today this species occupies almost the entire southeastern United States (Porter and Savignano 1990). RIFA arrived in occupied APC range circa 1970 (Allen et al. 1995, <u>http://www.extension.org/pages/14911/texas-quarantine-map</u>). The disruptive impacts of RIFA on native invertebrate communities are well documented (e.g., Porter and Savignano 1990, Allen et al. 2001, Wojcik et al. 2001). Specifically, Porter and Savignano (1990) found that species richness of non-ant arthropods was 30% lower in sites infested by RIFA, and numbers of individuals were 75% lower.

Adverse impacts of RIFA on a wide variety of fauna besides invertebrates also are well documented (e.g., Porter and Savignano 1990, Drees 1994, Allen et al. 1995, Allen et al. 1997, Mueller et al. 1999, Allen et al. 2001, Wojcik et al. 2001, Allen et al. 2004). Allen et al. (1995) found that Texas northern bobwhite quail (*Colinus virginianus*) populations consistently declined after RIFA infestation, whereas no significant trend existed before infestation, and no consistent population trends were apparent in uninfested counties. These investigators also found that bobwhite densities were more than 2 times greater on sites where RIFA were suppressed. Mueller et al. (1999) documented 2.7 times greater survival of bobwhite chicks to 21 days when RIFA were suppressed around nests. Allen et al. (2001) found that insect biomass and loggerhead shrike (*Lanius ludovicianus*) abundance were negatively correlated with RIFA infestation. APC populations also consistently declined during the 25-year period following invasion of APC habitat by RIFA circa 1970 (Figure 1). Therefore, it is clear that RIFA have negatively impacted the Texas coastal prairie ecosystem and ecosystems throughout the southeastern U.S. since their arrival in the 1930's (Allen et al. 2004).

During 2011–2012, we investigated the relationship between invertebrate abundance and APC brood survival and the effects of RIFA on invertebrate communities over a broad area at multiple large-scale treatment sites during the APC's early brooding season (May–mid-June). Additionally, we collected information on hen source (i.e., captivity or wild-reared) and age to evaluate potential impacts of the captive-rearing environment on the ability of hens to successfully rear young. Finally, we examined impacts of grazing, a ubiquitous land management practice within current APC range, on invertebrate abundance during APC brooding season. Specific objectives were to:

(1) evaluate the impacts of RIFA on APC brood habitat quality as indicated by invertebrate abundance,

(2) evaluate the role of grazing on invertebrate populations during APC brood rearing periods,

(3) evaluate the influence of invertebrate abundance with respect to APC brood survival, and

(4) evaluate the influence of hen age and time since release from captivity on APC brood survival.

# METHODS

# Study Sites

This 2-year study was conducted at 5 replicate sites spanning approximately 150 miles of the Texas gulf coast prairie ecosystem (Hatch et al. 1990) (Figure 2). All 5 sites consisted of managed grassland and rangeland where dominant grass species included little bluestem (*Schizichyrium scoparium*) or cordgrass (*Spartina* spp.) Species like brownseed paspalum (*Paspalum plicatulum*), dropseed (*Sporobolus* spp.), or Texas wintergrass (*Stipa leucotricha*) increase in prevalence at these sites under heavy grazing pressure.

<u>APCNWR</u> - On the northern edge of the gulf prairie region approximately 60 miles west of Houston in Colorado County, Texas, study sites on APCNWR were dominated by loamy and sandy ecological sites with a small percentage of lowland and claypan inclusions (Brown 2006). Little bluestem is the dominant

grass in the climax community of this site, accompanied by a wide diversity of primarily native grasses and forbs. Grasslands were managed for a heterogeneous landscape by burning 40–70-acre patches on a 4-year rotation (Fuhlendorf and Engel 2001, Fuhlendorf et al. 2006). Light to moderate grazing occurred throughout the refuge. For the 2010–2012 spring seasons (January–June), grazing was deferred in both treated and untreated control areas on APCNWR to eliminate grazing as a variable. Individual plant treatment and limited spot-treatment of brush and non-native plant species with herbicides occurred on study sites within the refuge.

<u>Texas City Prairie Preserve (TCPP)</u> - Situated along the shore of Galveston Bay about 40 miles south of Houston in Galveston County, Texas, TCPP encompasses approximately 2,300 acres of mixed grassland and wetland habitat. The study site was located in salty prairie, however blackland prairie ecological sites were present as well (Crenwelge 1988). Gulf cordgrass, little and seacoast (*S. s. littorale*) bluestems are the dominant grasses (Pratt 2010). Burning has historically been conducted at TCPP as a management tool; however, no burning was done just prior to or during this study. Heavy, continuous grazing was conducted during the duration of the study (A. Tjelmeland, Texas City Prairie Preserve, personal communication). Treatment of brush and non-native species occurred in fall of 2011, when approximately 25% of the treated area was aerially sprayed with Plateau® herbicide for deep-rooted sedge (*Cyperus entrerianus*). During the study, other spot applications of Plateau® were made in scattered locations within the study site (K. Feuerbacher, The Nature Conservancy of Texas, personal communication).

<u>*Midwell*</u> - Approximately 50 miles north of Corpus Christi in eastern Goliad County, the Midwell study site was located on a private cattle ranch consisting of mostly claypan prairie ecological sites (Wiedenfeld 2010; C. Carter, Ranch Consultant, NRCS retired, personal communication). The dominant climax species is little bluestem. Prescribed burning was conducted shortly before the first application of ant bait in 2010 to half of the study area, which resulted in half of the treated and half of the control area being burned. No other burns were conducted during the study. Short-duration rotational grazing consisted of approximately 21 days of grazing followed by 85 days of rest for each pasture and resulted in light– moderate grazing pressure. This entire study site was aerially treated with 2,4-D, a broadleaf herbicide in late April–early May of 2012 in a strip pattern which resulted in 75-90% coverage to suppress forbs and minimize competition to forage grasses (C. Carter, personal communication).

<u>W. Clarkson</u>– In Goliad County, about 10 miles east of the Midwell site and with similar vegetation, W. Clarkson is a privately-owned ranchland with mixed sandy prairie and claypan prairie ecological sites (Wiedenfeld 2010; C. Carter, personal communication). The dominant climax species is little bluestem. This area was not burned before or during the study. The site was grazed in a 3-pasture rotational system at moderate–heavy levels during the study, partially due to drought conditions during the summer of 2011 (Figure 3). Like the Midwell site, this entire study site was strip-sprayed with 2,4-D in the spring of 2012 which resulted in about 75-90% coverage of the area.

<u>*Tivoli*</u>– In Refugio County, and also on private ranchland, this site was approximately 20 miles east of the 2 Goliad County sites and consisted of almost entirely heavy clay soils (blackland range site). The climax vegetation for this ecological site is primarily little bluestem (Guckian 1988). The Tivoli site had no history of prescribed burning for >25 years. This site received moderate–heavy continuous grazing

during the study. Especially in the spring of 2012, the grazing level was heavy due to historic drought conditions which began in fall–winter 2010 (C. Carter, personal communication) (Figure 4).

# Climate

Extreme drought conditions existed at all 5 study sites during 2011 (Figures 3–6). Extremely dry and hot conditions prevailed throughout the region until fall of that year, when some areas began to receive rain. By the end of 2011, rainfall totals were 25, 28, 14, and 19 inches (or 41, 49, 57, and 43%) below the long-term average for APCNWR, TCPP, Goliad County (2 sites), and Tivoli, respectively. By the fall and winter of 2011–2012, rainfall returned to near long-term average monthly amounts. However, a large multi-year rainfall deficit remained at the end of this study at all 5 sites (Figures 3–6).

## Objective 1

To assess the impact of RIFA on APC brood habitat quality as indicated by invertebrate abundance, we used an impact-reference design with 5 sets of replicates in space and 3 replicates in time during each of 2 years. At each of the 5 replicate sites, 1 area was randomly assigned to treatment and 1 to untreated control. Within each area, 14 sampling plots (0.5-acre) were randomly chosen. Sampling plots were >200 feet from treatment edges, and were stratified by ecological site and time since burn to the extent practicable. The 440–725-acre treated areas received an application of Extinguish® Plus brand fire ant bait to reduce RIFA abundance. Extinguish® Plus was applied at the recommended label rate of 1.5 lbs/acre by helicopter during early November 2010 and again in late September 2011 during weather conditions appropriate for application (no moisture on vegetation, temperatures > 70°F). Observations on the ground during application verified the bait was picked up by RIFA. Sampling began the last week in April and continued for 3 consecutive bi-weekly periods through early-June each year.

Sampling at each plot consisted of (1) collecting an invertebrate sample and (2) assessing RIFA activity. Invertebrate samples were obtained by randomly choosing a direction to sweep and then walking 10 steps from the plot center. The vegetation was vigorously swept with a heavy duty, 38-cm diameter sweep net 25 times toward the perimeter of the circle. Each of the 25 sweeps was a single motion to one side or the other. Samples were placed in a plastic bag, labeled, and placed in a freezer for at least 24 hours until sorting could be performed. At that time, all invertebrates were separated from vegetation into groups, counted, dried for approximately 24 hours at 140°F, weighed to the nearest 0.0001 gram, and stored in a vial with alcohol. Invertebrates were placed in 1 of 2 groups to differentiate between those that were (1) older/larger/winged and therefore possibly could have migrated into the plot, from (2) invertebrates that were younger/smaller/unwinged and therefore less likely to have migrated from outside the treatment areas. Furthermore, these 2 groups likely represented functional groups for APC chicks since the larger more mobile insects (Group 1) were less likely to be fed upon by chicks than the smaller, less mobile, younger (Group 2) invertebrates (Table 1). Grasshoppers and katydids were considered adults when the wings protruded beyond the posterior tip of the abdomen. Nymphs with wings not protruding beyond the abdomen tip were assigned to Group 2.

RIFA and native ant activity were assessed within each sampling plot by placing 10 fatty lures (approximately 0.5-inch hot dog slices) on the ground using surveyor's flags, one in the center and 9

spaced roughly equidistantly around the perimeter of the 0.5-acre plot. After 45-60 minutes the number of RIFA and native ants on the top and sides of each hot dog slice was estimated and recorded (Porter and Tschinkel 1987). Number of ants present were estimated in increments of 10 up to a maximum of 100. Even if more than 100 RIFA were present, it was recorded as 100.

Analysis of data was conducted for Group 1, Group 2, and Group 1 + Group 2 (i.e., total) numbers and dry weights using R 2.15.2 (http://www.r-project.org/) statistical packages. Data were transformed using a Box-Cox transformation to better approximate a normal distribution (Crawley 2007). Fit to the normal distribution was assessed using quantile-comparison plots available in R. A suitable transformation was not found for Group 1 because so many observations were grouped at 0. Therefore, tests for treatment effects on Group 1 number and weight were done using a non-parametric Friedman's rank sum test for each year using site as a block (Daniel 1978). The  $H_0$ : treatment samples contained more numbers (or weight) of invertebrates than untreated control samples for each year was evaluated with the friedman.test in the R stats package. For this test, samples were summed by each site-treatment-year combination to remove pseudoreplication associated with the 3 replicate time periods within a year and plots within study site. The remaining data for Group 2 and total were subjected (separately for each response variable) to linear mixed effects regression (LMER) available in the R package lme4 to control for pseudoreplication (Crawley 2007). Initial models were of the form:

#### *Response Variable* ~ *TRT*\**year* + (*period/site/sample*)

where *Response Variable* = Group 2 or total number or dry weights for each sample, *TRT*=treatment or control (untreated), year=2011 or 2012, period=biweekly sampling period within year, site=study site, and *sample*=plot within study site. *TRT* and *year* were fixed effects, while the (*period/site/sample*) term specified the random effects of sampling period on samples collected at plots within study sites. TRT was the primary variable of interest. Year was included as a fixed effect for 2 reasons: (1) the 2 years of the study were dramatically different, so we wanted to evaluate any possible TRT\*year interactions, and (2) because there were only 2 levels of year, the LMER would have been unable to obtain a good estimate of variation if year were included as a random term (Crawley 2002). Models were simplified by sequential removal of non-significant (P > 0.05) TRT<sub>\*</sub>year interaction and removal of uncorrelated or near-zero random intercept or regression coefficient terms (Bates 2010). Maximum likelihood estimates of each sequential model version were tested against the previous model using ANOVA from the R stats package (Crawley 2007). Final models were evaluated using model critique plots contained within the R package LMERConvenienceFunctions. Model fit was improved by trimming observations where residuals were >2.5 standard deviations from 0 using the Exclude Outliers function of LMERConvenienceFunctions. Trimming was done only 1 time, and resulted in the maximum exclusion of 18 (2%) observations. In no case did dataset trimming result in substantive changes in model results. Significance values for LMER analyses were extracted using pamer.fnc within the LMERConvenienceFunctions package.

# Objective 2

To evaluate the role of grazing on invertebrate abundance during APC brood rearing season, invertebrates were collected at APCNWR only by sweep netting in the manner described for Objective 1. For this

assessment, grazed and ungrazed plots were stratified by ecological site within areas not treated to suppress RIFA and chosen at random. Severe drought conditions in 2011 (Figure 5) required removal of most cattle on APCNWR during that year to protect rangeland resources and APC habitat. Therefore, samples were collected for this objective only during 2012. We supplemented those data with similar data we collected in 2010 on APCNWR. Ungrazed areas had not been grazed since late January 2010, and early January 2012. Invertebrate samples were collected in May 2010 and June 2012. Once collected, samples were processed as described for Objective 1, except only total numbers and dry weights for each sample were determined. Differences in vegetative cover and structure were also assessed to determine if there were any correlations between invertebrate abundance and vegetative characteristics. Percent cover of grasses, forbs, and bare ground were measured by the Daubenmire (1959) method using a 0.25-m<sup>2</sup> frame at the center and each of the plot corners. Vegetative height was measured by taking photographs at each of the 5 points/plot while using a 4-foot square white dotboard as a constant background (Newell 1987, Toepfer 2003, Hunt et al. 2005). Maximum and effective height of vegetation intersecting the dotboard was determined at 10 points on the board for each photo. The repeated measures for each photo and plot were averaged and compared using the median test (Daniel 1978).

## **Objective 3**

To evaluate the influence of invertebrate abundance with respect to APC brood survival, invertebrate samples were collected by sweep-netting as described for objective 1 once daily at APC brood sites during the first 2 weeks after hatch. Brood sites were determined by triangulating radioed hens at APCNWR during 2009–2012, and at Goliad sites during 2011–2012. Samples were collected 1–2 days later to minimize potential disturbance to the brood. Samples were processed as described for Objective 2. At 2 weeks post-hatch, the radioed hen was observed at dawn (i.e., before leaving the night roost and while they should still be brooding their chicks) to determine if any chicks were still alive. A brood was considered successful if at least 1 chick was observed. No attempt was made to obtain a total count of chicks present. Median measures of invertebrate abundance/sample (i.e., number, dry weight) were determined for each brood unit to remove pseudoreplication associated with multiple samples/brood. These medians were then subjected to the Wilcoxon rank-sum test contained in the R stats package using the 1-tailed  $H_0$  that invertebrate abundance measures for successful broods were less than or equal to those for unsuccessful broods versus  $H_A$  that abundance measures were greater for successful broods.

#### **Objective 4:**

The influence of hen characteristics on APC brood survival was evaluated by comparing the success of broods as described for Objective 3 with hen age (second year (SY) versus after second year(ASY)), hen source (captive or wild), and for hens released from captivity, time since release. Additionally, we evaluated whether broods hatched in RIFA suppressed areas survived better than those hatched in untreated control areas. Chi-square tests of independence contained in the R stats package were used to evaluate the hypothesis:  $H_0$ : distribution of brood success was independent of hen characteristics (i.e., age, years from captivity, hen source).

# RESULTS

#### **Objective** 1

RIFA activity indices (i.e., RIFA/fatty lure) were lower (P < 0.03) on treated areas compared to untreated controls for both years of the study (Figure 7). The RIFA activity index on treated sites ranged from 0–65% of those observed on untreated control plots (i.e., 35–100% suppression) across sites and years, but more suppression was achieved during the second year of the study (after second year of treatment) (Figure 7). The index of RIFA activity was generally lower for both treated and untreated control plots in 2012 compared to 2011 (Figure 7), and was especially low for untreated controls for all sites but APCNWR in 2012. All sites except APCNWR averaged only 3–4 RIFA/fatty lure in untreated control plots in 2012, whereas APCNWR averaged 71 RIFA/fatty lure on untreated controls. The RIFA activity observed at APCNWR was typical of the average 75 RIFA/fatty lure observed for untreated controls across all sites in 2011. It is not clear why RIFA activity was so low on untreated controls except at APCNWR in 2012. However, regardless of the absolute RIFA activity index, the relative RIFA activity for treated plots compared to untreated controls was consistent across sites for both years, and averaged 47% and 16% in 2011 and 2012, respectively (Figure 7).

After removing 9 samples with missing or uncertain observations, a total of 831 invertebrate samples were available for analysis: 168 each for Midwell, W. Clarkson, and Tivoli, 166 for APCNWR, and 161 for TCPP. Measures of invertebrate abundance were highly variable across years and sites (Tables 2–4, Figures 8–19). However, median invertebrate data were generally more or less consistent across sites except for APCNWR totals (number and biomass) and Group 1 (number and biomass) during 2012 (Figures 8–19). In that year, these parameters at APCNWR were substantially higher *for the untreated control plots*. Because of this anomaly, in addition to analyzing all 5 sites together, data were also analyzed for APCNWR by itself and for the other 4 sites excluding APCNWR. Treatment<sub>\*</sub>year interactions were not significant (P > 0.05) in any of the final LMER models.

*Total invertebrate numbers/sample.* Overall, median total invertebrates/sample was 1.4 times higher for treated sites compared to untreated controls. The linear mixed effects regression indicated significant (P < 0.0001) fixed effects for both treatment and year (Table 5). Similar to the overall median effect, the back-transformed regression coefficient for treatment indicated a 42% increase in total invertebrate numbers/sample on RIFA suppressed sites, while that for year indicated an average 53% reduction in 2012 numbers/sample compared to 2011. Removing APCNWR from the dataset resulted in a regression coefficient of 1.60 for treatment (60% increase in numbers due to RIFA suppression) and 0.28 for year (72% reduction in 2012) (Table 6). For APCNWR alone, the LMER coefficients showed the opposite - a negative treatment effect and a substantial increase in 2012 compared to 2011 (Table 6). However, the APCNWR treatment coefficient was not different from 0 (P = 0.29), whereas the 3.36 coefficient for year indicated a highly significant (P < 0.0001) increase in invertebrate numbers for 2012 on APCNWR (Figures 8, 9).

*Total invertebrate biomass/sample.* Median dry weight of invertebrates/sample overall was 1.6 times higher for treated sites compared to untreated controls. The LMER analyses for all sites resulted in regression coefficients of 1.28 (P < 0.0001) and 0.94 (P = 0.10) for treatment and year, respectively (Table

5). Thus, dry sample biomass was 28% higher on treated sites, and 6% lower in 2012. Removing APCNWR resulted in regression coefficients of 1.38 (P < 0.0001) for treatment and 0.87 (P = 0.002) for year, while those for APCNWR alone were 0.92 (P = 0.63) and 1.20 (P < 0.01) for treatment and year, respectively (Figures 10,11).

*Group 1 invertebrate numbers and weight/sample.* Because a transformation could not be found for Group 1 parameters that resulted in an approximately normal distribution, LMER analyses could not be conducted for these data. Friedman's rank sum tests on Group 1 numbers indicated a significant difference due to treatment in 2011 (P = 0.025), but not for 2012 (P > 0.17) (Figures 12,13). For Group 1 dry weight, the Friedman's test was not significant for either year (P > 0.17) (Figures 14,15).

*Group 2 invertebrate numbers/sample.* The LMER analysis for Group 2 numbers/sample resulted in a regression coefficient 1.68 (P < 0.0001) for treatment and 0.24 (P < 0.0001) for year (Table 5, Figures 16, 17). This indicates that on average, RIFA suppressed plots had 68% more Group 2 invertebrates/sample, and 2012 had 76% fewer/sample than 2011. Removing APCNWR resulted in regression coefficients of 1.77 (P < 0.0001) and 0.16 (P < 0.0001) for treatment and year, respectively, while those for APCNWR alone were 1.02 (P = 0.94) for treatment and 1.25 (P = 0.14) for year (Table 6, Figures 16,17).

*Group 2 invertebrate biomass/sample.* The LMER analyses for Group 2 dry weight/sample for all sites yielded regression coefficients of 1.27 (P < 0.0001) and 0.95 (P = 0.12) for treatment and year, respectively (Table 5, Figures 18,19). Removing APCNWR, the respective coefficients were 1.34 (P < 0.0001) and 0.91 (P < 0.02) (Table 6). For APCNWR alone, LMER regression coefficients were 0.97 (P = 0.82) and 1.10 (P < 0.07) for treatment and year, respectively, indicating no treatment effect and only a marginal year effect (Table 6, Figures 18,19).

# **Objective 2**

Data for this objective were collected at APCWNR only, and only during 2012. Because of severe drought conditions in 2011 (Figure 5), cattle numbers were drastically reduced on APCNWR. Cattle numbers remained substantially reduced in 2012 to allow pastures to recover from 2011 drought conditions. Grazing pressure in pastures used in Objective 2 assessments were estimated at light (<20% estimated forage removed) from March (beginning of the growing season) to mid-June when these assessments were made. Because we were unable to collect data for this objective in 2011, we included data we previously collected in 2010 (Table 7). Grazed pastures in 2010 received light-moderate (estimated 12–38% forage removed) pressure from March–May when data were collected. In 2010, median insect numbers were 46% higher on ungrazed sites, but were reversed in 2012 (57% higher on grazed sites). The same relationships held for median dry weight, with the magnitude of differences 23% and 72% for 2010 and 2012, respectively. Similar relationships were observed for vegetation parameters, except for percent bare ground which was higher at grazed sites in both years (Table 7). However, only effective height was significantly different (P < 0.07) between grazed and ungrazed sites in 2010, whereas in 2012 percent grasses (less for grazed, P = 0.001), percent forbs (higher for grazed, P < 0.06), and percent bare ground (higher for grazed, P = 0.01) were significantly different. There was no difference (P > 0.21) in effective height between grazed and ungrazed sites in 2012. Sample sizes for grazed sites were substantially increased in 2012 (Table 7).

## **Objective 3**

Brood survival data were collected from 34 broods at APCNWR from 2009–2012, and 10 broods from Goliad County (Midwell and adjacent properties) in 2011–2012. Of these, 21 (48%) were successful (i.e., still had chicks at 2 weeks post-hatch). Overall, median invertebrate numbers were 2.1 times higher (P < 0.001) at successful brood sites compared to unsuccessful sites (128 versus 60, respectively). For APCNWR and Goliad, successful brood site median invertebrate numbers were 1.7 (P = 0.02) and 3 (P < 0.01) times higher, respectively than at unsuccessful sites (Figure 20). Similarly, median sample dry weights were 1.7 times higher (P = 0.01) for successful (0.761 g) compared to unsuccessful (0.449 g) broods. For APCNWR, the dry weight difference was less (1.2 times for successful broods, P>0.12) compared to Goliad (3.8 times, P < 0.02) (Figure 21). Of the 271 total invertebrate samples collected from brood sites, only 34% were from treated areas. No difference (P > 0.34) in invertebrate number or dry weight was detected between brood site samples collected in treated versus untreated control areas.

# **Objective 4**

Twenty-seven of 44 brood hens (61%) nested in areas treated to suppress RIFA. In general, more brood hens were SY (i.e., young hens) (57%) as opposed to ASY (adults), released from captivity (84%) (as opposed to hatched in the wild), had not nested previously (64%), and had not previously fledged chicks (84%). Chi-square tests of independence detected no differences (P > 0.52) between successful and unsuccessful broods attributable to hen age, hen source, whether hens nested in treated areas, or whether hens had previously nested or fledged chicks.

# DISCUSSION

Data collected in this study clearly demonstrate that availability of invertebrates during the first 2 weeks post-hatch is a major factor limiting survival of young APCs (Figures 20,21). Abundance measures for both invertebrate numbers and biomass were roughly 2 times higher at successful brood sites than those in which all chicks ultimately perished during this period. These observations are consistent with numerous other studies affirming the importance of invertebrates to galliform chicks during the first few weeks of life (Savory 1995), and specifically for prairie-chickens (e.g., Lehmann 1941, Jones 1963, Savory 1989, Hagen et al. 2005). Hagen et al. (2005) specifically highlighted the importance of insects during the first 2 weeks of life for galliform chicks. Green (1984) and Hill (1985) found that survival of partridge (*Alectoris rufa, Perdix perdix*) and ring-necked pheasant (*Phasianus colchicus*) chicks during the first 20 days were correlated with arthropod abundance. Hill (1985) also observed that the abundance of insects within the home range of pheasant broods explained 75% of the variation in chick survival. Lehmann (1941:26) observed that the stomachs of 3 juvenile APC contained 88.5% insects.

No other attributes of hens (age, released from captivity or wild-hatched, years since release for captivereared hens, previous nesting experience or success with fledging chicks, or nesting within RIFA suppressed areas) hypothesized to affect brood success were significant (P > 0.52). Some have hypothesized that the captive-rearing environment and the associated lack of experience with their own mothers make captive-reared galliforms behaviorally unfit to rear their own chicks (Rymesova et al. 2013). Our data do not support that hypothesis. The proportion of captive-reared hens (49%, n = 37) with broods that successfully survived 2 weeks post-hatch was not different (P > 0.77) than that for wild, parent-reared hens (43%, n = 7). Buner et al. (2011:599) made a similar conclusion for grey partridge leading them to conclude: "...that despite many generations of captive breeding, released stock with a game farm background maintains its natural breeding potential." While nesting in RIFA suppressed areas should logically provide an advantage to newly hatched broods, based on the work of Mueller et al. (1999) who documented higher survival of bobwhite chicks that hatched from nests where RIFA were suppressed, we have routinely treated areas surrounding APC nests for several years. We did not change that protocol for this study, so the immediate areas (< 0.1-acre) surrounding all active nests were treated to suppress RIFA with Amdro Pro® or Extinguish Plus®, both of which are labeled for pasture use. This also included nests located in untreated control pastures, which likely explains the lack of a treatment effect relative to nest location.

Data collected in this study also clearly demonstrate that the invasive RIFA has substantially reduced invertebrate abundance within historic and extant APC habitats, despite considerable variation among sites and between years (Tables 5,6; Figures 8–19). Magnitude of treatment effects indicated by LMER analyses, while still highly significant (P < 0.0001) when all 5 study sites were included, were substantially higher when APCNWR was excluded (Table 5,6). No treatment effects (P > 0.29) were identified by the LMER analyses when data for APCWNR were analyzed separately (Table 6). Several possible explanations for lack of treatment effect at APCWNR are: (1) climatic conditions were dramatically different between years as evidenced by rainfall data (Figure 5) and the strong year effect for invertebrate numbers and biomass at APCNWR (Table 6), (2) APCNWR has actively managed for increased insects for several years, and (3) cattle were removed from APCNWR study pastures from approximately January 1 – July 1during both years of the project to minimize grazing impacts as a variable. APCNWR maintained the highest cumulative rainfall deficit of the 5-sites as of June 2012 (Figures 3–6).

Patch burning at APCNWR was implemented in 2003 because research indicated such systems showed promise for maintaining a diverse insect community (e.g., Roper 1999, Doxon 2009, Doxon et al. 2011). In addition, fire breaks on APCNWR are disked to encourage early successional stage forbs which often support high insect numbers (Jones 1963, Green 1984, Hill 1985). Disking fire breaks also occurs at the Midwell and W. Clarkson sites, whereas ground disturbance is minimized at TCPP. Much of the increased abundance for APCWNR in 2012 was due to high Group 1 numbers, and was mostly attributable to large numbers of a very small, winged Psyllid (Psyllidae). It is likely that environmental conditions were favorable in 2012 for this species at APCNWR and populations exploded, overwhelming any treatment effect. A similar phenomenon with grasshoppers occurred at APCNWR in 2010 (APCNWR, unpublished data) and at some locations in Goliad County in 2012 (Figure 20,21) (J. Kelso, The Nature Conservancy of Texas, personal communication). Therefore, while undoubtedly RIFA are having an overall suppressive effect on insect abundance during the May-mid-June APC brooding period, there are likely times when environmental conditions are favorable for eruptive invertebrate population growth which are able to overcome the suppressive impacts of RIFA. Studies of longer duration than the 2 years of this study would be necessary to evaluate that hypothesis. Finally, removal of grazing during spring months, and reduction in overall grazing pressure due to the 2011 drought likely also explain at

least part of the anomalous responses at APCNWR compared to other sites. Cattle are highly selective for forage based on palatability and availability, and have had substantial impacts on spring forbs (e.g., *Coreopsis* spp.) known to support high insect numbers at APCNWR in the past (M. Morrow, APCNWR, personal observation). Quinn and Walgenbach (1990) observed that grazed sites supported higher numbers of obligate grass-feeding grasshoppers, while ungrazed grasslands were dominated by mixed forb- and grass-feeding species. This implies that grazing may have reduced forb abundance in that study.

Excluding APCNWR, the magnitude of treatment effects for total invertebrate numbers/sample (60%) (Table 6) and biomass (38%) (Table 6) are consistent with the findings of Porter and Savignano (1990) who reported a 75% reduction in non-ant arthropod numbers following RIFA invasion, and the 57% higher insect volumes collected from sites by Allen et al. (2001) where RIFA were suppressed. It is also consistent with the 70% fewer insects in samples collected by Pratt et al. (2003) from APC brood sites compared to GPC brood habitat where RIFA are absent. Hays and Hays (1959) observed that insects, with larval forms preferred, make up the bulk of the diet of *Solenopsis saevissima richteri*, which is now known as S. invicta (i.e., RIFA) (Ebeling 1975). This suggests the likely mechanism of RIFA impacts on invertebrates, and likely explains the large treatment response (68 and 76% increase for all 5 sites and APCWNR excluded, respectively) for measures of Group 2 invertebrate abundance (Tables 5,6; Figures 16,17). Group 2 invertebrates consisted of immature grasshoppers and katydids as well as other insects hypothesized to be less mobile (e.g., leafhoppers, beetles, spiders). We also hypothesized based on professional judgment that Group 2 invertebrates would be more likely to serve as prey for young APC chicks because of their more limited mobility. Lehmann (1941) highlighted the importance of grasshoppers and beetles to APC diets, and Hagen et al. (2005) observed that short-horned grasshoppers (Acrididae) as well as other invertebrate biomasses were higher at lesser prairie-chicken (T. *pallidicinctus*) brood sites. The fact that we did not observe higher invertebrate abundance for brood sites located in treated areas probably reflects the fact that most (77%) of the broods were observed at APCNWR where treatment effects on invertebrate abundance were not significant.

While reports of adverse direct impacts of RIFA to native fauna are numerous (Allen et al. 2004), few studies to our knowledge address the indirect impacts of this invasive species. Allen et al. (1995) documented significant population level effects for northern bobwhites, and hypothesized that reduced insect abundance resulting from RIFA invasion might be one of the causative mechanisms involved. Allen et al. (2001) observed that both insect biomass and loggerhead shrike abundance were negatively correlated with RIFA abundance. Lymn and Temple (1991) observed a significant (P < 0.0001) negative correlation with shrike abundance indices and years since invasion of a county by RIFA. These authors hypothesized that this relationship was due at least in part to direct competition between shrikes and RIFA for insect prey. Allen et al. (2004) hypothesized that declines of Texas horned lizards (Phrynosoma *cornutum*) may be related to decreased availability of its harvester ant (*Pogonomyrmex* spp.) prey resulting from RIFA invasion. The endangered Houston toad (Bufo houstonensis), found in the same general geographic locale as APC and thought by some to have once occupied similar Texas coastal prairie habitats, also are insectivorous. The Houston toad spotlight species action plan states: "Redimported fire ants (Solenopsis invicta) threaten Houston toads by killing toadlets emerging from ponds....They have also been known to drastically reduce the abundance of native insect species that serve as the Houston toad's food source." (http://ecos.fws.gov/docs/action\_plans/ doc3049.pdf).

Many factors, including the ecology of individual species, affect invertebrate species abundance within a grassland ecosystem (Quinn and Walgenbach 1990, Panzer 2002, Debano 2006). Weather, especially temperature and rainfall, are particularly important (Uvarov 1977). The 2 years of this study were very different, with historic drought conditions prevailing not only on our study sites, but throughout the entire state of Texas. This undoubtedly explains the highly significant year effects resulting from the LMER analyses (Tables 5,6). Yet despite dramatically different climatic conditions between the 2 years of this study, RIFA treatment effects were stronger except at APCNWR.

We also evaluated the impacts of spring grazing on insect abundance at APCNWR with mixed results (Table 7). Drought conditions during 2011 certainly impacted this evaluation. Most cattle were removed from APCNWR in 2011 to avoid damage to grassland communities, to protect APC habitat, and to avoid ill-effects to cattle herds. Stocking levels remained light into 2012. Therefore, grazing levels in 2012 were not typical of APCNWR which intentionally stocks at light to moderate levels, and certainly were not typical of generally more intense grazing levels within the region. Grazing pressure on pastures evaluated in 2010, while still rated at light-moderate levels, were greater than in 2012. In that year, insect numbers were higher (P = 0.063) on ungrazed sites, while in 2012 numbers and biomass were higher (P < 0.063) 0.10) on grazed sites (Table 7). However, this difference can likely be explained by vegetation parameters. Grazing pressure in 2012 was not high enough to even result in a difference (P > 0.21) in vegetation effective height, whereas in 2010 effective height was lower (P < 0.07) on grazed sites. However, in 2012 the percentage of forbs was higher (P < 0.06) on grazed sites than ungrazed sites, likely as a result of increased disturbance associated with grazing activity. Forb dominated communities often support higher invertebrate abundance (Jones 1963, Green 1984, Hill 1985). Rambo and Faeth (1999) and Debano (2006) found decreased insect abundance in grazed compared with ungrazed pastures, whereas Knutson and Campbell (1974) observed more grasshoppers with increased grazing pressure. Because of the confounding effects of the 2011 drought, our evaluation is certainly not definitive, and should be repeated in future years and at other locations. However, if moderate grazing does prove to impact insect abundance, its removal or reduction during periods critical to invertebrate life history could be used to mitigate the impacts of RIFA on invertebrate abundance.

# CONCLUSIONS

- 1. Invertebrate abundance as indicated by both numbers and biomass are essential to APC brood survival.
- 2. RIFA clearly have an adverse impact on invertebrate numbers and biomass.
- 3. Captive-reared hens were just as successful at rearing young as wild-produced hens.
- 4. Hen age, previous nesting experience, or previous success with fledging chicks were not associated with brood success.
- 5. Additional research is needed to evaluate the impacts of spring grazing on invertebrate abundance.

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Table 1. List of typical Group 1 and Group 2 invertebrates. Grasshopper and katydids were considered adults when the wings protruding beyond the posterior tip of the abdomen, while numphs with wings not protruding beyond the abdomen tip were assigned to Group 2.

Group 1	Group 2
Grasshoppers/katydids, adult	Grasshoppers/katydids, juvenile
Flies/gnats/green flies	Leafhoppers
Dragonflies	Beetles/weevils/grubs
Butterflies/moths	Spiders
Bees/wasps	Crickets
	Caterpillars/inch worms
	Mantids/walking sticks
	Ants

Site   Year   Period   Treated   Control   Treated   Control     APCNWR   2011   1   34   30   0.0783   0.0850     2   31   32   0.1167   0.0787     3   31   26   0.1051   0.0994     overall   32   28   0.0937   0.0929     2012   1   53   76.5   0.0804   0.0678     2012   1   53   76.5   0.1545   0.1725     3   34.5   70.5   0.1160   0.1446     overall   40.0   73.5   0.1139   0.1287     Midwell   2011   1   23   20.5   0.116   0.1446     2   15   21   0.1335   0.0442   3   6.5   0.0772   0.0326     overall   20.5   18   0.1139   0.1274   0.0326   0.0556     3   6.5   6.5   0.0772   0.0326   0.0556				÷	-		
APCNWR   2011   1   34   30   0.0783   0.0850     2   31   32   0.1167   0.0787   3   31   26   0.1051   0.0994     overall   32   28   0.0937   0.0929   2012   1   53   76.5   0.0804   0.0678   2   46   74.5   0.1545   0.1725     3   34.5   70.5   0.1162   0.1640   overall   40.0   73.5   0.1139   0.1287     Midwell   2011   1   23   20.5   0.1116   0.1446     2   15   21   0.1335   0.0442     3   22   13   0.1041   0.1411     overall   20.5   18   0.1139   0.1274     2012   1   38   15   0.0068   0.0412     2   2   9   6   0.1255   0.0556     3   6.5   6.5   0.072   0.0326     overall							
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3   31   26   0.0151   0.0994     overall   32   28   0.037   0.0929     2012   1   53   76.5   0.0804   0.0678     2   46   74.5   0.1545   0.1725     3   34.5   70.5   0.1162   0.1640     overall   40.0   73.5   0.1139   0.1287     Midwell   2011   1   23   20.5   0.1116   0.1446     2   15   21   0.1335   0.0422   3   22   13   0.1041   0.1411     overall   20.5   18   0.1139   0.1274   2012   1   38   15   0.068   0.0412     2   9   6   0.1255   0.0556   3   6.5   0.5   0.0772   0.0326     overall   16.5   8.5   0.1091   0.0454   2   20   0.158   0.0518   0.0518   0.0518   0.0518   0.0518   0.159 <t< td=""><td>APCNWR</td><td>2011</td><td></td><td></td><td>30</td><td>0.0783</td><td>0.0850</td></t<>	APCNWR	2011			30	0.0783	0.0850
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2012   1   53   76.5   0.0804   0.0678     2   46   74.5   0.1545   0.1725     3   34.5   70.5   0.1162   0.1640     overall   40.0   73.5   0.1139   0.1287     Midwell   2011   1   23   20.5   0.1116   0.1446     2   15   21   0.1335   0.0442   3   22   13   0.1041   0.1411     overall   20.5   18   0.1139   0.1274   3   22   9   6   0.1255   0.0556     3   6.5   6.5   0.0772   0.0326   0verall   16.5   8.5   0.1091   0.0454     Texas City   2011   1   72   39   0.1640   0.0741     2   20   19.5   0.0307   0.0556   3   43.5   21   0.1288   0.0318     overall   42.5   26.5   0.0868   0.0518   3   313 <t< td=""><td></td><td></td><td>3</td><td></td><td></td><td>0.1051</td><td>0.0994</td></t<>			3			0.1051	0.0994
2   46   74.5   0.1545   0.1725     3   34.5   70.5   0.1162   0.1640     overall   40.0   73.5   0.1139   0.1287     Midwell   2011   1   23   20.5   0.1116   0.1446     2   15   21   0.1335   0.0442     3   22   13   0.1041   0.1411     overall   20.5   18   0.1139   0.1274     2012   1   38   15   0.1068   0.0412     2   9   6   0.1255   0.0326     overall   16.5   8.5   0.1091   0.0454     Texas City   2011   1   72   39   0.1640   0.0741     2   20   19.5   0.0307   0.0556   3   43.5   21   0.1288   0.0318     overall   42.5   26.5   0.0868   0.0518   13   11   0.0678   0.0597     12   45 </td <td></td> <td></td> <td></td> <td></td> <td></td> <td>0.0937</td> <td></td>						0.0937	
Midwell   3   34.5   70.5   0.1162   0.1640     widwell   2011   1   23   20.5   0.1116   0.1446     2   15   21   0.1335   0.0442     3   22   13   0.1041   0.1411     overall   20.5   18   0.1139   0.1274     2012   1   38   15   0.1068   0.0412     2   9   6   0.1255   0.0556     3   6.5   6.5   0.0772   0.0326     overall   16.5   8.5   0.1091   0.0454     1   72   39   0.1640   0.0711     2   20   19.5   0.0307   0.0556     3   43.5   21   0.1288   0.0318     overall   42.5   26.5   0.0864   0.0518     2012   1   34.5   21   0.1285   0.0518     overall   34.5   22   0.1060   0.0684		2012				0.0804	
Midwell   2011   1   23   20.5   0.1139   0.1287     Midwell   2011   1   23   20.5   0.1116   0.1446     2   15   21   0.1335   0.0442     3   22   13   0.1041   0.1411     overall   20.5   18   0.1139   0.1274     2012   1   38   15   0.1068   0.0412     2   9   6   0.1255   0.0556     3   6.5   6.5   0.0772   0.0326     overall   16.5   8.5   0.1091   0.0454     1   72   39   0.1640   0.0741     2   20   19.5   0.0307   0.0556     3   43.5   21   0.1288   0.0318     overall   42.5   26.5   0.0868   0.0518     2012   1   34.5   22.5   0.1272   0.0781     2   45   22.5   0.1266   <				46	74.5	0.1545	
Midwell   2011   1   23   20.5   0.1116   0.1446     2   15   21   0.1335   0.0442     3   22   13   0.1041   0.1411     overall   20.5   18   0.1139   0.1274     2012   1   38   15   0.1068   0.0412     2   9   6   0.1255   0.0556     3   6.5   6.5   0.0772   0.0326     overall   16.5   8.5   0.1091   0.0454     Texas City   2011   1   72   39   0.1640   0.0741     2   20   19.5   0.0307   0.0556   3   43.5   21   0.1288   0.0318     overall   42.5   26.5   0.0868   0.0518   2012   1   34.5   22   0.1266   0.0831     3   13   11   0.0678   0.0159   0.0684   15   0.0810   0.0239     overall   34 </td <td></td> <td></td> <td>3</td> <td>34.5</td> <td>70.5</td> <td>0.1162</td> <td>0.1640</td>			3	34.5	70.5	0.1162	0.1640
2   15   21   0.1335   0.0442     3   22   13   0.1041   0.1411     overall   20.5   18   0.1139   0.1274     2012   1   38   15   0.1068   0.0412     2   9   6   0.1255   0.0556     3   6.5   6.5   0.0772   0.0326     overall   16.5   8.5   0.1091   0.0454     12   20   19.5   0.0307   0.0556     3   43.5   21   0.1288   0.0318     overall   42.5   26.5   0.0868   0.0518     2012   1   34.5   22.5   0.1272   0.0781     2012   1   34.5   22.5   0.1272   0.0781     2012   1   34.5   22.5   0.1272   0.0781     3   13   11   0.0678   0.0159     overall   34   19   0.1096   0.0684			overall	40.0	73.5	0.1139	0.1287
3   22   13   0.1041   0.1411     overall   20.5   18   0.1139   0.1274     2012   1   38   15   0.1068   0.0412     2   9   6   0.1255   0.0556     3   6.5   6.5   0.0772   0.0326     overall   16.5   8.5   0.1091   0.0454     Texas City   2011   1   72   39   0.1640   0.0741     2   20   19.5   0.0307   0.0556   3   43.5   21   0.1288   0.0318     overall   42.5   26.5   0.0868   0.0518   2012   1   34.5   22.5   0.1272   0.0781     2012   1   34.5   22.5   0.1272   0.0781     2012   1   34.5   22.5   0.1272   0.0781     2011   1   45.5   41.5   0.0891   0.0436     15   0.0211   1   7.5   10.5 </td <td>Midwell</td> <td>2011</td> <td>1</td> <td>23</td> <td>20.5</td> <td>0.1116</td> <td>0.1446</td>	Midwell	2011	1	23	20.5	0.1116	0.1446
overall   20.5   18   0.1139   0.1274     2012   1   38   15   0.1068   0.0412     2   9   6   0.1255   0.0556     3   6.5   6.5   0.0772   0.0326     overall   16.5   8.5   0.1091   0.0454     Texas City   2011   1   72   39   0.1640   0.0741     2   20   19.5   0.0307   0.0556   3   43.5   21   0.1288   0.0318     overall   42.5   26.5   0.0868   0.0518   2012   1   34.5   22.5   0.1272   0.0781     2012   1   34.5   22.5   0.1272   0.0781     2012   1   34.5   22.5   0.1272   0.0781     2012   1   34.5   22.5   0.1272   0.0781     2011   1   45.5   41.5   0.0819   0.0436     2011   1   45.5   0				15	21	0.1335	0.0442
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			3	22	13	0.1041	0.1411
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			overall	20.5	18	0.1139	0.1274
3   6.5   6.5   0.0772   0.0326     overall   16.5   8.5   0.1091   0.0454     Texas City   2011   1   72   39   0.1640   0.0741     2   20   19.5   0.0307   0.0556   3   43.5   21   0.1288   0.0318     overall   42.5   26.5   0.0868   0.0518   0.0741     2   45   22.5   0.1272   0.0781     2   45   22   0.1206   0.0831     3   13   11   0.0678   0.0159     overall   34   19   0.1096   0.0684     Tivoli   2011   1   45.5   41.5   0.0819   0.0239     overall   37   18.5   0.0832   0.0383     2012   1   7.5   10.5   0.1540   0.0957     2   11   6   0.1268   0.0327     3   6   4   0.0346   0.0055 </td <td></td> <td>2012</td> <td>1</td> <td>38</td> <td>15</td> <td>0.1068</td> <td>0.0412</td>		2012	1	38	15	0.1068	0.0412
overall   16.5   8.5   0.1091   0.0454     Texas City   2011   1   72   39   0.1640   0.0741     2   20   19.5   0.0307   0.0556     3   43.5   21   0.1288   0.0318     overall   42.5   26.5   0.0868   0.0518     2012   1   34.5   22.5   0.1272   0.0781     2012   1   34.5   22.5   0.1272   0.0781     2012   1   34.5   22.5   0.1272   0.0781     3   13   11   0.0678   0.0159     overall   34   19   0.1096   0.0684     1   45.5   41.5   0.0819   0.0239     overall   34   19   0.0967   0.0321     3   37.5   11.5   0.0832   0.0383     2012   1   7.5   10.5   0.1540   0.0957     2   11   6   0.126			2	9	6	0.1255	0.0556
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			3	6.5	6.5	0.0772	0.0326
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			overall	16.5	8.5	0.1091	0.0454
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Texas City	2011	1	72	39	0.1640	0.0741
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			2	20	19.5	0.0307	0.0556
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			3	43.5	21	0.1288	0.0318
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			overall	42.5	26.5	0.0868	0.0518
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		2012	1	34.5	22.5	0.1272	0.0781
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$			2	45	22	0.1206	0.0831
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			3	13	11	0.0678	0.0159
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			overall	34	19	0.1096	0.0684
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Tivoli	2011	1	45.5	41.5	0.0819	0.0436
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$			2	24.5	15.5	0.0597	0.0321
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			3	37.5	11.5	0.0981	0.0239
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			overall	37	18.5	0.0832	0.0383
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		2012	1	7.5	10.5	0.1540	0.0957
overall860.10830.0391W. Clarkson2011131.5410.19810.1401236200.14180.102833019.50.16000.2010overall31.522.50.16760.13412012135.5200.20900.1312211.5110.10280.048636.515.50.02810.0979			2	11	6	0.1268	0.0327
W. Clarkson 2011 1 31.5 41 0.1981 0.1401   2 36 20 0.1418 0.1028   3 30 19.5 0.1600 0.2010   overall 31.5 22.5 0.1676 0.1341   2012 1 35.5 20 0.2090 0.1312   2 11.5 11 0.1028 0.0486   3 6.5 15.5 0.0281 0.0979			3	6	4	0.0346	0.0055
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			overall	8	6	0.1083	0.0391
33019.50.16000.2010overall31.522.50.16760.13412012135.5200.20900.1312211.5110.10280.048636.515.50.02810.0979	W. Clarkson	2011	1	31.5	41	0.1981	0.1401
overall31.522.50.16760.13412012135.5200.20900.1312211.5110.10280.048636.515.50.02810.0979			2	36	20	0.1418	0.1028
2012135.5200.20900.1312211.5110.10280.048636.515.50.02810.0979			3	30	19.5	0.1600	0.2010
211.5110.10280.048636.515.50.02810.0979			overall	31.5	22.5	0.1676	0.1341
3 6.5 15.5 0.0281 0.0979		2012	1	35.5	20	0.2090	0.1312
			2	11.5	11	0.1028	0.0486
overall 11.5 16 0.1129 0.0972			3	6.5	15.5	0.0281	0.0979
			overall	11.5	16	0.1129	0.0972

Table 2. Medians of total number and dry weight of invertebrates/25-sweeps by site.

			Number		Dry Weight (g)	
Site	Year	Period	Treated	Control	Treated	Control
APCNWR	2011	1	4	2	0.0047	0.0042
		2	3	2	0.0016	0.0008
		3	3	3.5	0.0039	0.0025
		overall	3	3	0.0034	0.0024
	2012	1	19	45	0.0082	0.0131
		2	24	44	0.0151	0.0166
		3	10.5	17	0.0093	0.0414
		overall	15.5	40	0.0102	0.0146
Midwell	2011	1	2	1	0.0167	0.0012
		2	2.5	3	0.0731	0.0007
		3	2.5	2	0.0138	0.0797
		overall	2	2	0.0177	0.0044
	2012	1	11.5	5.5	0.0019	0.0010
		2	1.5	0.5	0.0022	0.0008
		3	1.5	0	0.0001	0.0000
		overall	2	1	0.0015	0.0009
Texas City	2011	1	9	6.5	0.0032	0.0044
		2	2	2	0.0018	0.0013
		3	3	0.5	0.0066	0.0002
		overall	4	2.5	0.0035	0.0014
	2012	1	5.5	1.5	0.0033	0.0020
		2	8	5	0.0036	0.0036
		3	3.5	3	0.0152	0.0007
		overall	5	3	0.0034	0.0023
Гivoli	2011	1	5.5	4.5	0.0014	0.0017
		2	4	0.5	0.0009	0.0001
		3	3.5	0.5	0.0025	0.0001
		overall	4	2	0.0017	0.0008
	2012	1	1	3	0.0023	0.0101
		2	1	0.5	0.0021	0.0011
		3	0	0	0.0000	0.0000
		overall	1	1	0.0012	0.0017
W. Clarkson	2011	1	4	2	0.0182	0.0012
		2	2	1	0.0007	0.0001
		3	2.5	2	0.0036	0.0912
		overall	3	2	0.0021	0.0016
	2012	1	2	3.5	0.0030	0.0063
		2	1.5	1	0.0110	0.0012
		3	0	1	0.0000	0.0062
		overall	1	1	0.0016	0.0020

Table 3. Medians of Group 1 number and dry weight of invertebrates/25-sweeps by site.

			ЪT		D	<b>-let</b> (c)
0.4	V	р <sup>.</sup> .	Number		Dry Weight (g)	
Site	Year	Period	Treated	Control	Treated	Control
APCNWR	2011	1	33	25	0.0740	0.0831
		2	30.5	29.5	0.0852	0.0728
		3	28	21.5	0.0825	0.0865
		overall	29	25	0.0743	0.0831
	2012	1	32	28.5	0.0546	0.0477
		2	22.5	23.5	0.1296	0.0896
		3	23	27.5	0.1047	0.1043
		overall	26.5	27	0.0933	0.0777
Midwell	2011	1	22	19	0.0938	0.0457
		2	11.5	16	0.0638	0.0442
		3	20.5	11	0.0873	0.0303
		overall	18.5	14	0.0809	0.0416
	2012	1	28	10	0.1026	0.0405
		2	6.5	5	0.1087	0.0439
		3	6	5.5	0.0648	0.0320
		overall	13	6	0.1001	0.0392
Texas City	2011	1	63	34.5	0.1451	0.0605
		2	17.5	17.5	0.0271	0.0502
		3	41	19	0.1184	0.0313
		overall	36.5	22	0.0833	0.0421
	2012	1	28	20	0.1240	0.0753
		2	35	17	0.1183	0.0761
		3	9.5	8	0.0506	0.0141
		overall	27	16	0.1050	0.0628
Tivoli	2011	1	41.5	38.5	0.0786	0.0416
		2	18.5	15.5	0.0538	0.0313
		3	33.5	11	0.0943	0.0215
		overall	32	17	0.0766	0.0335
	2012	1	5.5	7.5	0.1330	0.0834
		2	10.5	5	0.1159	0.0271
		3	5	3.5	0.0333	0.0055
		overall	6.5	5	0.1061	0.0254
W. Clarkson	2011	1	28.5	37.5	0.1679	0.1137
		2	30	19.5	0.1178	0.0955
		3	26	16.5	0.1264	0.1077
		overall	28	21	0.1304	0.1086
	2012	1	31	15.5	0.2062	0.0863
		2	9.5	8	0.0857	0.0341
		3	6	15	0.0281	0.0616
		overall	10	14	0.1021	0.0688

Table 4. Medians of Group 2 number and dry weight of invertebrates/25-sweeps by site.

Response Variable <sup>a</sup>	$\begin{array}{c} \text{Box-}\\ \text{Cox}\\ \lambda^{\text{b}} \end{array}$	Fixed Effects	Back- transformed β	t	lower density $P^c$
Total Number	0.15	TRT	1.42	4.66	< 0.0001
		year	0.47	9.38	< 0.0001
Group 2 Number	0.23	TRT year	1.68 0.24	5.63 14.49	< 0.0001 < 0.0001
Total Weight	0.2	TRT	1.28	4.94	< 0.0001
	·. <b>_</b>	year	0.94	1.63	0.10
Group 2 Weight	0.25	TRT	1.27	5.85	< 0.0001
<u> </u>		year	0.95	1.56	0.12

Table 5. Linear mixed effects regression (LMER) results using data from all 5 sites. Treatment (TRT) effects are relative to untreated control plots, and year effects are for 2012 relative to 2011.

<sup>a</sup>Group 2 consists of generally smaller, less mobile, immature invertebrates as opposed to Group 1 (data not shown) which consisted of larger, more mobile individuals. Total = Group 1 + Group 2. <sup>b</sup>Response variables were transformed using the Box-Cox transformation  $(y^{\lambda}-1)/\lambda$  to normalize data. <sup>c</sup>Conservative estimate of *P* from pamer.fnc of the R LMERConvenienceFunctions package.

Response	Box- Cox	Fixed	Back- transformed		lower
Variable <sup>a</sup>	$\lambda^{\mathrm{b}}$	Effects	β	t	density P <sup>c</sup>
APCNWR					
Total Number	0.15	TRT	0.74	1.07	0.29
		year	3.36	8.41	< 0.0001
Group 2 Number	0.23	TRT	1.02	0.05	0.94
		year	1.25	1.48	0.14
Total Weight	0.2	TRT	0.92	0.47	0.63
		year	1.20	2.94	< 0.01
Group 2 Weight	0.25	TRT	0.97	0.23	0.82
		year	1.10	1.83	< 0.07
Other 4 sites					
Total Number	0.15	TRT	1.60	6.39	< 0.0001
		year	0.28	15.04	< 0.0001
Group 2 Number	0.23	TRT	1.77	6.52	< 0.0001
		year	0.16	16.03	< 0.0001
Total Weight	0.2	TRT	1.38	6.77	< 0.0001
		year	0.87	3.07	< 0.01
Group 2 Weight	0.25	TRT	1.34	8.21	<.0001
- <b>v</b>		year	0.91	2.46	< 0.02

Table 6. Separate linear mixed effects regression (LMER) results for APCNWR alone versus those for the other 4 sites (Midwell, Tivoli, TCPP, and W. Clarkson) combined. Treatment (TRT) effects are relative to untreated control plots, and year effects are for 2012 relative to 2011.

<sup>a</sup>Group 2 consists of generally smaller, less mobile, immature invertebrates as opposed to Group 1 (data not shown) which consisted of larger, more mobile individuals. Total = Group 1 + Group 2. <sup>b</sup>Response variables were transformed using the Box-Cox transformation  $(y^{\lambda}-1)/\lambda$  to normalize data. <sup>c</sup>Conservative estimate of *P* from pamer.fnc of the R LMERConvenienceFunctions package.

2010 2012 Ungrazed Grazed Ungrazed Grazed 104.5 (n=18) 163 (n=19) Number 91 (n=6) 62.5 (n=20) Р 0.06 0.10 Dry Wt. (g) 0.475 (n=6) 0.587 (n=20) 0.233 (n=18) 0.403 (n=19) Р 1 0.05 Eff. Ht. (in) 7.6 (n=15) 9.9 (n=20) 5.1 (n=15) 11.1 (n=20) Р 0.07 0.21 23 (n=20) % Grasses 33 (n=14) 30 (n=15) 15.5 (n=20) Р 1 0.001 % Forbs 41 (n=14) 31 (n=15) 26 (n=20) 33 (n=20) Р 0.06 0.18 % Bare Ground 34 (n=14) 41 (n=15) 54 (n=20) 50 (n=20) Р 0.01 0.18

Table 7. Comparison of medians for invertebrate number and dry weight, effective vegetation height, and cover of grasses, forbs and bare ground from grazed and ungrazed sites on APCNWR. Grazed plots were not included in analyses evaluating the impacts of RIFA on invertebrate abundance. Neither grazed nor ungrazed plots were treated to suppress RIFA.

Figure 1. Attwater's prairie-chicken (APC) population trends 1937–2012. The dotted line indicates the 25-year average population trend following invasion of APC range by red imported fire ants circa 1970.

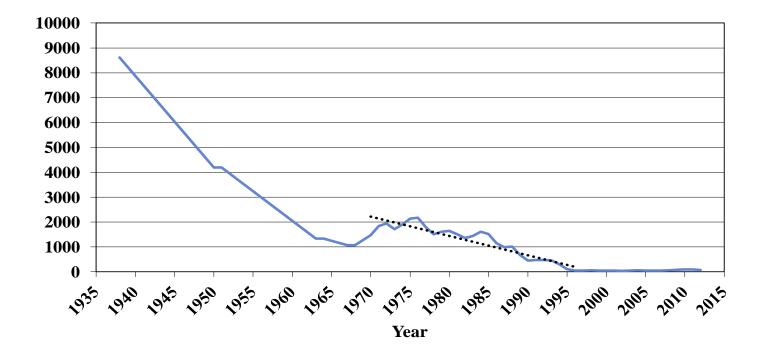


Figure 2. Vicinity map of 5 study sites located in Colorado, Galveston, Goliad, and Refugio counties, Texas. The gray area indicates the historic range of Attwater's prairie-chicken (Lehmann and Mauermann 1963).

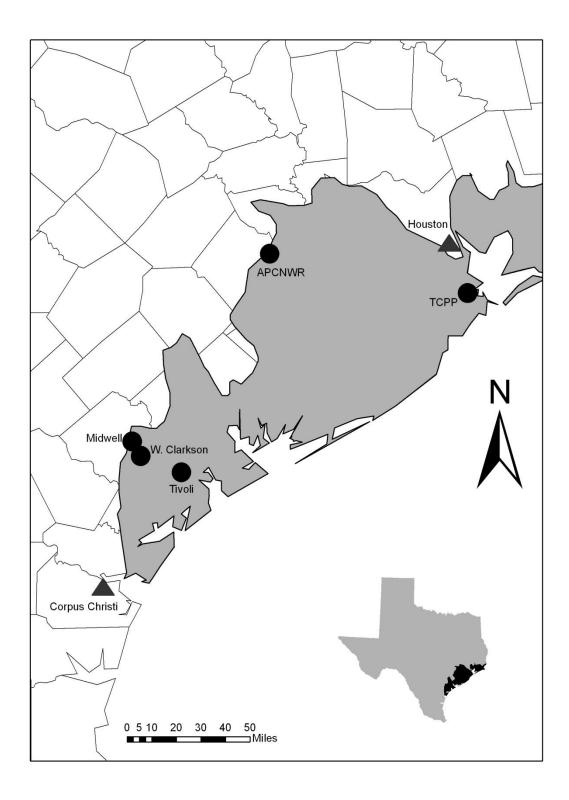


Figure 3. Cumulative precipitation (PPT) and long-term average (LTA) rainfall (<u>http://www.ncdc.noaa.gov</u>) for Goliad (2.4 miles southeast), Goliad County, Texas near the Midwell and W. Clarkson study sites.

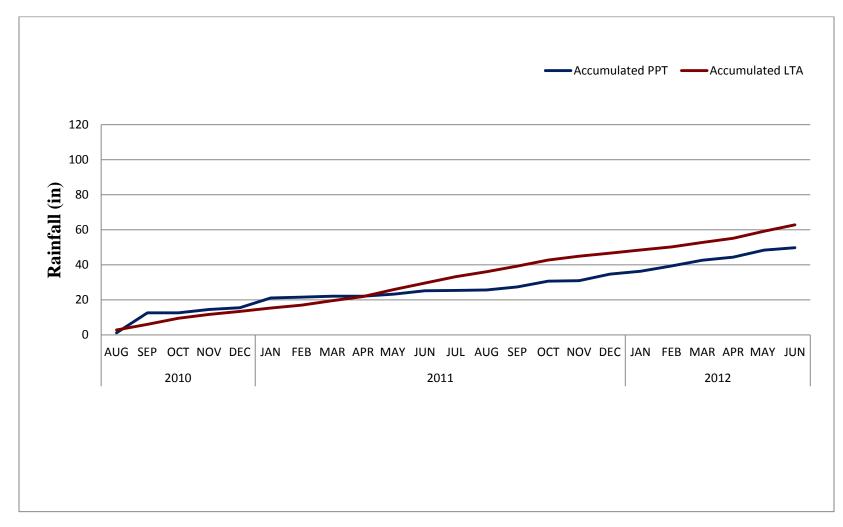


Figure 4. Cumulative precipitation (PPT) and long-term average (LTA) rainfall (<u>http://www.ncdc.noaa.gov</u>) for Refugio (3 miles southwest), Refugio County, Texas near the Tivoli study site.

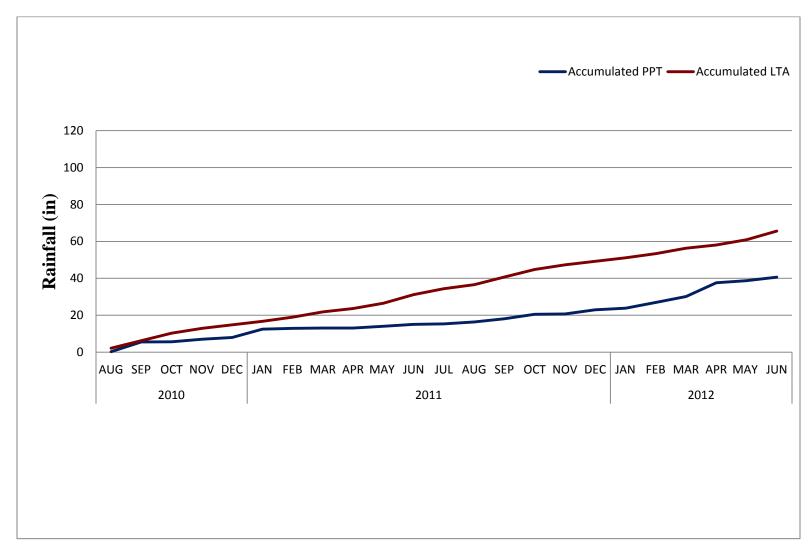


Figure 5. Cumulative precipitation (PPT) and long-term average (LTA) rainfall (<u>http://www.ncdc.noaa.gov</u>) for Sealy, Austin County, Texas near the APCNWR study site.

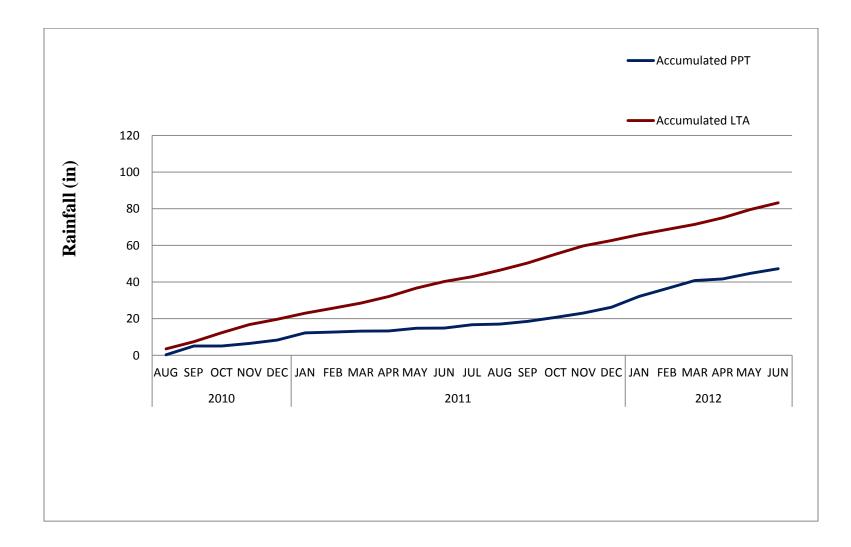
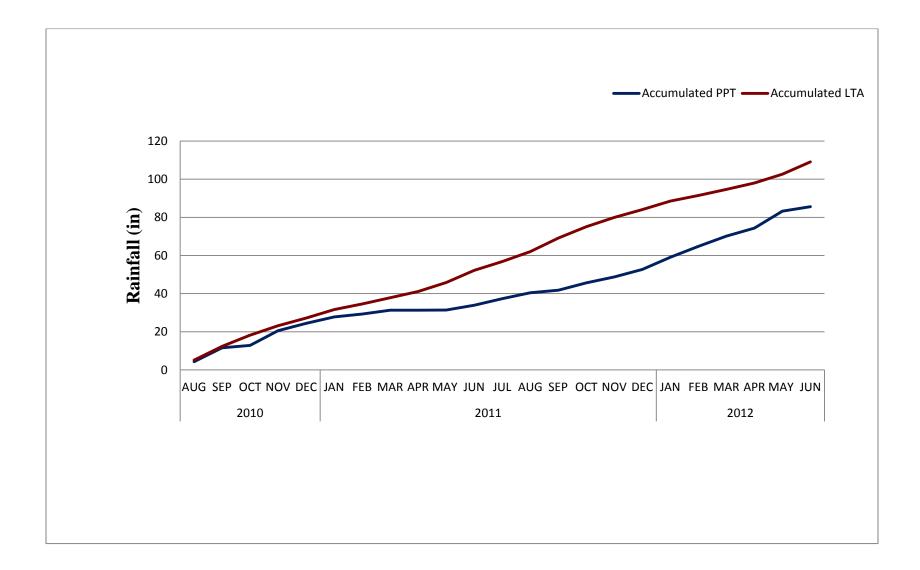
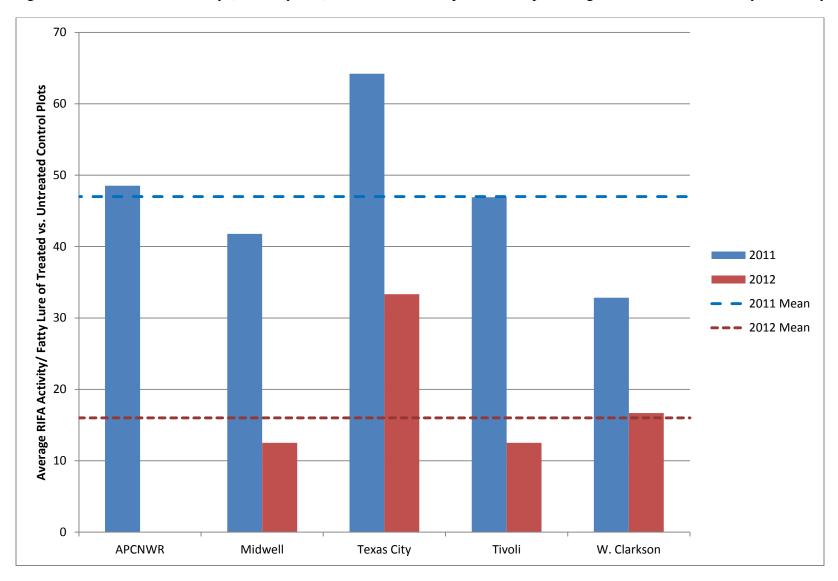
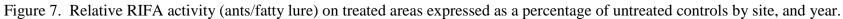


Figure 6. Cumulative precipitation (PPT) and long-term average (LTA) average rainfall (<u>http://www.ncdc.noaa.gov</u>) for League City, Galveston County, Texas near the TCPP study site.







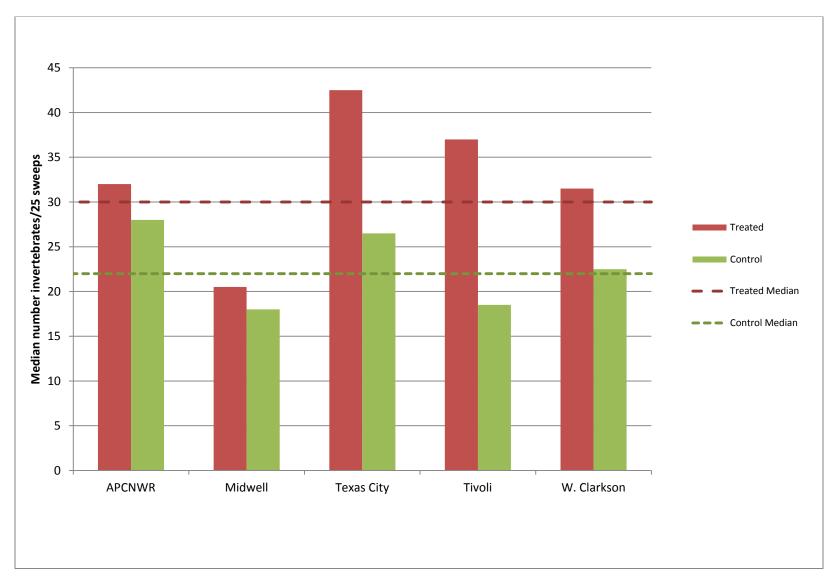


Figure 8. Median total (Group 1 + Group 2) invertebrates/25 sweeps by treatment and site for 2011.

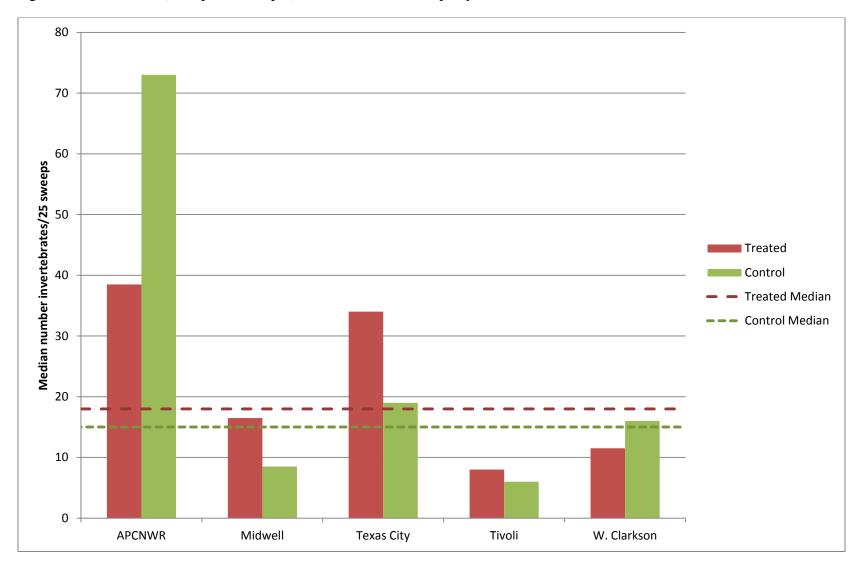


Figure 9. Median total (Group 1 + Group 2) invertebrates/25 sweeps by treatment and site for 2012.

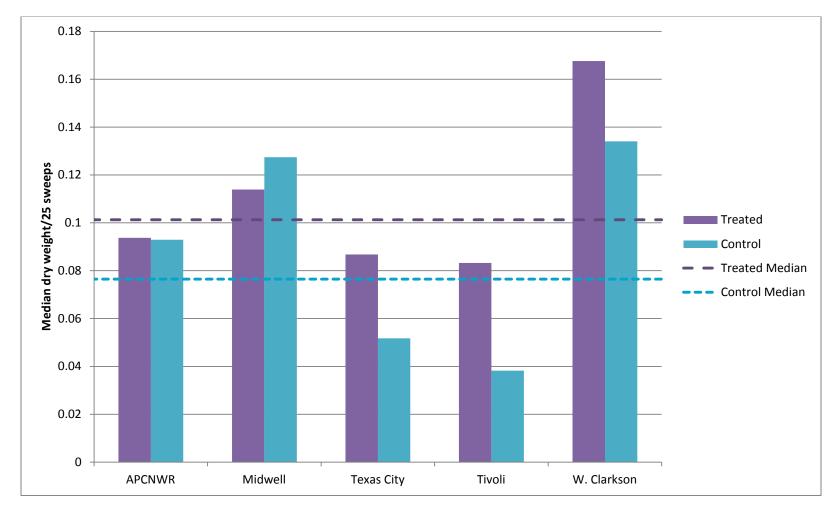


Figure 10. Median total (Group 1 + Group 2) dry weight (g)/25 sweeps by treatment and site for 2011.



Figure 11. Median total (Group 1 + Group 2) dry weight (g)/25 sweeps by treatment and site for 2012.

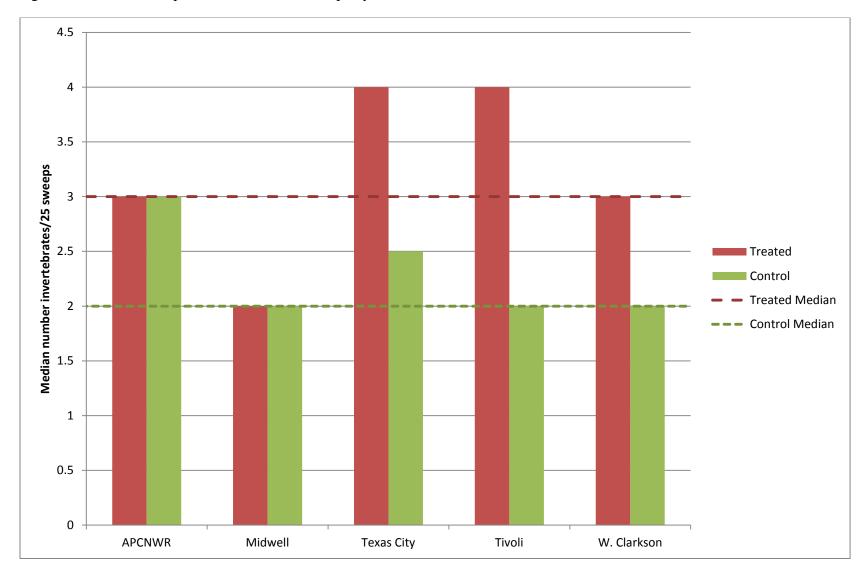


Figure 12. Median Group 1 invertebrates/25 sweeps by treatment and site for 2011.

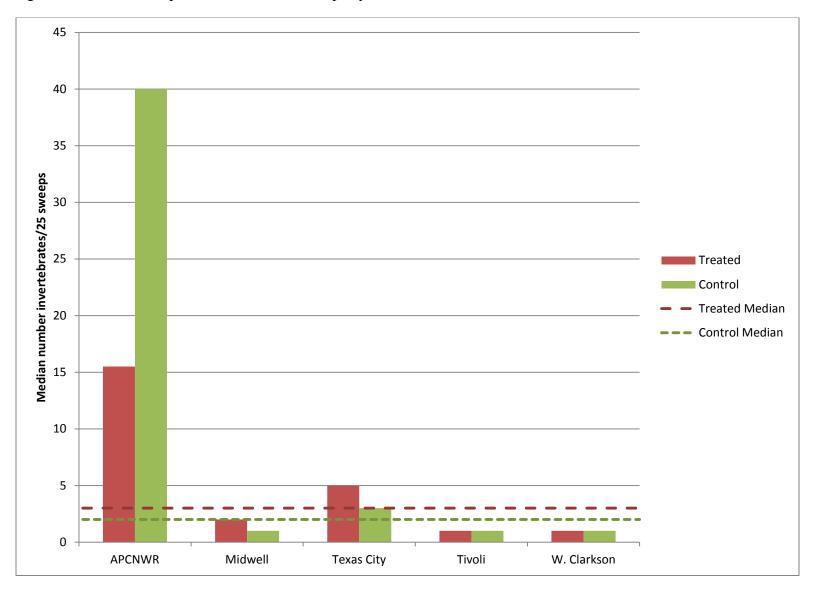


Figure 13. Median Group 1 invertebrates/25 sweeps by treatment and site for 2012.

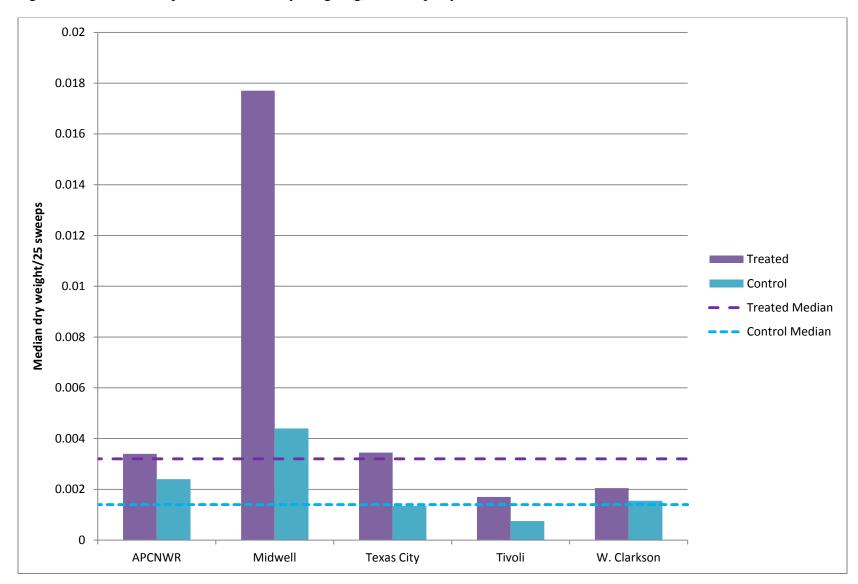


Figure 14. Median Group 1 invertebrate dry weight (g)/25 sweeps by treatment and site for 2011.

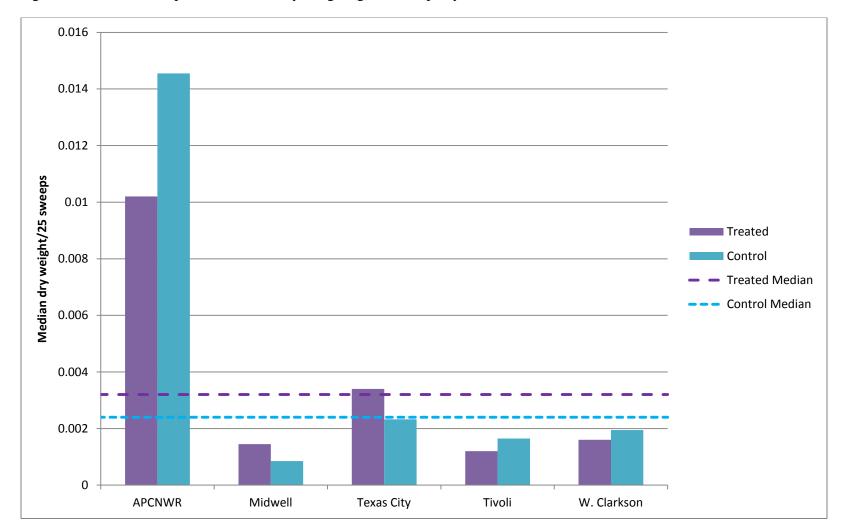


Figure 15. Median Group 1 invertebrate dry weight (g)/25 sweeps by treatment and site for 2012.

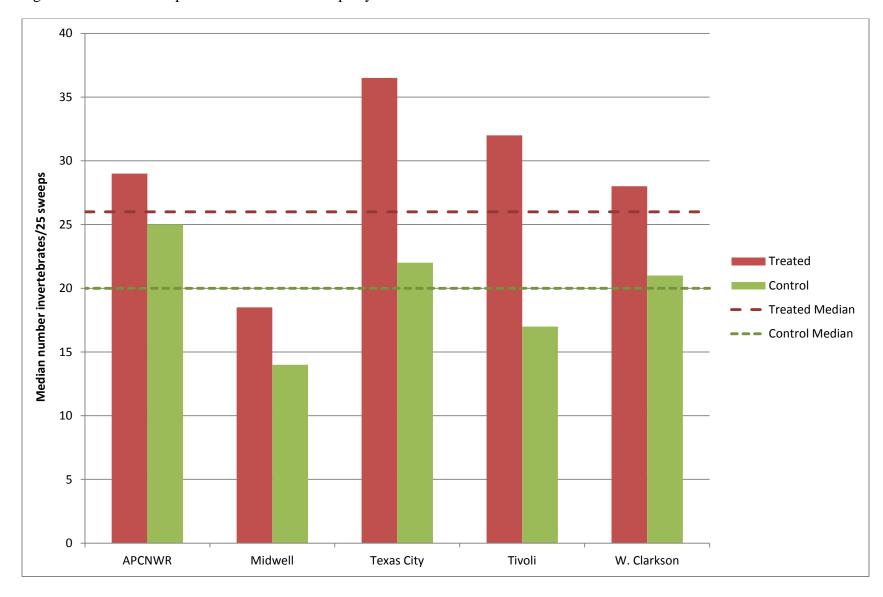
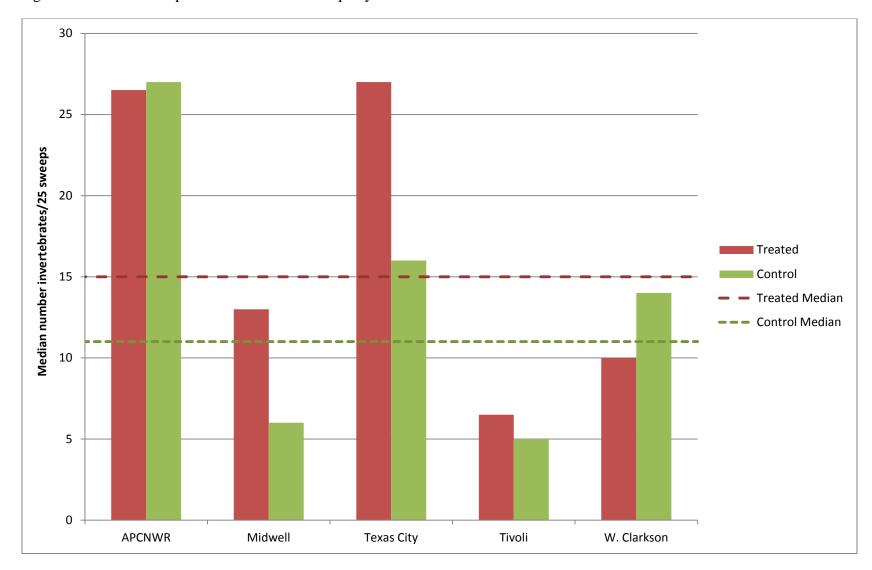
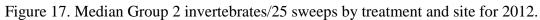


Figure 16. Median Group 2 invertebrates/25 sweeps by treatment and site for 2011.





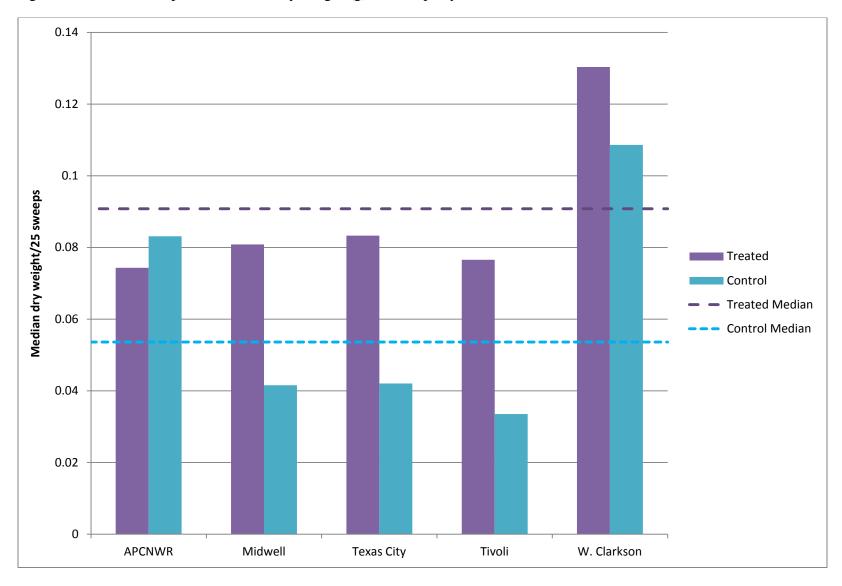
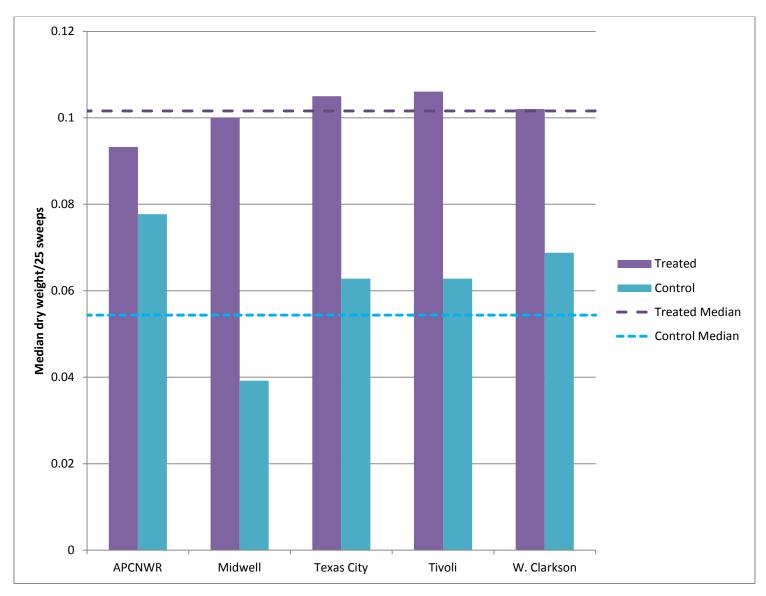
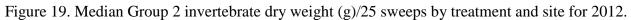


Figure 18. Median Group 2 invertebrate dry weight (g)/25 sweeps by treatment and site for 2011.





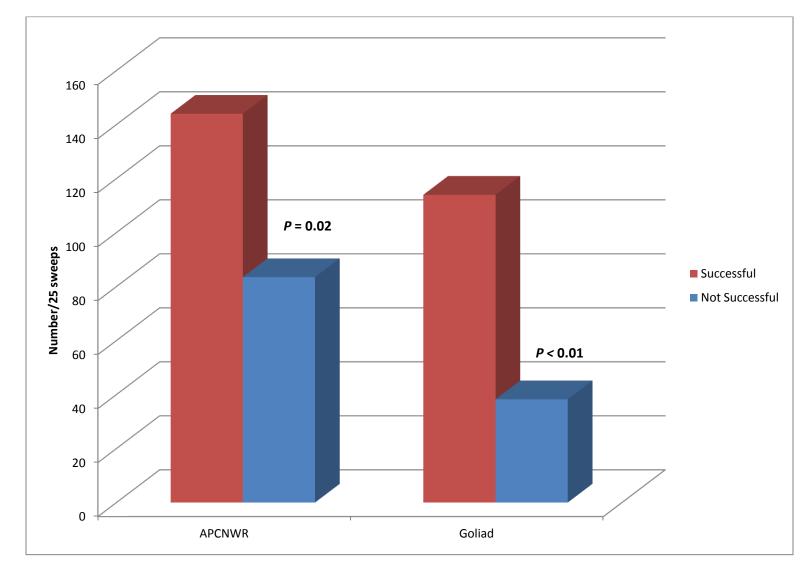
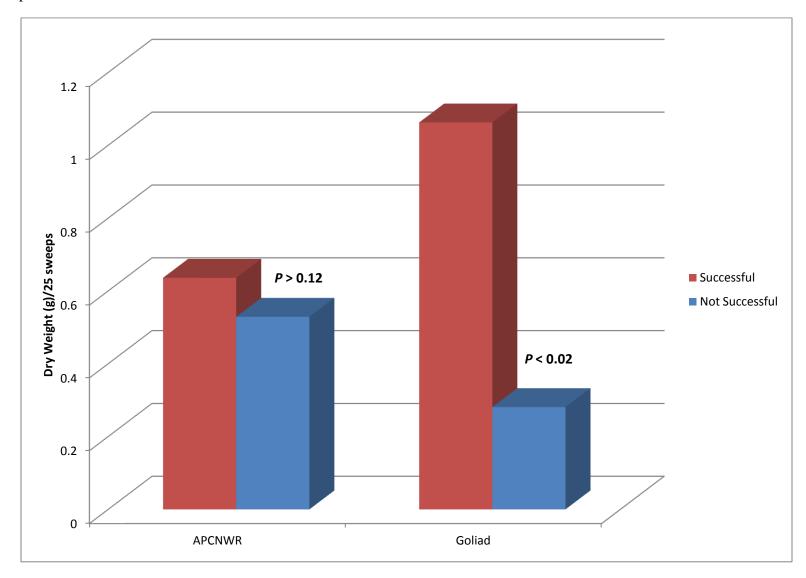


Figure 20. Median number of invertebrates at APC brood sites by brood fate at APCNWR (2009-2012) (n = 34) and private rangeland in Goliad County, Texas (2011-2012) (n = 10). Brood success was determined by presence of at least one chick at 2 weeks post-hatch.

Figure 21. Median dry weight (g) of invertebrates at APC brood sites by brood fate at APCNWR (2009-2012) (n = 34) and private rangeland in Goliad County, Texas (2011-2012) (n = 10). Brood success was determined by presence of at least one chick at 2 weeks post-hatch.



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