Research Article

Indirect Effects of Red Imported Fire Ants on Attwater's Prairie-Chicken Brood Survival

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ABSTRACT The invasive red imported fire ant (*Solenopsis invicta*) has negatively affected a host of taxonomic groups throughout its acquired North American range. Many studies have hypothesized indirect trophic impacts, but few documented those impacts. We evaluated invertebrate abundance as a factor limiting juvenile survival of the endangered Attwater's prairie-chicken (*Tympanuchus cupido attwateri*), and whether fire ants reduce invertebrate numbers and biomass. From 2009–2013, we monitored survival of Attwater's prairie-chicken broods ($n = 63$) with radio telemetry during the first 2 weeks post-hatch and collected daily invertebrate samples at brood sites. Broods located in areas with the highest median invertebrate count (338 invertebrates/25 sweeps) had a survival probability of 0.83 at 2 weeks post-hatch compared to 0.07 for broods located in areas with the lowest median invertebrate count (18 invertebrates/25 sweeps). During 2011–2012, we evaluated the reduction of fire ants on invertebrate numbers and biomass by aerially treating areas with Extinguish Plus™ in an impact-reference study design. Treated fields had 27% more individual invertebrates and 26% higher invertebrate biomass than reference fields. Our results clearly document that invertebrate abundance affects Attwater's prairie-chicken brood survival and that fire ants may indirectly contribute to low brood survival by suppressing invertebrate abundance. We posit that within the fire ant's acquired North American range, fire ants are likely contributing to declines of other insectivorous species. Published 2015. This article is a U.S. Government work and is in the public domain in the USA.

KEY WORDS Attwater's, brood survival, fire ants, indirect effects, invertebrates, prairie-chicken, *Tympanuchus cupido attwateri*.

Red imported fire ants (*Solenopsis invicta*) were accidentally introduced to the United States at the port of Mobile, Alabama, circa 1930 (Allen et al. 1995), and arrived in Attwater's prairie-chicken (*Tympanuchus cupido attwateri*; hereafter Attwater's) range circa 1970 (Allen et al. 1995, eXtension 2008). Today these fire ants occupy almost the entire southeastern United States (Porter and Savignano 1990). Fire ants affect a host of fauna, including insects (e.g., Porter and Savignano 1990, Drees 1994, Mueller et al. 1999, Wojcik et al. 2001, Allen et al. 2004). Although there is considerable evidence documenting direct adverse effects at the individual level, few studies address population-level impacts, and fewer still provide experimental evidence regarding functional mechanisms of indirect impacts.

The Attwater's prairie-chicken is endemic to prairies of the Texas and Louisiana gulf coast (Bendire 1894), and was listed as an endangered species in 1967 (U.S. Fish and Wildlife Service [USFWS] 2010). Historically approaching 1 million individuals on 2.4 million ha of habitat (Lehmann 1968), fewer than 110 have existed in the wild since 1995 despite intensive management (USFWS 2010). 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). The Attwater's Prairie-Chicken 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 the total loss of 8 broods <12 days post-hatch. Several dead or dying chicks were found with brood hens at night roosts (USFWS 2010). Necropsy of those chicks attributed cause of death to inanition and dehydration.

Invertebrates are a critical food source for young prairie-chickens, including Attwater's (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 *T. cupido* <5–8 weeks old. From 2004–2012, we confined broods (i.e., hen and chicks) at nest sites for 2 weeks post-hatch and provided locally collected insects ad libitum. Most (83% of 547) chicks treated in this manner survived the critical 2-week period. These observations led us to hypothesize that reduced invertebrate abundance resulting from fire ant invasion was limiting Attwater's chick survival. In this study, we investigated the relationship between invertebrate abundance and Attwater's brood survival and the effects of fire ants on invertebrate abundance at multiple large-scale treatment sites during the Attwater's early brooding season (May–mid-June).

STUDY AREA

We conducted our study at 5 locations in Texas within the Attwater's prairie-chicken's current or historical range. The 970 ha Texas City Prairie Preserve (TCPP; Galveston County) was owned and managed by The Nature Conservancy of Texas. The 4,265-ha APCNWR (Colorado County) was part of the USFWS National Wildlife Refuge System, and was established specifically to maintain habitat for Attwater's prairie-chickens. The Midwell (Goliad County), West Clarkson (Goliad County), and Tivoli (Refugio County) study sites were located on private cattle ranches situated within approximately 20,445 ha of relatively contiguous grasslands (USFWS 2010). All 5 sites were within the gulf prairies and marshes vegetational area of Texas (Hatch et al. 1990) and consisted of managed open grasslands dominated by mid–tall native grass species. Four of the sites had wild Attwater's populations, which had been supplemented with captive-reared birds (Morrow et al. 2004, USFWS 2010).

Climate of the region was subtropical, and dominated by warm, moist air masses derived from the Gulf of Mexico (Smeins et al. 1991). Annual precipitation ranged from approximately 864 mm at the western-most site (Midwell) to 1,118 mm for the eastern-most site (TCPP; Hatch et al. 1990). During 2011, extreme drought conditions prevailed throughout the region, and rainfall totals were only 41–57% of long-term averages for our study areas (National Oceanic Atmospheric Association 2013).

All sites were grazed by cattle during our study. Grazing at APCNWR and Midwell was at light to moderate levels, West Clarkson and Tivoli received moderate to heavy grazing, and TCPP received heavy grazing (M. E. Morrow, Attwater Prairie Chicken National Wildlife Refuge, unpublished data; C. Carter, Ranch Consultant, and A. Tjelmeland, The Nature Conservancy, personal communication). Grazing pressure was exacerbated during drought conditions. All sites used prescribed burning as a management tool except Tivoli, which had no history of prescribed burning for more than 25 years. The Midwell and West Clarkson sites were aerially treated in a strip pattern (approx. 75–90% coverage) by ranch managers with a broadleaf herbicide (2,4-D) in late April–early May of 2012 to suppress forbs and minimize competition with forage grasses (C. Carter, personal communication).

METHODS

We treated a 308-ha block at APCNWR by helicopter with 1.7 kg/ha (1.5 lbs/ac) of Extinguish Plus^{TM} (0.365% hydramethylnon, 0.25% s-methoprene; Central Life Sciences, Schaumburg, IL) fire ant bait to suppress fire ants as a pilot project during April and November 2009. Bait products such as Extingush Plus are specific to ants, and have few environmental risks compared to contact insecticides (Barr et al. 2005, Knutson and Campos 2008). Although other ant species are susceptible to this product, fire ants dominate the insecticide-laden bait product to their detriment while allowing native ant population increases (Calixto et al. 2007). During November 2010 and again in late-September 2011, we treated 527, 294, 204, 259, and 179 ha similarly at APCNWR, Midwell, Texas City, Tivoli, and West Clarkson, respectively. We treated only APCNWR in September 2012. We applied Extinguish Plus during weather conditions appropriate for application (no moisture on vegetation, temperature >21°C). We made on-the-ground observations during application to verify that the bait was picked up by fire ants.

Brood Survival

From 2009–2013, we evaluated the relationship between brood survival and invertebrate abundance at the APCNWR and Midwell study sites by monitoring wild Attwater's broods. Attwater's hens were equipped with poncho-mounted radio transmitters with tuned-loop antennas (Telemetry Solutions, Walnut Creek, CA; Advanced Telemetry Systems, Isanti, MN) incorporated into the poncho (Amstrup 1980, Toepfer 2003). A limited number of transmitters (American Wildlife Enterprises, Monticello, FL) were equipped with whip antennas. We trimmed antennas on these transmitters to extend only approximately 6 cm beyond the poncho to avoid potential interference with flight (Marks and Marks 1987). We placed transmitters (≤3% of body mass) on hens several months in advance of nesting.

We tracked broods (hen with chicks) using telemetry approximately 10 times (i.e., daily during the 5-day work week) during the first 2 weeks post-hatch, except when adverse weather hindered obtaining these data or hen movements (successive >1.6 km) indicated probable brood loss. We determined brood hen locations either by triangulation using a vehicle-mounted 6-element yagi antenna system and computer software (DogTrack, Blacksburg, VA; Locate III, Tatamagouche, NS, Canada), or by circling brood hens at a distance of 10–20 m with a hand-held telemetry system and recording location data with a global positioning system (GPS) unit. We classified brood locations with respect to fire ant treatment (i.e., located in an area treated within the past year or not), and determined the proportion (%) of locations occurring within treated areas. Radiomarking of hens and brood monitoring activities were authorized by Federal Fish and Wildlife Permit TE051839

and Texas Parks and Wildlife Department Scientific Research Permit SPR-0491-384.

We collected invertebrate samples at brood locations 1–4 days later to avoid disturbance to the broods. We made 25 vigorous sweeps of vegetation along an approximately 20-m transect in a random direction from the pre-determined brood location with a 38-cm diameter, canvas sweep-net. Each sweep consisted of a single approximately 2-m arc of the net. We did not collect samples when vegetation was wet. We labeled invertebrate samples and froze them until we could determine counts of individuals for each sample. We then dried samples for 24 hours at 60 °C and weighed them to the nearest 0.0001 g on a digital analytical scale. We determined median invertebrate numbers and dry mass for each brood to control for pseudoreplication within brood unit (Crawley 2007). We censored broods if the hen died prior to 14 days post-hatch.

We approached radioed hens with hand-held telemetry equipment 14 days post-hatch and observed them at dawn, before leaving night roosts, to determine if any chicks remained with the hen. We encouraged hens to move only if necessary to be absolutely certain of chick presence. We considered a brood successful if we observed ≥1 chick with the hen. To reduce disturbance to the hen and brood, we did not attempt to obtain a total count of chicks.

Impact of Fire Ant Suppression on Invertebrate Abundance

During 2011–2012, we assessed invertebrate abundance and biomass following fire ant suppression using an impact-reference design replicated at 5 locations (APCNWR, Midwell, TCPP, Tivoli, West Clarkson) with 3 temporal replicates during each year. We randomly assigned treated and untreated reference areas at each of the 5 sites. Each treated area received 2 applications of Extinguish Plus (autumn 2010 and 2011) as described previously. The pilot project area at APCNWR was not included in this portion of the study.

We sampled invertebrates and fire ant activity on each location replicate at 14 0.2-ha randomly located plots within treated and reference areas (28 sampling plots/site). Sampling plots were >61 m from treatment edges, and were stratified by ecological site and time since burn. We sampled areas each year on 3 occasions separated by about 2 weeks starting in late April–early May following the autumn applications of Extinguish Plus. We assessed fire ant activity within each sampling plot by placing 10 fatty lures (approx. 0.5-inch hot dog slices) on the ground using surveyor's flags, 1 in the center and 9 spaced roughly equidistantly around the perimeter of the 0.2-ha plot. After 45–60 minutes, we estimated and recorded the number of fire ants on the top and sides of each hot dog slice (Drees 2002). We estimated numbers of ants present in increments of 10 up to a maximum of 100. We obtained invertebrate samples by sweep-netting in a random direction using the procedure described previously for prairie-chicken brood sites and froze samples for 24 hours. Invertebrates were separated into 2 groups, counted, dried, weighed to the nearest 0.0001 g, and

stored in alcohol. We sorted invertebrates into 2 groups to differentiate between those that were more prone to flight and therefore could have moved into the plot, from those that were more typically ground-dwelling and less likely to have immigrated from outside treatment areas. Invertebrates more prone to flight included adult (indicated by wings protruding behind the posterior tip of the abdomen) grasshoppers or katydids (Orthoptera), Dipterans, adult Lepidopterans, and bees or wasps (Hymenoptera). Ground-dwelling invertebrates included juvenile grasshoppers or katydids; leafhoppers (Cicadellidae); beetles, weevils, and grubs (Coleoptera); crickets (Gryllidae); larval Lepidopterans, mantids or walking sticks (Mantodea, Phasmatodea); ants (Formicidae); and snails (Gastropoda). These 2 groups (hereafter mobile-flier and ground-dwelling, respectively) also likely represented functional groups for Attwater's chicks because mobile-fliers would be more difficult for chicks to catch than the ground-dwelling group. More than 85% of the invertebrate species found in Attwater's diets by Lehmann (1941) would likely have been classified as ground-dwelling based on the season of collection. We removed 9 samples with missing or uncertain observations, resulting in a total of 831 invertebrate samples for analysis ($n = 168$ each for Midwell, West Clarkson, and Tivoli; 166 for APCNWR; and 161 for TCPP).

Data Analyses

Brood survival.—We determined the relationship of invertebrate abundance (counts and biomass) and fire ant treatment with brood survival (S_b) using generalized linear mixed effects models. The response variable was presence or absence of at least 1 chick at 2 weeks post-hatch. Explanatory variables included the median count and biomass of invertebrate samples collected during the first 2 weeks after hatch for each brood, year, site, and the percentage of observations for each brood that were in treated fields. We used hen ID to specify a random effect (intercept). By including hen as a random variable, we avoided pseudo-replication resulting from the inclusion of hens with more than one brood in successive years. We built models using the glmer function in the package lme4 (Bates et al. 2014) in the R statistical system (R Version 3.2.0, www.r-project.org, accessed 27 May 2015) and specified a binomial distribution with a logit link. We identified top models using change in Akaike's Information Criterion values (ΔAIC; Akaike 1973) and associated Akaike weights (*w*; Burnham and Anderson 2002). We report 90% rather than 95% confidence intervals because 1) given that the Attwater's prairie-chicken is a critically endangered species, we considered the risk of mistakenly concluding no treatment effect when there was one (type II error) a worse error than the risk of concluding there was a treatment effect when there was not (type I error); and 2) the small size of the remaining population of Attwater's prairie-chickens meant that the brood sample size was also small.

Effect of fire ant suppression on invertebrate abundance.—We determined the effect of fire ant treatment on invertebrate count and biomass using linear and generalized linear mixed

effects models. Random effects in these models accounted for lack of independence among samples collected from the same plots. Although differences among sites were not of particular interest to us and therefore could have have been included as random effects, we included site as a fixed effect because sample size ($n = 5$) was too small to include this variable as a random effect (Bolker et al. 2009). The basic procedure for model fitting was the same for both linear and generalized linear mixed effects models. We first fit competing models of random effects using the same global model of fixed effects for all models (Zuur et al. 2009). We compared 4 competing models of random effects: a random intercept of the plot only model and 3 models with a random intercept of plot and a random slope of plot across time (year, day of sample, and sample period). The fixed effects in the global model were year, site, treatment, and an interaction between site and year. We used the random effects component in the model with the lowest AIC in subsequent models comparing fixed effects. Our candidate set of fixed effects included 9 models using combinations of treatment, site, year, the interaction between site and year, and the interaction between treatment and year. We included the interaction between treatment and year to account for possible greater treatment effects in the second year, after 2 years of fire ant suppression. We identified top models using ΔAIC and w values. We ran all analyses in the R statistical system.

Response variables for count analyses included counts of individuals for the mobile-flier and ground-dwelling invertebrate groups, and for total invertebrates. Using generalized models for count data is preferred because they can model dispersion and eliminate the possibility of predicting negative counts (O'Hara and Kotze 2010). For all count data sets, variances of the counts were much larger (range 2–72× for all site and year combinations) than the mean; therefore, we used a negative binomial distribution to account for this overdispersion. We visually inspected for outliers and removed 4 (0.5%) deviant observations. We compared data sets with outliers removed to unaltered data sets to verify that removing outliers did not alter the magnitude or direction of parameter estimates. We built count data analysis models using the glmmadmb function in the package glmmADMB (Fournier et al. 2012, Skaug et al. 2012).

For biomass data, we used linear mixed effects models for ground-dwelling and total invertebrates. We Box-Cox transformed data to approximate normality (Box and Cox 1964). We selected the type of transformation for each data set using the powerTransform function in the car package (Fox and Weisberg 2011). We visually inspected transformed data using quantile–quantile plots to verify normality and did not detect any outliers. We built models using the lmer function in the package lme4 (Bates et al. 2013).

Biomass data of mobile-flier invertebrates had an excess amount of zeros and we could not find a transformation that would approximate normality. Therefore, we used a hurdle model for this data set (Cragg 1971). The log-normal hurdle model consists of 2 parts. The first part is a binomial model

that divides the data into its zero and non-zero components; the second part models the positive range of the data set. This model allows for the possibility that the mechanisms that determine the zero and non-zero components can differ (Ridout et al. 1998). In other words, the hurdle model tests whether fire ant treatment affects the presence of mobile-flier invertebrates within a field, and then separately, given that mobile-fliers are present, tests the effect that fire ant treatment has on mobile-flier biomass. We built hurdle models using the glmmadmb function in the package glmmADMB.

RESULTS

Brood Survival

We collected brood survival data from 36 hens with 44 broods at APCNWR and 17 hens with 19 broods at Midwell. Of these 63 broods, 28 (44%) successfully maintained chicks until 14 days post-hatch. Twenty-one (33%) broods were in treatment areas at some point during observation, although most broods remained entirely within treatment (27%) or reference fields (67%). Of 427 brood locations, 29% were in fields treated to suppress fire ants. We made a median 7 observations on individual broods (range 1–12).

The median number of total invertebrates at brood sites was the best predictor of 2-week brood survival (S_b), with 6 times more support for this model than for the next best model (Table 1; Fig. 1). The effect of median number of invertebrates ($\beta = 1.45$, 90% CI 0.43–2.41) on brood survival was positive; broods located in areas with the highest median invertebrate counts (338 invertebrates/sample) were more than 11 times more likely to have chicks at 2 weeks post-hatch ($S_b = 0.83$, 90% CI 0.56–0.95) than those areas with the lowest count (18 invertebrates/sample; $S_b = 0.07$, 90% CI 0.01–0.31). With considerably less support, invertebrate biomass ($\beta = 1.09$, 90% CI 0.13–1.99) was the next best predictor of brood survival. As with invertebrate counts, broods located in areas with the highest median invertebrate biomass (2.6 g/sample) were more than 7 times more likely to have chicks at 2 weeks post-hatch ($S_b = 0.78$, 90% CI 0.47–0.94) than those with the lowest dry weight (0.1 g/sample; $S_b = 0.10$, 90% CI 0.02–0.41; Fig. 1). The 2 models with invertebrate variables had a cumulative w of 0.87, indicating

Table 1. Results for binomial mixed effects modeling of factors affecting brood survival at 2 weeks for Attwater's prairie-chicken at 2 sites in the Texas gulf coast prairie ecosystem during 2009–2013. K is the number of parameters estimated, AIC is Akaike's Information Criterion, ΔAIC is the difference in AIC from the best model, and w is the model weight.

Model	K	AIC	ΔAIC	w	Σw
Invertebrate count	3	81.85	0.00	0.75	0.75
Invertebrate biomass	3	85.46	3.61	0.12	0.87
Brood locations[a]	3	87.06	5.21	0.06	0.93
Year	6	88.05	6.20	0.03	0.96
Null	2	88.99	7.14	0.02	0.99
Site	3	89.67	7.83	0.01	1.00

[a] Indicates the percentage of brood locations that were in a treated field during the first 2 weeks.
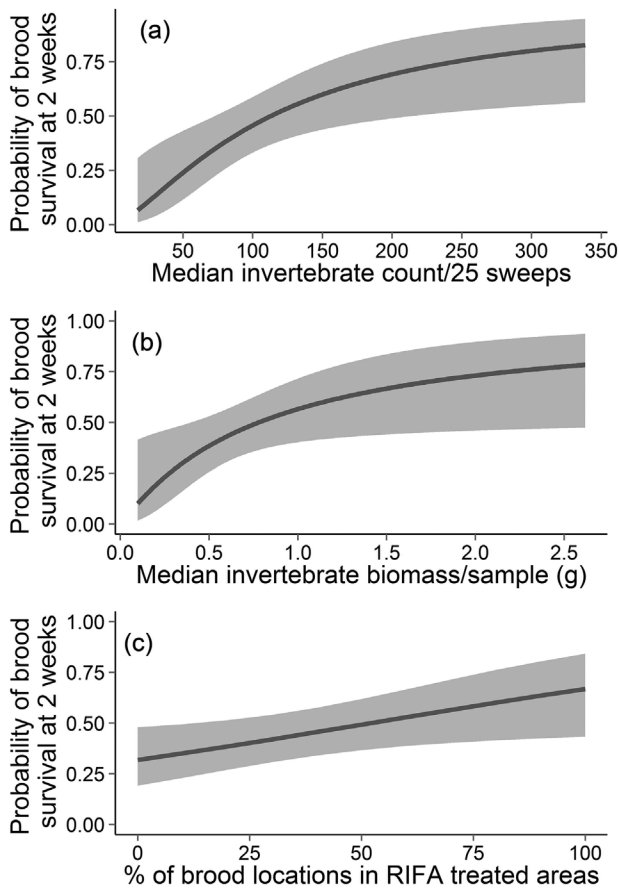
Figure 1. Effect of (a) median invertebrate count, (b) median invertebrate biomass, and (c) the percentage of brood observations in fields treated to suppress red imported fire ants (RIFA) on probability of Attwater's prairie-chicken brood survival to 2 weeks. Gray area indicates 90% confidence intervals.

considerably less support for the remaining 3 models (Table 1). Although the percent of brood observations within fire ant treated areas had substantially less support than median invertebrate numbers, the β value (β = 1.46, 90% CI 0.14–2.78) indicated that the estimated probability of chicks being observed at 2 weeks was more than doubled if a brood was always observed in a treated field ($S_b = 0.67$, 90% CI 0.43–0.84) compared to a brood never observed in a treated field ($S_b = 0.32$, 90% CI 0.19–0.38; Fig. 1).

### Impact of Fire Ant Suppression on Invertebrate Abundance

We observed a reduction in fire ant activity as measured by the fatty lure counts across all sites treated with Extinguish Plus compared to reference sites; in 2011, fire ant counts on treated sites averaged 53% lower than reference site counts (range 35–68%) and in 2012 fire ant counts on treated sites averaged 84% lower than reference site counts (range 64–100%). Although invertebrate abundance and biomass were highly variable across years and sites, 8 of 10 comparisons (5 sites, 2 years) for each dataset showed positive treatment responses (Fig. 2). The best supported models for the total, mobile-flier, and ground-dwelling invertebrate counts included year, site, an interaction between year and site,

and a treatment effect (Table 2). For mobile-fliers, the model selection results for counts also indicated an interaction between treatment and year. Residuals of top models were approximately normally distributed with no strong pattern of overdispersion or heteroscedasticity. The confidence intervals of the relative treatment effect did not overlap 1 in any of the top count models (Table 3). For all invertebrates combined, count per plot for treated fields was an estimated 1.27 (95% CI 1.16–1.40) times higher than reference fields across all sites and years. Mobile-flier and ground-dwelling invertebrates had similar responses to treatment (Table 3), although there was support for differences in mobile-flier treatment effects by year.

For biomass, treatment was in the top models for ground-dwelling invertebrates and total invertebrates with less support for a treatment effect for mobile-flier invertebrates (Table 2). The best transformations for total and ground-dwelling invertebrate biomasses were the 0.20 and 0.24 power transformations, respectively. Residuals of the top models were approximately normally distributed with no strong pattern of overdispersion or heteroscedasticity. Total invertebrate biomass had a nearly identical response to treatment as total count; total biomass in the treatment fields was an estimated 1.26 (95% CI 1.15–1.39) times higher than the biomass in reference fields across all sites and years. For mobile-flier biomass, there was evidence that fire ant treatment slightly increased the probability that mobile-flier invertebrates would be present at a plot in 2011 but not in 2012, and there was no evidence that fire ant treatment increased the biomass of mobile-flier invertebrates at a plot, given that they were present (Table 3). For ground-dwelling invertebrate biomass, there was evidence of a treatment and year interaction (Tables 2, 3). Contrary to our expectation, there was no clear evidence of treatment effects increasing during the second year of the study (Table 3); ground-dwelling invertebrate biomass was the only 1 of the 6 analyses to exhibit a larger treatment effect in the second year of treatment.

DISCUSSION

Juvenile grouse (subfamily Tetraoninae) experience highest mortality during the first 2 weeks of life (Hannon and Martin 2006). Data collected in this study clearly demonstrate that availability of invertebrates during this period was a major factor limiting survival for young Attwater's prairie-chickens. Our observations were consistent with other studies affirming the importance of invertebrates to galliform chicks during the first weeks of life, and for prairie-chicken species in particular (Lehmann 1941, Jones 1963, Savory 1989, Hagen et al. 2005). Lehmann (1941:26) observed that stomachs of 3 juvenile Attwater's prairie-chickens contained 88.5% insects. Population simulations suggest that to reach Attwater's prairie-chicken recovery goals, brood survival must increase to levels comparable to stable greater prairie-chicken (*Tympanuchus cupido*) populations and those of historical wild Attwater's prairie-chickens (Pratt 2010). Pratt (2010) reported 69% survival of greater prairie-chicken
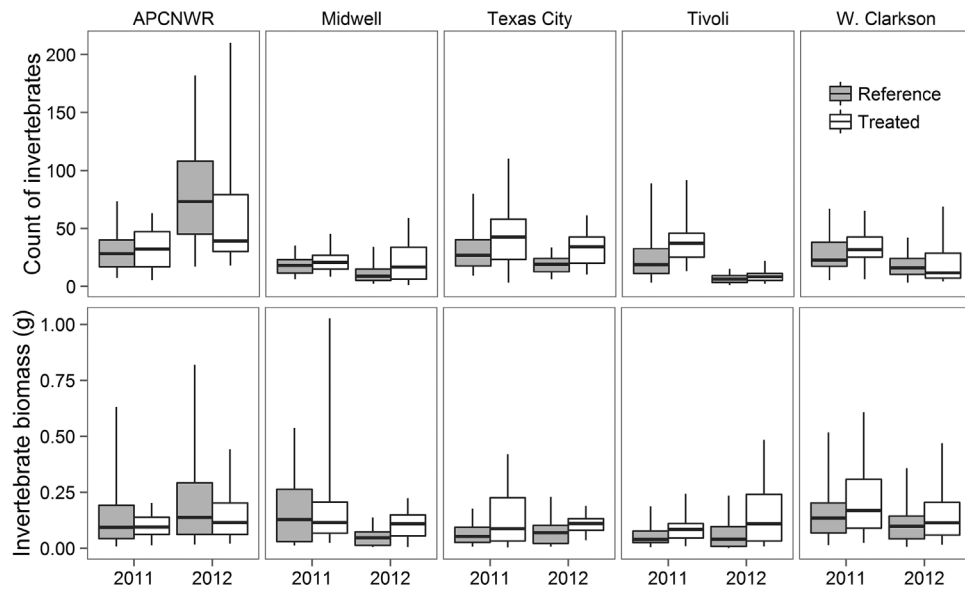
Figure 2. Median invertebrate counts and biomass/25 sweeps at 5 locations in Texas during 2011–2012 including Attwater Prairie Chicken National Wildlife Refuge (APCNWR). The dark line indicates median value with boxes representing the 75% and 25% quantiles and the lines representing the 2.5% and 97.5% quantiles.

broods to 2 weeks post-hatch. Our analyses suggest that Attwater's brood habitat would have to support invertebrate populations resulting in approximately 190 invertebrates/25 sweeps to achieve this brood survival (Fig. 1). Invertebrate abundances of this level or higher were observed only for 16% of broods, suggesting that much of Attwater's brood habitat supported less than the desired levels of invertebrate abundance. However, broods with all of their locations within treated fields had survival estimates at 2 weeks of 67% (90% CI 43–84%), suggesting that widespread fire ant control could result in Attwater's prairie-chicken brood

survival comparable to that observed for stable greater prairie-chicken populations (Pratt 2010).

Data collected in this study also clearly demonstrate that fire ants have caused a substantial reduction in invertebrate abundance within historical and extant Attwater's habitats, despite considerable variation among sites and between years (Fig. 2). Insects, especially immature forms, make up the bulk of fire ant diets (Hays and Hays 1959). This suggests a likely mechanism of fire ant impacts on invertebrates, and likely explains the more consistent increases of ground-dwelling invertebrates due to treatment for both count and

Table 2. Results for linear and generalized linear mixed effects modeling of the effect of fire ant treatment on invertebrate counts and biomass at 5 sites in the Texas gulf coast prairie ecosystem during 2011–2012. K is the number of parameters estimated, ΔAIC is the difference in Akaike's Information Criterion (AIC) from the best model, and w is the model weight. Only models with ΔAIC <10 are shown.

Data set[a]	Fixed effects[b]	K	ΔAIC	w
Count				
Mobile-fliers	Site + year + site × year + treatment + treatment × year	14	0.0	0.75
	Site + year + site × year + treatment	13	2.3	0.24
	Site + year + site × year	12	9.6	0.01
Ground-dwelling	Site + year + site × year + treatment	13	0.0	0.70
	Site + year + site × year + treatment + treatment × year	14	1.7	0.30
Total	Site + year + site × year + treatment	13	0.0	0.73
	Site + year + site × year + treatment + treatment × year	14	2.0	0.27
Biomass				
Mobile-fliers (presence)	Site + year + site × year + treatment + treatment × year	13	0.0	0.78
	Site + year + site × year + treatment	12	2.6	0.21
	Site + year + site × year	11	9.0	0.01
Mobile-fliers (if present)	Site + year + site × year	12	0.0	0.66
	Site + year + site × year + treatment	13	1.5	0.25
Ground-dwelling	Site + year + site × year + treatment + treatment × year	14	0.0	0.61
	Site + year + site × year + treatment	13	1.1	0.36
	Site + treatment	8	6.1	0.03
Total	Site + year + site × year + treatment	15	0.0	0.58
	Site + year + site × year + treatment + treatment × year	16	0.6	0.42

[a] Mobile-fliers = more mobile, flying invertebrates; ground-dwelling = less mobile invertebrates; and total = those 2 groups combined.
[b] A random intercept of sampling plot was included in all models, except total biomass which had a random intercept of sampling plot and a random slope across the 3 sampling periods.

Table 3. Treatment effects (relative to untreated reference plots) of red imported fire ant suppression on insect abundance across 5 sites within the Texas gulf coast prairie during 2011–2012 as estimated from linear and general linear mixed effects models.

Type	Data set[a]	Year[b]	Treatment effect[c]
Count	Mobile-fliers	2011	1.53 (1.22–1.91)
		2012	1.10 (0.82–1.50)
	Ground-dwelling	Both	1.28 (1.15–1.43)
	Total	Both	1.27 (1.16–1.40)
Biomass	Mobile-fliers presence	2011	1.12 (1.00–1.17)
		2012	1.00 (0.86–1.14)
	Mobile-fliers if present	NA[d]	NA
	Ground-dwelling	2011	1.19 (1.07–1.31)
		2012	1.34 (1.17–1.52)
	Total	Both	1.26 (1.15–1.39)

[a] Mobile-fliers = more mobile, flying invertebrates; ground-dwelling = less mobile invertebrates; and total = those 2 groups combined.

[b] If the model selection process supported the inclusion of a year × treatment interaction, treatment effects were given by year.

[c] The treatment effect is relative to the reference plots (e.g., a treatment effect of 1.53 indicates treated plots had 1.53 × the abundance or biomass of the reference plots). A treatment effect of 1 indicates no treatment difference, <1 or >1 indicates a decrease or increase in abundance or biomass due to treatment, respectively. Numbers in parentheses indicate 95% confidence intervals.

[d] No evidence of treatment effects.

biomass, and between years of our study, compared to mobile-fliers (Table 3). Ground-dwelling invertebrates consisted of immature Orthopterans as well as others we hypothesized to be less mobile (e.g., leaf hoppers, beetles, spiders) and therefore more vulnerable to Attwater's and fire ant predation.

A likely explanation for lack of treatment effects and substantially higher invertebrate abundance at APCNWR in 2012 (Fig. 2) is that APCNWR was actively managed to increase insect abundance in Attwater's brood habitat for several years prior to our study because Attwater's are the focus of management at this site. Management practices directed at increasing invertebrate abundance have included patch burning (e.g., Fuhlendorf and Engle 2001, Roper 2003, Fuhlendorf et al. 2006, Doxon 2009, Doxon et al. 2011) and removal of cattle during January–June. Fire breaks on APCNWR are disked to encourage early successional forbs, which often support high insect numbers (Jones 1963, Green 1984, Hill 1985). Disking fire breaks also occurred at the Midwell and West Clarkson but not at TCPP or Tivoli.

Removal of grazing during the first half of the growing season, and reduction in overall grazing pressure due to the 2011 drought, also likely 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 (M. E. Morrow, personal observation). Quinn and Walgenbach (1990) observed that grazed sites supported higher numbers of obligate grass-feeding grasshoppers, whereas ungrazed grasslands were dominated by mixed forb- and grass-feeding grasshoppers, implying that grazing may have reduced forb abundance and thus forb-feeding grasshoppers in that study.

Other 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 our study were very different, with drought conditions prevailing during 2011 but not during 2012. This likely explains why year was included in all of the top models in the linear and generalized linear mixed effects models of invertebrate abundance (Table 2). It may also explain the smaller treatment effects we observed (1.27 and 1.26× reference plots for invertebrate numbers and biomass, respectively) across all sites compared to other studies (Porter and Savignano 1990, Allen et al. 2001). Much of the increased invertebrate abundance for APCNWR in 2012 (Fig. 2), irrespective of treatment, was 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 (J. Kelso, The Nature Conservancy of Texas, personal communication). Although undoubtedly fire ants have an overall suppressive effect on insect abundance during the May–mid-June Attwater's brooding period, there are likely times when environmental conditions are favorable for eruptive invertebrate population growth that are able to overcome the suppressive impacts of fire ants. Studies of longer duration than 2 years would be necessary to evaluate that hypothesis. This high level of spatial and temporal variation in invertebrate densities may result in a lack of an observed treatment effect of fire ant suppression in some years or locations.

Reports of adverse direct impacts of fire ants to native fauna are numerous (Allen et al. 2004), but to our knowledge few studies address indirect impacts of this invasive species. Indirect effects related to a reduction in invertebrates due to fire ants have been hypothesized for diverse groups of species including northern bobwhites (*Colinus virginianus*; Allen et al. 1995), loggerhead shrikes (*Lanius ludovicianus*; Lymn and Temple 1991), Texas horned lizards (*Phrynosoma cornutum*; Allen et al. 2004), and the endangered Houston toad (*Bufo houstonensis*; USFWS 2009). Allen et al. (2001) observed that both insect biomass and loggerhead shrike abundance were negatively correlated with fire ant abundance.

MANAGEMENT IMPLICATIONS

Our study suggests that for Attwater's recovery to be successful, fire ants must be controlled or other methods found to mitigate their effects such that invertebrates used as food by Attwater's chicks are present in sufficient numbers to support high brood survival. Given the Attwater's high potential reproductive rate (Pratt 2010, USFWS 2010), improving brood success should result in substantial progress toward recovery of this critically endangered species. Results of our study indicate that the ecological disruption caused by

invasive fire ants goes beyond direct impacts to individual species, and may contribute to a trophic cascade affecting other species occurring throughout the acquired range of the red imported fire ant. Indeed, long-term population declines observed for northern bobwhites and wild turkeys (*Meleagris gallopavo*; North American Bird Conservation Initiative 2009, Nickens 2013) may be partially attributable to indirect impacts of fire ants, as chicks of both species depend on insect-rich diets (Palmer et al. 2001, Randel et al. 2007). We strongly suggest that additional research is needed to fully elucidate the indirect impacts of fire ants on other species and to provide guidance for corrective management actions.

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