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# Regal Fritillary (Speyeria idalia) Sex Ratio in Tallgrass Prairie: Effects of Survey Timing and Management Regime

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ABSTRACT.—The regal fritillary, *Speyeria idalia* (Drury), was once a common inhabitant of North American grassland communities. Regal fritillary populations are commonly reported to have a male biased adult sex ratio (ASR) throughout their range. We assessed the observed ASR of regal fritillary throughout an annual flight period, investigated how the overall density of both sexes changed, and tested effects of prescribed fire, grazing and haying management treatments on male and female density. We found that regal fritillary exhibited an observed 2:1 male biased ASR across the entire emergence period. Our analysis also revealed that male density peaked earlier than female density in the flight period. Point estimates of density indicated sites that received prescribed burning at the moderate fire-return interval supported $\geq$1.3 times greater density of males and $\geq$5.6 times greater density of females versus sites burned with short and long fire-return intervals. Additionally, this effect was enhanced when combined with grazing which showed males were $\geq$1.9 times and females had $\geq$1.2 times greater point estimates of density in sites that were grazed and burned at a moderate fire-return interval versus other sites. The relatively stable status of regal fritillary within our study region suggests that a 2:1 male to female ASR may be considered the model composition of populations throughout their range. Likewise, the dynamic nature of the ASR throughout the flight period highlights the importance of conducting surveys across the flight period. Finally, these results corroborate an increasing number of research results that reveal common prairie management practices, such as prescribed fire can be applied within sites that contain regal fritillary and continue to support stable populations.

## INTRODUCTION

Adult sex ratio (ASR) is an important concept in population biology and can vary greatly among species and across spatio-temporal scales (Bessa-Gomes *et al.*, 2004; Ewen *et al.*, 2011; Székely *et al.*, 2014). For instance biased ASRs have been reported in populations of birds (Donald, 2007), fish (McKellar *et al.*, 2009), tetrapods (Le Galliard *et al.*, 2005; Pipoly *et al.*,

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2015) and butterflies (Nagel *et al.,* 1991; Kopper *et al.*, 2001a; Vlašánek *et al.,* 2009; among others). Consequently, understanding patterns, causes, and consequences of variance in ASR estimates is important, given its implications for population demography and biodiversity conservation (Baguette *et al.*, 1996; Underwood and Shapiro, 1999; Vlašánek *et al.,* 2009; among others). Although butterfly populations in the wild often exhibit male biased ASRs (Tabashnik, 1980; Ehrlich, 1989; Kopper *et al.*, 2001a; Vlašánek *et al.*, 2009; among others), the sex ratio of laboratory reared butterflies is typically 1:1 (reported as M:F hereafter; Ehrlich *et al.*, 1984). A number of hypotheses have been proposed to explain male-biased ASRs in butterfly populations, including: differing mortality rates during larval development; protandry resulting in sampling bias; and differences in detection due to sex related alterations in behavior that ultimately lead to sample bias (Brussard and Ehrlich, 1970; Clayton and Petr, 1992; Scriber *et al.*, 1998; among others).

The ASR of a population is linked to a wide range of behaviors and ultimately fitness (Baguette *et al.*, 1996; Underwood and Shapiro, 1999; Dyson and Hurst, 2004; Vlašánek *et al.*, 2009, among others). For example in populations that are male biased, breeding behavior and male to male competition may be exaggerated (Leftwich *et al.*, 2012). A male biased ASR may also lead to increased aggression and harassment towards females and result in increased female mortality (Le Galliard *et al.*, 2005). Furthermore, if one sex is more abundant, a lower number of individuals of the opposite sex contribute disproportionately to the next generation (Vlašánek *et al.*, 2009). This situation is particularly precarious for threatened or endangered species that already occur in small numbers by further diminishing the genetic diversity and overall viability of populations (Frankham, 1995; Wedekind, 2002). The regal fritillary, *Speyeria idalia* (Drury), is commonly reported to have a male biased ASR (Nagel *et al.,* 1991; Kelly and Debinski, 1998; Kopper *et al.,* 2001a; Swengel and Swengel, 2005).

As a prairie specialist butterfly, the regal fritillary was historically a common sight within North American grassland communities (Hammond and McCorkle, 1983; NatureServe, 2005), and is often considered an indicator or flagship species of pristine native prairie (Hammond and McCorkle, 1983). Regal fritillary's range once extended from eastern Colorado eastward to Maine, U.S.A., and as far north as southern Canada and south into Oklahoma, U.S.A (NatureServe, 2005; Selby, 2007). Unfortunately, the regal fritillary has suffered sharp declines in abundance and marked range contraction (NatureServe, 2005; Selby, 2007; Sims, 2017; among others). It is estimated that eastern populations have been in decline since at least the 1940s (Wagner *et al.*, 1997); 40% of the regal fritillary's historical range has been lost since the 1970s (NatureServe, 2005). Currently, the regal fritillary is being considered for federal listing under the 1973 Endangered Species Act (USFWS, 2015). Although habitat loss, fragmentation, and conversion are largely thought to be the primary causes for the range wide declines of the regal fritillary (Hammond and McCorkle, 1983; NatureServe, 2005; Selby, 2007; among others), a number of studies have suggested that prescribed fire also may be harmful to the species (Swengel and Swengel, 2001; Vogel *et al*, 2007; Swengel *et al.,* 2011; among others). Likewise, intensive, unmanaged grazing (Hammond and McCorkle, 1983; Royer and Marrone, 1992), improperly timed haying (Selby, 2007), and degradation of remaining habitat are also thought to be threats to remaining populations (Sims, 2017; Henderson *et al.*, 2018).

The vast, relatively connected expanses of grasslands within the Flint Hills ecoregion of Kansas, U.S.A., affords a unique opportunity to study the regal fritillary within a landscape scale prairie framework, which contrasts most previous work that is often conducted in small isolated prairie remnants (Ferster and Vulinec, 2010; Moranz *et al.*, 2014; Henderson *et al.*, 2018; among others). Additionally, Kansas is one of the few places where the species is reported to be at least locally secure (NatureServe, 2005; Powell *et al.*, 2007; Selby, 2007). Given the strongly male biased ASRs detected among populations in other studies, the changes in sex ratio throughout the adult flight period as a result of protandry and female reproductive diapause, and the ambiguity regarding effects of prairie management practices, such as fire, grazing, as well as haying, our objectives for this study were threefold. First, we assessed the observed ASR of regal fritillary throughout the flight period. Second, we determined how the overall male and female density changed throughout the flight period. Third, we quantified effects of overall management regime (burned, grazed, and hayed) and fire-return interval (short, 1 to 2 y; moderate, 3 to 5 y; or long ≥10 y) on male and female density.

Information pertaining to the ASR of regal fritillary populations from a region where the species is considered locally secure and documenting how the ASR changes through time may provide conservation managers with a baseline value from a stable population. In turn, this baseline could be used to assess the ASR and status of other more precarious populations. Similarly, knowledge of potential effects of common prairie management practices on both male and female regal fritillary may help guide land management decisions. Finally, the regal fritillary is an ideal candidate for studies pertaining to ASRs. Their large size, distinguishable markings, sexual dimorphism, and generally unobstructed view of their grassland habitat enable them to be sexed in the field with relative ease (Swengel and Swengel, 2005).

## METHODS

### STUDY AREA

The information contained within this study and the respective analyses and results were derived from a larger study investigating the effects of management regime and subsequent habitat response on regal fritillary across four years (2012, 2014–2016; McCullough *et al.*, 2019). In the aforementioned study, data were analyzed collectively for both males and females across the study period, in contrast to the information and results presented in this study, which focus on sex specific data collected in 2015. This study was conducted in the Flint Hills ecoregion of northeastern Kansas at the Fort Riley Military Reserve (FRMR; 39°03′N, 96°49′W; Geary and Riley counties) and the Konza Prairie Biological Station (KPBS; 39°05′N, 96°35′W; Riley County; Fig. 1) during 2015. Approximately 29,000 ha of the FRMR's 40,000 ha are managed for multiple uses that include military training, conservation, and outdoor recreation activities. Grasslands comprise nearly 67% of the FRMR and are managed using a combination of burning and haying regimes. Prescribed burns are generally conducted during spring and fall; however, wildfires from live fire military training can happen throughout the year. Many grassland fields on the FRMR are leased for hay harvest, which occurs from 15 July–15 August each year. The KPBS is a 3487 ha native tallgrass prairie preserve jointly owned by The Nature Conservancy and Kansas State University. The KPBS has been a National Science Foundation Long Term Ecological Research site since 1981 and watersheds are experimentally managed with various grazing and burning regimes (Knapp *et al.*, 1998). Prescribed burns are applied at 1, 2, 3, 4, and 20 y intervals, with most fires ignited during spring. Grazing treatments include the use of native bison [*Bison bison* (L.)], cattle [*Bos taurus* (L.)], or no grazing.

Watersheds at the KPBS grazed by cattle are stocked with cow-calf pairs at a light to moderate stocking rate typical of the Flint Hills region of one pair per 3.24 ha for

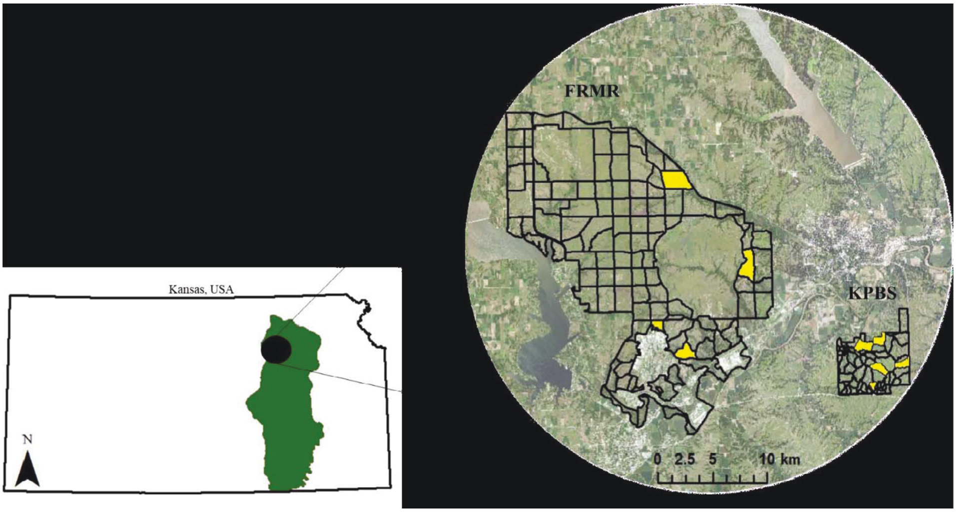

FIG. 1.—Study area and surrounding landscape in the Flint Hills of northeastern Kansas, U.S.A. The green region spanning across the eastern portion of Kansas highlights the Flint Hills ecoregion. The location of the Fort Riley Military Reserve (FRMR) and Konza Prairie Biological Station (KPBS) study sites are indicated by the black circle within the Flint Hills. The enlargement displays the training area and watershed boundaries of the FRMR and KPBS study sites, respectively. The yellow highlighted areas within the FRMR and KPBS depict where regal fritillary (*Speyeria idalia*) adult sex ratio survey transects were located during 2015

approximately five months each year during the growing season (Owensby, 2010; KDA, 2017). Watersheds occupied by bison are grazed year round at a rate of approximately one bison per 6.07 ha. Although bison and cattle differ with respect to their grazing and movement patterns (Kohl et al., 2013), studies have demonstrated that their effects on the vegetation community are comparable, with differences likely attributable to how they are managed rather than to species (Towne *et al.*, 2005; Fuhlendorf *et al.*, 2018). The differences in stocking rate and duration of grazing between cattle and bison at the KPBS make it difficult to determine whether variation in the tallgrass prairie's response and, in turn, any effect on regal fritillary is a reflection of the species of grazer or their respective management. Therefore, for the purposes of this study, bison and cattle units were treated collectively as grazed to broadly demonstrate the response of male and female regal fritillary to the presence of a large herbivore on the landscape.

### REGAL FRITILLARY SURVEYS

We surveyed nine transects randomly distributed throughout the FRMR and KPBS for male and female regal fritillary. Transects were 500 m to >1 km in length and stratified by overall management regime (burned, grazed, and hayed) and fire-return interval (short, 1 to 2 y; moderate, 3 to 5 y; or long ≥10 y; Table 1). The distance between transects ranged from 700 m to >28 km with a mean distance of >13 km separating transects from one another. Transects were surveyed six times (June 8–23, June 24–July 1, July 6–14, July 16–22, July 23–30, and July 31–August 8; Table 1) during the course of the study and successive survey bouts did not begin until all transects for the current bout had been surveyed. Surveys were

Table 1.—The number of male (♂) and female (♀) regal fritillary (*Speyeria idalia*) detected along the nine transects surveyed for sex ratios at the Fort Riley Military Reserve (FRMR) and Konza Prairie Biological Station (KPBS) in northeastern Kansas, U.S.A., during 2015. Included is the transect name, transect location (FRMR or KPBS), treatment (burned, grazed, and hayed) and fire return interval (short, 1 to 2 y; moderate, 3 to 5 y; or long ≥10 y), number of males and females detected at each transect by sample bout, and size of each watershed or training area (ha) in which each transect was located

| | | | No. regal fritillary by sample bout | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | June 8–June 23 | | June 24–July 1 | | July 6–July 14 | | July 16–July 22 | | July 23–July 30 | | July 31–Aug 8 | | |
| Transect | Location | Treatment | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | Area (ha) |
| K1A | KPBS | Burned + short | 3 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 113 |
| 15 | FRMR | Burned + moderate | 1 | 0 | 1 | 0 | 2 | 2 | 0 | 6 | 0 | 2 | 0 | 1 | 95 |
| 20B | KPBS | Burned + long | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 |
| C1SB | KPBS | Grazed + short | 3 | 0 | 3 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 68 |
| C3B | KPBS | Grazed + moderate | 5 | 0 | 4 | 0 | 3 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 226 |
| N20B | KPBS | Grazed + long | 1 | 1 | 4 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 84 |
| 97 | FRMR | Hayed + short | 0 | 0 | 1 | 0 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 385 |
| 29 | FRMR | Hayed + moderate | 3 | 1 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 301 |
| 11 | FRMR | Hayed + long | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 191 |
| | | | **17** | **2** | **16** | **2** | **8** | **8** | **3** | **8** | **3** | **3** | **1** | **1** | |

conducted between 0830 and 1830 CST, under sunny and warm conditions when weather was appropriate for butterfly flight (temperatures were ≥17 C if the sky was overcast, and winds <20 km/h; Pollard and Yates, 1993). Surveys were conducted by two people walking the transect centerlines. While one person recorded the perpendicular distance from the centerline to each regal fritillary detected, the second person identified the sex of ≥25% of regal fritillaries detected. The distance at which each regal fritillary was first detected from either side of the transect centerline was estimated within width binned intervals of 0–5 m, >5 m–10 m, >10 m–20 m, and >20 m–30 m. Sex was determined by visual observation and based on the color of the outermost row of spots on the dorsal hindwing, which is orange on males and white on females (Scudder, 1889; Figs. 2A, B). When not in flight (*e.g.*, nectaring and resting), the individual was observed until it opened its wings and sex could be determined. Individuals that were flying were followed until they stopped to rest or nectar or were captured with a butterfly net to accurately determine sex.

### STATISTICAL ANALYSES

We estimated ASRs for each of the six survey bouts as well as combined the ASRs across sample bouts in order to obtain an overall estimate of the regal fritillary's ASR throughout the flight period. For the purposes of this study, ASRs were estimated based on the ratio of the number of observed, sexed individuals. We performed a chi-square test to compare the overall observed ASR to an expected ASR of 1:1 with $\alpha = 0.05$. To determine male and female densities across sample bouts and estimate male and female densities as a function of grassland management practices (Royle *et al.*, 2004), we conducted distance sampling (Buckland et al., 1993; Brown and Boyce, 1998; Moranz *et al.*, 2014) using function distsamp

FIG. 2.—Male and female adult regal fritillary (*Speyeria idalia*). (**A**) displays a male regal fritillary with the outer most row of spots on the dorsal hind wing being orange and (**B**) which displays a female regal fritillary with the outer most row of spots on the dorsal hind wing being white

in package Unmarked (Fiske and Chandler, 2011) in Program R (R Core Team, Version 3.2.2, 2018). Due to changing plant composition and structure throughout the flight period, dynamic nature of the regal fritillary's relative abundance, and their movement capabilities, transects were considered independent between sample bouts. In order to account for survey effort, variation in transect length, and to treat each sample bout as an independent survey event, density estimates were weighted by transect length in the models. To identify which models best supported observed patterns in density, we used an information theoretic framework to compare, rank, and select models best supported by these data (Burnham and Anderson, 2002). We used the second order variant of Akaike's Information Criterion adjusted for small sample sizes ($AIC_c$) to rank alternative models. We compared $AIC_c$ values and implemented a parametric bootstrap test ($n = 1000$ simulations) for goodness of fit on candidate models using the key functions uniform, half normal, and hazard rate to determine the best fitting detection function. We calculated delta $AIC_c$ ($\Delta AIC_c$) and Akaike weights ($w_i$), to evaluate relative support for each model (Burnham and Anderson, 2002). We used $AIC_c$ to rank models and selected the best fitting models as those with the lowest $AIC_c$ scores (Buckland *et al.*, 2001). We considered all models with a $\Delta AIC_c < 2$ from the top ranked model to have support.

We developed four ecologically relevant models to evaluate the effects of overall management regime (burned, grazed, hayed) and fire-return interval (short, 1 to 2 y; moderate, 3 to 5 y; or long $\geq$10 y) on male and female density: null (no effect), fire-return interval, overall management, and global (management + fire-return interval). In the fire-return interval model, management other than burning (grazing or haying) was ignored and density was estimated for the three levels of fire-return interval. In the overall management model, fire-return interval was ignored and density was estimated for the three overall management treatments. In the global model, density was estimated for overall management and the three levels of fire-return interval. All data were modeled with the uniform detection function because summary statistics from the parametric bootstrap test for goodness of fit, including SSE, chi-square, and Freeman Tukey supported its use (all fit statistics $P >> 0.05$) and this function best fit these data (Table 2; Figs. 3A–C).

TABLE 2.—Comparison of models with different detection functions used to estimate the density of male and female regal fritillary (*Speyeria idalia*) at the Fort Riley Military Reserve and Konza Prairie Biological Station in northeastern Kansas, U.S.A., during 2015. Models include null (no effect), fire return interval in which management other than fire (*i.e.*, grazing and haying) was ignored, and density was estimated based on the three levels of fire return interval (short, 1 to 2 y; moderate, 3 to 5 y; or long ≥10 y), management in which fire-return interval, was ignored and density was estimated based on the overall management regime, and management + fire-return interval in which density was estimated based on the overall management regime and fire-return interval of the site. The bold italicized numbers represent the model results for females, whereas the regular font denotes the model results for males

| Model | Detection function | K | | $AIC_c$ | | $\Delta AIC_c$ | | $w_i$ | |
|---|---|---|---|---|---|---|---|---|---|
| Null | Uniform | ***1*** | 1 | ***162.54*** | 239.96 | ***0.00*** | 0.00 | ***0.62*** | 0.68 |
| | Half normal | ***2*** | 2 | ***164.21*** | 242.11 | ***1.67*** | 2.15 | ***0.27*** | 0.23 |
| | Hazard rate | ***3*** | 3 | ***165.89*** | 244.15 | ***3.35*** | 4.19 | ***0.12*** | 0.08 |
| Fire return interval | Uniform | ***3*** | 3 | ***147.35*** | 239.90 | ***0.00*** | 0.00 | ***0.64*** | 0.71 |
| | Half normal | ***4*** | 4 | ***149.20*** | 242.23 | ***1.84*** | 2.33 | ***0.26*** | 0.22 |
| | Hazard rate | ***5*** | 5 | ***151.07*** | 244.46 | ***3.71*** | 4.56 | ***0.10*** | 0.07 |
| Management | Uniform | ***3*** | 3 | ***162.46*** | 240.02 | ***0.00*** | 0.00 | ***0.64*** | 0.71 |
| | Half normal | ***4*** | 4 | ***164.30*** | 242.35 | ***1.84*** | 2.33 | ***0.26*** | 0.22 |
| | Hazard rate | ***5*** | 5 | ***166.17*** | 244.58 | ***3.71*** | 4.56 | ***0.10*** | 0.07 |
| Management + fire return interval | Uniform | ***5*** | 5 | ***149.44*** | 236.70 | ***0.00*** | 0.00 | ***0.67*** | 0.73 |
| | Half normal | ***6*** | 6 | ***151.48*** | 239.22 | ***2.04*** | 2.53 | ***0.24*** | 0.21 |
| | Hazard rate | ***7*** | 7 | ***153.56*** | 241.67 | ***4.13*** | 4.97 | ***0.09*** | 0.06 |

* K = number of parameters, $AIC_c$ = Second order variant of Akaike's information criterion adjusted for small sample sizes, $\Delta AIC_c$ = difference in $AIC_c$, $w_i$ = model weight

## RESULTS

### ADULT SEX RATIO AND DENSITY

We observed 92 regal fritillaries along the nine transects surveyed throughout the study. While we detected males at nine of nine (100%) transects, females were only detected at seven of nine (77%) transects (Table 1). We were able to identify the sex of 72 individuals across all survey periods, recording 48 males and 24 females, which differed from an expected 1:1 sex ratio ($\chi2 = 4.11$, DF = 1, P = 0.04; Table 1). Male density peaked early in the adult flight during sample bouts one and two (~June 8–July 1; Fig. 4); whereas, female density peaked slightly later in the flight period during sample bouts three and four (~July 6–July 22; Fig. 4). Initially during sample bouts one (June 8–23) and two (June 24–July 1), the ASR was male biased at 8.5:1 and 8:1, respectively. However, during sample bouts three (July 6–14), five (July 23–30), and six (July 31–August 8) male density began to decline and the ASR was 1:1. In contrast during sample bout four (July 16–July 22), the ASR was female biased at 0.375:1.

### MANAGEMENT AND FIRE-RETURN INTERVAL (♂)

We detected males along transects in all treatment categories, but the highest ranked model testing the effect of grassland management and fire-return interval on density was the global model (management + fire-return interval; $AIC_c = 236.70$, $\Delta AIC_c \geq 3.21$, $K = 5$, $w_i = 0.63$). This model revealed that male densities were greatest in areas that were grazed and

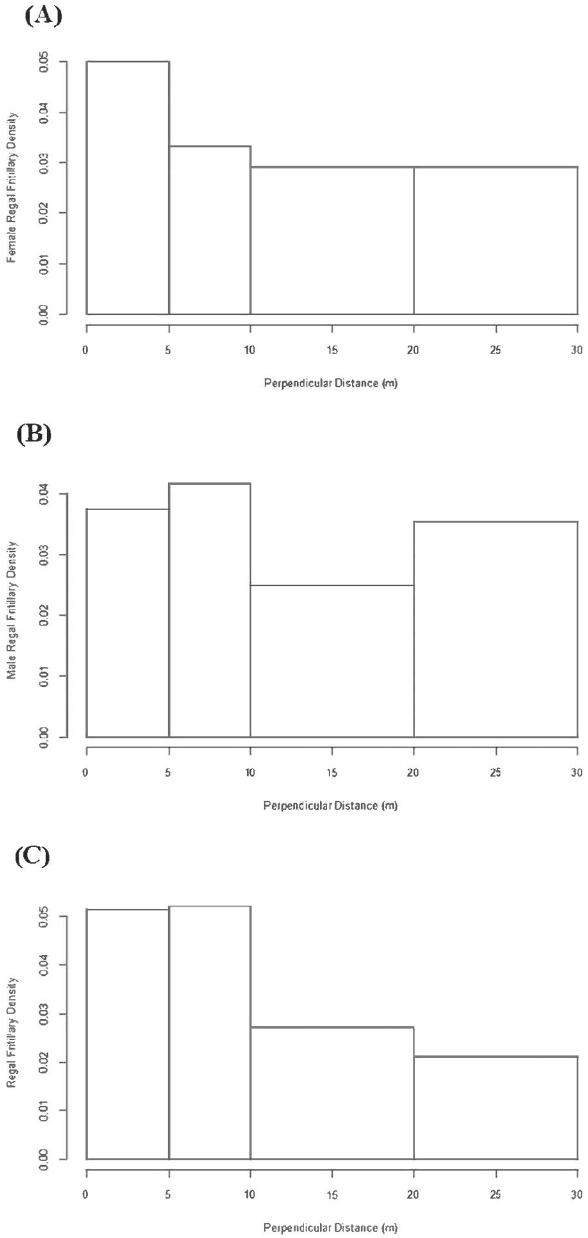

FIG. 3.—Histogram of distances for regal fritillary (*Speyeria idalia*) where (**A**) displays the histogram of distance for female regal fritillary, (**B**) male regal fritillary, and (**C**) male and female regal fritillaries collectively. Histograms of distances were produced using function distsamp in package Unmarked in Program R from regal fritillary surveys conducted in 2015 at the Fort Riley Military Reserve and Konza Prairie Biological Station in northeastern Kansas, U.S.A.

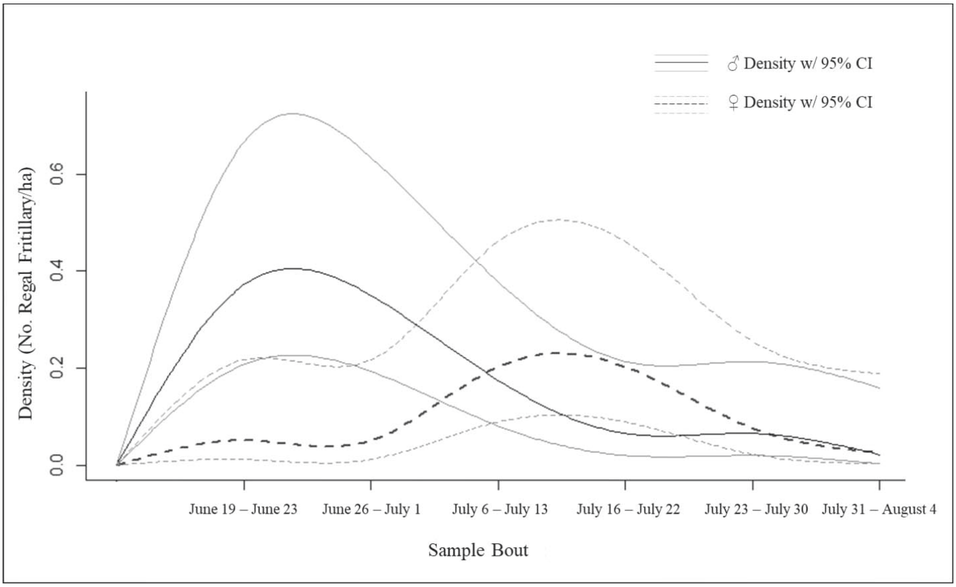

FIG. 4.—Density estimates (no./ha) of male and female regal fritillary (*Speyeria idalia*) with 95% confidence intervals across six sample bouts from sex ratio surveys conducted in 2015 at the Fort Riley Military Reserve and Konza Prairie Biological Station in northeastern Kansas, U.S.A. Density estimates and 95% confidence intervals were calculated using function distsamp in package Unmarked in program R. Estimates were weighted by transect length (*i.e.*, survey effort)

burned with a moderate fire-return interval (Fig. 5A). Although the 95% confidence interval of the grazed + moderate fire-return interval treatment category did overlap with other treatments, the point estimate of male density for the grazed + moderate fire-return interval category was at least 47% greater than the other treatment categories (Fig. 5A). The fire-return interval only model indicated that male density did not differ among the fire-return interval treatments, nonetheless this model revealed that point estimates of male density were 22% and 59% greater in sites with moderate fire-return intervals than in sites with short and long fire-return intervals, respectively (Fig. 5B). Like the fire-return interval model density estimates of males did not differ among overall management regime; however, the point estimate of male density was at least 27% greater in sites that were grazed compared to sites burned or hayed (Fig. 5C).

### MANAGEMENT AND FIRE-RETURN INTERVAL (♀)

In contrast to males, females were not detected along transects in all treatment categories. Females were not detected along transects within the burned + long fire-return interval or the hayed + long fire-return interval treatment categories (Table 1). The highest ranked model testing the effect of fire-return interval and grassland management on female density was the fire-return interval model ($AIC_c = 147.35$, $K = 3$, $w_i = 0.74$). Point estimates of density for this model showed that female density was 79% and 91% greater in sites that were burned with a moderate fire-return interval versus sites with short and long fire-return intervals, respectively (Fig. 5D). Although it was not the top ranked model as was the case for

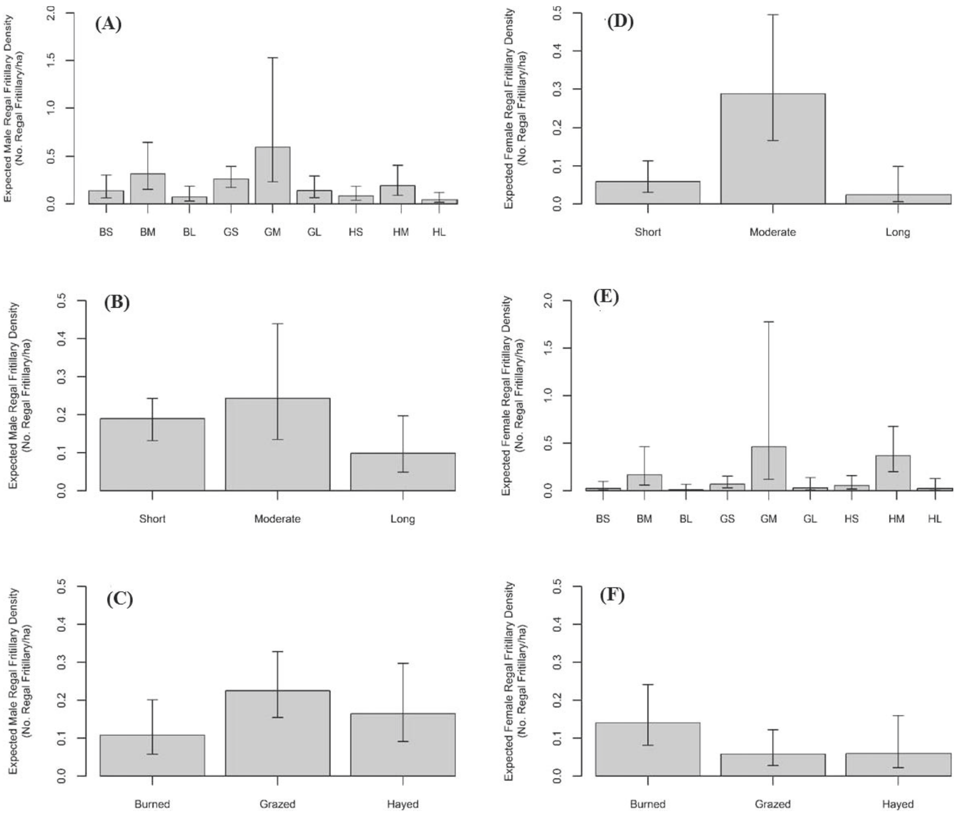

FIG. 5.—Density (no./ha) estimates of regal fritillary (*Speyeria idalia*) with 95% confidence intervals from adult sex ratio surveys conducted during 2015 at the Fort Riley Military Reserve and Konza Prairie Biological Station in northeastern Kansas, U.S.A. (**A**, **E**) Density estimates from the global model (management + fire return interval) for male (**A**) and female (**E**) regal fritillary. In this model, density estimates were grouped by both overall management regime (burned, grazed, hayed) and fire return interval (short, 1 to 2 y; moderate, 3 to 5 y; or long ≥10 y). The nine treatment categories for the global models are abbreviated as follows: BS = burned + short fire return interval, BM = burned + moderate fire return interval, BL = burned + long fire return interval, GS = grazed + short fire return interval, GM = grazed + moderate fire return interval, GL = grazed + long fire return interval, HS = hayed + short fire return interval, HM = hayed + moderate fire return interval, and HL = hayed + long fire return interval. (**B**, **D**) Density estimates from the fire return interval model for male (**B**) and female (**D**) regal fritillary. In this model, density estimates were grouped by fire return interval and other management (*i.e.*, grazing and haying) was ignored. (**C**, **F**) Density estimates from the overall management model for male (**C**) and female (**F**) regal fritillary. In this model fire return interval was ignored and density estimates were grouped by overall management regime. In all models density estimates and 95% confidence intervals were calculated using function distsamp in package Unmarked in Program R. Estimates were weighted by transect length (*i.e.*, survey effort).

males, the global model (management + fire-return interval) revealed that females displayed a similar pattern to males and point estimates of density indicated the female density was 20% greater in sites that were grazed and burned with a moderate fire-return interval than any other treatment category (Fig. 5E). Similar to males, density estimates of females did not differ among overall management regime; however, point estimates of density revealed female density was at least 56% greater in sites that were burned versus sites that were grazed or hayed (Fig. 5F).

## Discussion

### ADULT SEX RATIO AND DENSITY

Regal fritillary within our study sites exhibited an observed 2:1 male biased ASR across the entire emergence period. These findings are consistent with previous research that also found male biased ASRs among regal fritillary populations. For instance, Kopper *et al.*, (2001a) surveyed for regal fritillary in Kansas from early June through early September and reported an ASR of 2.5:1; Nagel *et al.* (1991) reported an ASR of 28:1 at Rowe Sanctuary in Nebraska through late July. Kelly and Debinski (1998) surveyed 16 different sites across four states and reported ASRs of 27:1, 0.55:1, 0.42:1, and 1.8:1 from Iowa, South Dakota, North Dakota, and Kansas, respectively. Swengel and Swengel (2005) surveyed 116 prairie sites dispersed across Wisconsin, Iowa, Minnesota, North Dakota, and Missouri. Summary statistics of surveys from this study during June–September where sites were grouped by subregion (subregion 1—Wisconsin, subregion 2—Iowa and Minnesota, subregion 3—Minnesota and North Dakota, and subregion 4—Missouri) revealed ASRs of 10.4:1, 6.7:1, 6.4:1, and 19.4:1, respectively. It is worth noting the female biased ASRs described by Kelly and Debinski (1998) from North and South Dakota were surveyed in August. During this time, females are emerging from post reproductive diapause and initiating oviposition flights and males are becoming scarce across the landscape as they begin to expire (Wagner *et al.*, 1997; Kopper *et al.*, 2001a, 2001b). Although the aforementioned studies also reported male biased ASRs, the difference in magnitude of the ratio was much smaller in our study. Actually, the magnitude of the male biased ASR appears to be consistently smaller within Kansas study sites compared to studies conducted elsewhere. Kopper *et al.* (2001a) and Kelly and Debinski (1998) are two of the previously mentioned studies that also had study sites in Kansas and reported ASRs of 2.5:1 and 1.8:1, respectively. Despite the 19-y difference in timing between these studies and ours, the ASR of regal fritillary within Kansas appears to have remained relatively consistent and may be indicative of a stable population within the state.

As in other protandrous butterflies, we found that the ASR of regal fritillary varied over time. Early in the flight period, during sample bouts one and two (~June 8–July 1), the ASR was male biased; however, the ASR shifted as the flight period progressed and was 1:1 during sample bouts three (July 6–14), five (July 23–30), and six (July 31–August 8). The ASR was female biased during sample bout four (July 16–July 22). We also found that male density peaked early in the adult flight period (~June 8–July 1), whereas female density peaked later (~July 6–July 22). This trend is comparable to the differences in peak abundances reported in other studies (Nagel *et al.*, 1991; Kopper *et al.*, 2001a; Swengel and Swengel, 2005). Likewise, the dynamic nature of the ASR exhibited by regal fritillary in this study and others is expected. The shifting ASR throughout the flight period is likely due to a combination of protandry and female reproductive diapause. It is well documented that males emerge before females (Scudder, 1889; Nagel *et al.*, 1991; Kopper *et al.*, 2001a; Swengel and Swengel, 2005); therefore, the male biased ASR early in the flight period is to be expected. Moreover, as time proceeds males begin to expire (approximately 2 wk after mating most males perish; Kopper, 1997; Kopper *et al.*, 2001b) and there is likely a shift in the magnitude when the male bias is not as great and may even transition to female biased as seen in this study and others, such as Kelly and Debinski (1998). Finally, as the flight period progresses, females enter a period of post reproductive diapause (Wagner *et al.*, 1997; Kopper *et al.,* 2001b; Zercher *et al.*, 2002; among others) and their presence on the landscape will appear scarce until they begin exploratory oviposition flights and begin depositing eggs in September (Wagner *et al.*, 1997; Kopper *et al.*, 2001a).

Although the dynamic nature of the ASR throughout the flight period appears to be well understood, the exact cause of the apparent overall male bias remains unclear. Two hypotheses have been proposed to explain the observed overall male biased ASR among regal fritillary populations. One hypothesis is that the male biased ASR detected among populations may be, in part, due to a female density dependent behavior (Swengel and Swengel, 2005). To avoid excessive harassment by courting males, female butterflies can become more evasive and secretive (Grossmueller and Lederhouse, 1987; Odendall *et al.*, 1989; Clayton and Petr, 1992). Swengel and Swengel (2005) also surmised that there may actually be more females present earlier in the flight period that go undetected due to this possible density dependent behavior. Alternatively, some have proposed that male biased ASRs may be due to host plant food limitation, as well as suboptimal host plant distribution (Kelly and Debinski, 1998; Bierzychudek and Warner, 2014; Hill *et al.*, 2018). Violets (*Viola* spp.) are the larval host plants for all members of the genus *Speyeria* (Klots, 1951, Hammond, 1974; Ferris and Brown, 1981; among others); however, regal fritillaries tend to be associated with specific violet species in different parts of their range (Selby, 2007). Kelly and Debinski (1998) found the ASR of regal fritillary increased to 10:1 when violet host plant densities were low. They postulated that because male regal fritillary larvae emerge at least 2 wk before females (Scudder, 1889; Nagel *et al.*, 1991; Kopper *et al.*, 2001a), male larvae may be able to find and consume host plants sooner and more readily, whereas females may struggle to find enough resources and perish during the larval stage.

While information pertaining specifically to regal fritillary larval ecology is largely lacking, research conducted on larvae of other members of the genus *Speyeria* appear to support the hypothesis that larval food limitation may disproportionately affect females. For instance, it is reported that male unsilvered fritillary, *Speyeria adiaste* (W.H. Edwards), larvae consume significantly less host plant leaf than their female counterparts (Hill *et al.*, 2018). Hill *et al.*, 2018) also suggested that the amount of host plant present was not necessarily equal to the amount of host plant available to a larva, because of host plant distribution and associated larval movement. *Speyeria* larvae are seemingly inefficient at finding host plants, and studies conducted on regal fritillary larvae indicated they were apparently unable to perceive violet host plants as close as 1 cm (Kopper *et al.*, 2001c). Additionally, a number of violet species are reported to contain proteins called cyclotides (Göransson *et al.*, 1999; Gruber *et al.*, 2008; Hashempour *et al.*, 2011; among others) that have been shown to defend the plant against herbivory by Lepidopterans (Barbeta *et al.*, 2008). If *Speyeria* larvae are in fact affected by these defenses, then host plants that had been previously grazed upon may be less desirable or even fatal (Hill *et al.,* 2018).

Assuming the aforementioned traits exhibited by other members of the genus *Speyeria* are generally applicable to regal fritillary, then the combined effect of these factors could have a number of consequences for the species. Differences in biomass consumed between male and female unsilvered fritillary larvae indicate that the number of males that can be supported by an area is likely greater than for females. These results have implications for female fecundity, ASR, and how many adult butterflies can be supported by a given area (Hill *et al.*, 2018). Fecundity has been linked to adult size among butterfly species (García-Barros, 2000), and in female mormon fritillary, *Speyeria mormonia* (Boisduval), food limitation was directly correlated with daily egg production (Boggs et al., 1993). Studies conducted on regal fritillary have noted that body size in females was smaller when host plant densities were low (Kelly and Debinski, 1998). Furthermore, the amount of violet host plants available to regal fritillary larvae may not only be reduced by their inability to detect host plants even at relatively close distances, but may also be further reduced by the apparent toxicity of previously grazed violets. Therefore, inadequate density or spatial arrangement of host plants may have a greater impact on females, leading to increased female mortality that reduces the numbers of females present on the landscape, contributing to a male biased ASR and ultimately exacerbating population declines (Hill *et al.*, 2018).

Our results are synonymous with other studies investigating ASR in protandrous butterflies and revealed survey timing affects ASR and sex specific density measurements (Schtickzelle *et al.*, 2002, Swengel and Swengel, 2005; Nowicki *et al.*, 2009; Trochet *et al.,* 2013; among others). Therefore, these results highlight the importance of assessing ASRs throughout the flight period. Failure to do so may lead to erroneous survey results, inaccurate conclusions, and misguided management recommendations. In addition to survey timing, secretive density dependent female behavior has also been proposed as a factor leading to the observed male biased ASR among populations. However, it would be expected that if protandry and density dependent female behavior were the only sources contributing to the bias that the observed number of females during oviposition would equal the number of males observed earlier in the flight period, thus resulting in an overall 1:1 ASR. Yet this is not necessarily the case as seen in this study and others, such as Kopper *et al.* 2001a, which surveyed for regal fritillary from the time males first emerged until females began oviposition flights and reported an observed ASR of 2.5:1. This result, coupled with the information within this study, previous research on regal fritillary, and the aforementioned studies conducted on other members of the genus *Speyeria* suggest violet host plant density, spatial arrangement, and overall habitat quality are also plausible culprits contributing to a male biased ASR. Nevertheless, more research across the regal fritillary's flight period and throughout their range are necessary to corroborate these findings and verify whether assumptions drawn from the aforementioned studies conducted on other members of the genus *Speyeria* are generally applicable.

### EFFECTS OF FIRE-RETURN INTERVAL AND MANAGEMENT

Point estimates of density indicated that sites with prescribed burning at a moderate (3 to 5 y) fire-return interval supported $\geq$1.3 times greater density of males and $\geq$5.6 times greater density of females versus sites burned with short and long fire-return intervals. In addition to the positive response to moderate (3 to 5 y) fire-return intervals by both males and females, our results also suggested additional positive responses when grazing was combined with moderate fire-return intervals, particularly among males. Point estimates of density revealed males had $\geq$1.9 times greater density and females had $\geq$1.2 times greater density in the grazed + moderate fire-return interval treatment category compared to other treatment combinations. Finally, we found that overall management, when underlying fire-return interval was ignored, had no observable effect on male or female densities. These results indicate that fire is the primary driving force behind both sexes' densities, with trends towards greater densities in the moderate (3 to 5 y) fire-return interval treatment, and add to growing body of evidence that suggests prescribed burning may not always be detrimental to regal fritillary.

Although previous studies have indicated that prescribed fire may be harmful to regal fritillary populations (Swengel, 1996, 1998; Powell *et al.*, 2007; Vogel *et al.*, 2010; among others), there is a growing body of evidence that indicates the application of prescribed fire, particularly patch burning, is not necessarily unfavorable (Moranz *et al.*, 2014; Henderson *et al.*, 2018; McCullough *et al.,* 2019; among others). In fact, recent research has suggested that a lack of fire may be more damaging to regal fritillary and their habitat (Henderson *et al.,* 2018; McCullough *et al.*, 2019). For example, Henderson *et al.* (2018) found that regal fritillary were more than twice as abundant in sites in which habitat quality was considered high regardless of burn history. Subsequently, they concluded that creating and maintaining high quality habitat that harbors abundant violets and a wide variety of nectar resources may be the most important management and conservation strategy for the species. Moreover, despite the relatively small sample size for both males ($n = 48$) and females ($n = 24$) within this study, these results are further bolstered by the analogous results found in McCullough *et al.* (2019), in which effects of prescribed burning and management on regal fritillary were examined collectively for both males and females.

A number of explanations have been proposed by those studies that support the use of fire in grasslands harboring regal fritillary to explain the interpretation inconsistencies when it comes to determining effects of fire on populations. One of the reasons that has been suggested is that many studies are likely measuring recovery rates rather than, or in addition to, treatment effects (Henderson *et al.,* 2018). A large majority of the studies that have examined the effects of fire on regal fritillary populations tend to focus on abundance in terms of burned versus unburned sites (Swengel, 1996, 1998; Huebschman and Bragg, 2000; Vogel *et al.,* 2010; among others). While establishing fire sensitivity and post-fire recovery times is essential, this information alone is limited in scope and does not provide a comprehensive understanding of how fire shapes long term population dynamics (Panzer, 2002). Likewise, many of the studies mentioned above were conducted over fairly short timescales (one to three years) and thus lack a broad picture of the complex interrelationships among population recovery, habitat, and fire effects (Henderson *et al.*, 2018). This is essential as fire interacts with habitat quality in many important ways that may not be apparent immediately (Henderson *et al.*, 2018).

Survey timing can also have important implications on the conclusions drawn when determining effects of fire on butterfly abundance (Latham *et al.*, 2007; Moranz *et al.*, 2014; McCullough *et al.*, 2019). It is well documented that in sites directly affected by a fire, prairie specialist butterflies, including the regal fritillary, typically decline initially (Swengel, 1996; Panzer, 2002). Conversely, in the time following the burn, the recovered population often grows larger (Schultz and Crone, 1998). While reported recovery times vary, recolonization can occur as soon as four weeks post-burn (Huebschman and Bragg, 2000) and numbers may reach pre-burn levels or greater within two to four years (Henderson *et al.*, 2018). Therefore, it is imperative to survey sites throughout the flight period, and, if possible, across several years to draw meaningful conclusions regarding the treatment effects of fire on butterfly populations.

Finally, another potential reason for inconsistency among studies is that interpreting burning effects depends strongly on the scale of the observation (Latham *et al.*, 2007; Moranz *et al.,* 2014; Henderson *et al.*, 2018; McCullough *et al.,* 2019). For example, in Swengel and Swengel (2001) that showed fire negatively impacted regal fritillary abundance, many of the prairies sampled were smaller than 30 ha. In our study, in which both male and female density was greater in sites experiencing burning at moderate fire-return intervals, areas were nearly 29,000 ha at the FRMR and 3487 ha at the KPBS with survey sites ranging from 23 ha to 385 ha and a mean sites size of 165 ha. Despite their strong flight capabilities and ability to disperse great distances (Nagel *et al.,* 1991; Zercher *et al.,* 2002), regal fritillaries have a strong propensity to remain in native prairie and are sensitive to habitat boundaries such as tree lines, roads, and row crops (Ries and Debinski, 2001; Caven *et al.*, 2017). This may explain why recolonization can happen in some contexts such as the contiguous grassland within the Flint Hills but not others (McCullough *et al.,* 2019).

Our overall management model did not demonstrate that one management method was most beneficial for either male or female regal fritillary. These results do, however, imply that prescribed conservation management using fire, grazing, or haying can be used in areas that contain regal fritillary without damaging effects on density of either sex. Our findings correspond with those of other studies that also found grazing and haying or mowing to be suitable management strategies for sites that contain regal fritillary (Swengel, 1996, 1998; Swengel *et al.*, 2011; Moranz *et al.,* 2014; among others). For instance, light to moderate grazing is thought to be favorable to regal fritillary and studies have noted increased violet density in grazed prairies (Mello, 1989; Debinski and Kelly, 1998; NatureServe, 2005). It has even been proposed that the elimination of livestock grazing in New England contributed to the loss and degradation of habitat and in turn the extirpation of regal fritillary among those sites (Dunwiddie and Sferra, 1991). Nonetheless, these management regimes should be applied with caution as they can also have negative consequences when applied at high frequencies or too aggressively. For example, heavy intensive grazing is thought to be a threat to regal fritillary populations by reducing or homogenizing plant structure, decreasing overall plant diversity, and trampling eggs and larvae could also be problematic (Hammond and McCorkle, 1983; Royer and Marrone, 1992; Fritz, 1997; Moranz et al., 2014; among others). Similarly, haying or mowing may remove crucial nectar resources when they are needed by adults, reduce larval host plants, and cutting an area too short may harm developing eggs and larvae (Selby, 2007).

Although historical habitat loss is widely accepted as the most probable cause for regal fritillary population decline, the degradation and loss of habitat quality among remaining prairie remnants has largely gone unchecked and even accelerated in recent decades (Alstad *et al.,* 2016) and is also a serious threat to remaining populations (Sim, 2017; Henderson *et al.,* 2018). In the absence of grazing and periodic fire, the host plants, habitat, and ultimately the butterflies that rely on them disappear as these processes provide the disturbance required for ecosystem function (Cushman and Murphy, 1993; Schultz and Crone, 1998; Pollick, 2004). Violets, for example, are a critical component of high quality regal fritillary habitat that often compete poorly with other plants, particularly woody species (Ferster and Vulinec, 2010). Studies have demonstrated that prescribed fire can temporarily boost the density of violet host plant species (Latham *et al.*, 2007), as well as increase flower production and prairie violet growth and seed production (Lovell *et al.,* 1983). Additionally, fire has been shown to promote the growth of essential nectar resources utilized by regal fritillary (Moranz *et al.*, 2014), suppress woody vegetation (Abrams and Hulbert, 1987; Briggs *et al.*, 2005; Lett and Knapp, 2005; among others), and encourage the growth of dominant warm season grasses such as big bluestem (Collins, 1990). In contrast, if fire return intervals are delayed by more than a few years, grasslands can transition to shrublands and a complete lack of fire can lead to the invasion of woody species and conversion from grasslands to woodlands (Ratajczak *et al.*, 2016).

## CONCLUSION

The Flint Hills status as home to the largest contiguous tract of tallgrass prairie remaining in North America (Reichman, 1987) and the regal fritillaries relatively stable state in the region, lends to the notion that the overall 2:1 male to female ASR observed within this study could be considered the model composition of populations across their range. Despite nearly two decades separating this study from previous sex ratio surveys conducted in Kansas, the consistent ASRs reported from this region further support this conclusion. Furthermore, our study is one of the first, to our knowledge, to examine the effects of prairie management practices (*e.g.,* fire, grazing, and haying) on male and female regal fritillary independently. Our results for both males and females mirror the growing body of research that indicates prescribed burning is not necessarily detrimental to regal fritillary, and may even be beneficial under certain conditions. Moreover, our results elucidate the importance of habitat quality for regal fritillary population persistence and indicate that degraded habitat quality may be more harmful to populations than fire. Due to the current conservation concerns for regal fritillary populations throughout its range, the information we provide in this study will not only allow for assessment of trends throughout the species range, focusing efforts in areas in most need of conservation and a better understanding of the ecology of this imperiled butterfly species, but may also serve as an indicator of health and viability of other grassland obligate species that are less readily sexed and identified in the field. As an indicator species, if the regal fritillary is failing, it is indicative that other grassland obligate species are likely also in peril and potentially the system as a whole is at risk. Accordingly, this information may provide a tool for monitoring and assessing the health, viability, and status of regal fritillary populations and may also have implications for other imperiled species.

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## LITERATURE CITED

ABRAMS, M.D. AND L.C. HULBERT. 1987. Effect of topographic position and fire on species composition in tallgrass prairie in northeast Kansas. *Am. Midl. Nat.*, **117**:442–445.

ALSTAD, A.O., E.I. DAMSCHEN, T.J. GIVNISH, J.A. HARRINGTON, M.K. LEACH, D.A. ROGERS, AND D.M. WALLER. 2016. The pace of plant community change is accelerating in remnant prairies. *Sci. Adv.*, **2**:e1500975.

BAGUETTE, M., I. CONVIÉ, AND G. NÈVE. 1996. Male density affects female spatial behavior in the butterfly *Proclossiana eunomia. Acta Oecol.*, **17**:225–232.

BARBETA, B.L., A.T. MARSHALL, A.D. GILLON, D.J. CRAIK, AND M.A. ANDERSON. 2008. Plant cyclotides disrupt epithelial cells in the midgut of lepidopteran larvae. *Proc. Natl. Acad. Sci. USA.*, **105**:1221–1225.

BESSA-GOMES, C., S. LEGENDRE, AND J. CLOBERT. 2004. Allee effects, mating systems and the extinction risk in populations with two sexes. *Ecol. Lett.*, **7**:802–812.

BIERZYCHUDEK, P. AND K.A. WARNER. 2014. Modeling caterpillar movement to guide habitat enhancement for *Speyeria zerene hippolyta*, the Oregon silverspot butterfly. *J. Insect Conserv.*, **19**:45–54.

BOGGS, C.L. AND C.L ROSS. 1993. The effect of adult food limitation on life history traits in *Speyeria mormonia* (Lepidoptera: Nymphalidae). *Ecology*, **74**:433–441.

Briggs, J.M., A.K. Knapp, J.M. Blair, J.L. Heisler, G.A. Hoch, M.S. Lett, and J.K. McCarron. 2005. An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. *Bioscience*, **55**:243–254.

Brown, J.A. and M.S. Boyce. 1998. Line transect sampling of Karner blue butterflies (*Lycaeides melissa samuelis*). *Environ. Ecol. Stat.*, **5**:81–91.

Brussard, P.F. and P.R. Ehrlich. 1970. The population structure of *Erebia epipsodea* (Lepidoptera: Satyrinae). *Ecology*, **51**:119–129.

Buckland, S.T., D.R. Anderson, K.P. Burnham, J.L. Laake. 1993. Distance sampling: estimating abundance of biological populations. Chapman and Hall, London, UK.

———, ———, ———, ———, D.L. Borchers, and L. Thomas. 2001. Introduction to distance sampling: estimating abundance of wildlife populations. Oxford University Press, Oxford, UK.

Burnham, K.P. and D.R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science and Business Media, New York, N.Y., U.S.A.

Caven, A.J., K.C. King, J.D. Wiese, and E.M. Brinley-Buckley. 2017. A descriptive analysis of regal fritillary (*Speyeria idalia*) habitat utilizing biological monitoring data along the big bend of the Platte River, NE. *J. Insect Conserv.*, **21**:183–205.

Clayton, D.L. and D. Petr. 1992. Sexual differences in habitat preference and behavior of *Oeneis chryxus* (Nymphalidae: Satyrinae). *J. Lepid. Soc.*, **46**:110–118.

Collins, S.L. 1990. Introduction: fire as a natural disturbance in tallgrass prairie ecosystems, p. 3–7. *In*: S.L. Collins and L.L. Wallace (eds.). Fire in North American tallgrass prairies. University of Oklahoma Press, Norman, Okla., U.S.A.

Cushman, J.H. and D.D. Murphy. 1993. Susceptibility of Lycaenid butterflies to endangerment. *Wings*, **17**:16–21.

Debinski, D.M. and L. Kelly. 1998. Decline of Iowa populations of the regal fritillary (*Speyeria idalia*) Drury. *J. Iowa Acad, Sci.*, **105**:16–22.

Donald, P.F. 2007. Adult sex ratios in wild bird populations. *Ibis*, **149**:671–692.

Dunwiddie, P.W. and N. Sferra. 1991. Loss of rare butterfly and plant species in coastal grasslands. *Nat. Area. J.* **11**:119–120.

Dyson, E.A. and D.D. Hurst. 2004. Persistence of an extreme sex-ratio bias in a natural population. *P. Natl. Acad. Sci. USA*, **101**:6520–6523.

Ehrlich, P.R., A. E. Launer, and D.D. Murphy. 1984. Can sex ratio be defined or determined? The case of a population of checkerspot butterflies. *Am. Nat.*, **124**:527–539.

Ehrlich, P. 1989. The structure and dynamics of butterfly populations. p. 25–40. *In*: R.I. Vane-Wright and P.R. Ackery (eds.). The Biology of Butterflies. Princeton University Press. Princeton, N.J., U.S.A.

Ewen, J.G., R. Thorogood, and D.P. Armstrong. 2011. Demographic consequences of adult sex ratio in a reintroduced hihi population. *J. Anim. Ecol.*, **80**:448–455.

Ferris, C.D. and F.M. Brown. 1981. Butterflies of the Rocky Mountain States. University of Oklahoma Press, Norman, Okla., U.S.A.

Ferster, B. and K. Vulinec. 2010. Population size and conservation of the last eastern remnants of the regal fritillary, *Speyeria idalia* (Drury) [Lepidoptera, Nymphalidae]; implications for temperate grassland restoration. *J. Insect Conserv.*, **14**:31–42.

Fiske, I.J. and R.B. Chandler. 2011. Unmarked: an R package for fitting hierarchical models of wildlife occurrence and abundance. *J. Stat. Softw.*, **43**:1–23.

Frankham, R. 1995. Conservation genetics. *Ann. Rev. Genet.*, **29**:305–327.

Fritz, M.I. 1997. Survey report for two rare invertebrate species; tawny crescent (*Phycoides batessii*) and the regal fritillary (*Speyeria idalia*) on the Nebraska National Forest, U.S. Forest Service. Unpublished report to the Nebraska National Forest, U.S. Forest Service, Chadron, Neb. 15+p.

Fuhlendorf, S.D., C.A. Davis, R.D. Elmore, L.E. Goodman, and R.G. Hamilton. 2018. Perspectives on grassland conservation efforts: should we rewild to the past or conserve for the future? *Philos. T. Roy. Soc. B.*, **373**:20170438.

GARCÍA-BARROS, E. 2000. Body size, egg size, and their interspecific relationships with ecological and life history traits in butterflies (Lepidoptera: Papilionoidea, Hesperioidea). *Biol. J. Linn. Soc.*, **70**:251–284.

GÖRANSSON, U., T. LUIJENDIJK, S. JOHANSSON, L. BOHLIN, AND P. CLAESON. 1999. Seven novel macrocyclic polypeptides from *Viola arvensis. J. Nat. Prod.*, **62**:283–286.

GROSSMUELLER, D.W. AND R.C. LEDERHOUSE. 1987. The role of nectar source distribution in habitat use and oviposition by the tiger swallowtail butterfly. *J. Lepid. Soc.*, **41**:159–165.

GRUBER, C.W., A.G. ELLIOT, D.C. IRELAND, P.G. DELPRETE, S. DESSEIN, U. GÖRANSSON, M. TRABI, C.K. WANG, A.B. KINGHORN, E. ROBBRECHT, AND D.J. CRAIK. 2008. Distribution and evolution of circular miniproteins in flowering plants. *Plant Cell*, **20**:2471–2483.

HAMMOND, P.C. 1974. An ecological survey of the nymphalid butterfly genus *Speyeria.* Thesis, University of Nebraska, Lincoln, Neb., U.S.A.

——— AND D.V. MCCORKLE. 1983. The decline and extinction of *Speyeria* populations resulting from human environmental disturbances (Nymphalidae: Argynninae). *J. Res. Lepidoptera*, **22**:217–224.

HASHEMPOUR, H., A. GHASSEMPOUR, N.L. DALY, B. SPENGLER, AND A. RÖMPP. 2011. Analysis of cyclotides in *Viola ignobilis* by Nano liquid chromatography Fourier transform mass spectrometry. *Protein Peptide Lett.*, **18**:747–752.

HENDERSON, R.A., J. MEUNIER, AND N.S. HOLOUBEK. 2018. Disentangling effects of fire, habitat, and climate on an endangered prairie-specialist butterfly. *Biol. Conserv.*, **218**:41–48.

HILL, R.I., C.E. RUSH, AND J. MAYBERRY. 2018. Larval food limitation in a *Speyeria* butterfly (Nymphalidae): how many butterflies can be supported? *Insects*, **9**:179.

HUEBSCHMAN, J.J. AND T.B. BRAGG. 2000. Response of regal fritillary (*Speyeria idalia* Drury) to spring burning in an eastern Nebraska tallgrass prairie, USA. *Nat. Areas J.*, **20**:386–388.

KDA (KANSAS DEPARTMENT OF AGRICULTURE). 2017. Bluestem pasture release 2017. https://www.agmanager.info/land-leasing/land-buying-valuing/land-use-value-research/bluestem-pasture-release-2017.

KELLY, L. AND D.M. DEBINSKI. 1998. Relationship of host plant density to size and abundance of the regal fritillary *Speyeria idalia* Drury (Nymphalidae). *J. Lepid. Soc.*, **52**:262–276.

KLOTS, A.B. 1951. A field guide to the butterflies of North America, east of the Great Plains. Houghton Mifflin Company, Boston, Mass., U.S.A.

KNAPP, A.K., J.M. BRIGGS, J.M. BLAIR, AND C.L. TURNER. 1998. Patterns and controls of above ground net primary production in tallgrass prairie. p. 193–221. *In:* A.K. Knapp, J.M Briggs, D.C. Hartnett, and S.L. Collins (eds.). Grassland dynamics: long-term ecological research in tallgrass prairie. Oxford University Press, New York, N.Y., U.S.A.

KOHL, M.T., P.R. KRAUSMAN, K. KUNKEL, AND D.M. WILLIAMS. 2013. Bison versus cattle: are they ecologically synonymous? *Rangeland Ecol. Manag.*, **66**:721–731.

KOPPER, B.J. 1997. Behavioral ecology of *Speyeria idalia* (Lepidoptera: Nymphalidae), in Kansas tallgrass prairie: reproductive diapause, factors influencing oviposition site selection, and larval foraging behavior. M.S. thesis, Kansas State University, Manhattan, Kan, U.S.A.

———, D.C. MARGOLIES, AND R.E. CHARLTON. 2001a. Life history notes on the regal fritillary, *Speyeria idalia* (Drury) (Lepidoptera: Nymphalidae), in Kansas tallgrass prairie. *J. Kansas Entomol. Soc.*, **74**:172–177.

———, S. SHU, R.E. CHARLTON, AND S.B. RAMASWAMY. 2001b. Evidence for reproductive diapause in the fritillary *Speyeria idalia* (Lepidoptera: Nymphalidae). *Phys. Biochem and Toxicol.*, **94**:427–432.

———, D.C. MARGOLIES, AND R.E. CHARLTON. 2001c. Notes on the behavior of *Speyeria idalia* (Drury) (Nymphalidae) larvae with implications that they are diurnal foragers. *J. Lepid. Soc.*, **54**:96–97.

LATHAM, R.E., D. ZERCHER, P. MCELHENNY, P. MOORESIDE, AND B. FERSTER. 2007. The role of disturbance in habitat restoration and management for the eastern regal fritillary (*Speyeria idalia idalia*) at a military installation in Pennsylvania. *Ecol. Rest.*, **25**:103–111.

LE GALLIARD, J.F., P. S. FITZE, R. FERRIÈRE, AND J. CLOBERT. 2005. Sex ratio bias, male aggression, and population collapse in lizards. *Proc. Natl Acad. Sci.*, **102**:18231–18236.

LEFTWICH, P.T., D.A. EDWARD, L. ALPHEY, M.J. GAGE, AND T. CHAPMAN. 2012. Variation in adult sex ratio alters the association between courtship, mating frequency and paternity in the lek-forming fruitfly *Ceratitis capitata. J. Evol. Biol.*, **25**:1732–1740.

Lett, M.S. and A.K. Knapp. 2005. Woody plant encroachment and removal in mesic grassland: production and composition responses of herbaceous vegetation. *Am. Midl. Nat.*, **153**:217–231.

Lovell, D.L., R.A. Henderson, and E.A. Howell. 1983. The response of forb species to seasonal timing of prescribed burns in remnant Wisconsin prairies. p. 11–15. *In*: R. Brewer (ed.). Proceedings of the eighth North American prairie conference, 1–4 August 1982. Western Michigan University, Kalamazoo, Mich., U.S.A.

McCullough, K., G. Albanese, D.A. Haukos, A.M. Ricketts, and S. Stratton. 2019. Management regime and habitat response influence abundance of regal fritillary (*Speyeria idalia*) in tallgrass prairie. *Ecosphere*, 10(**8**):e02845.

McKellar, A.E., M.M. Turcotte, and A.P. Hendry. 2009. Environmental factors influencing adult sex ratio in Trinidadian guppies. *Oecologia,* **159**:735–745.

Mello, M.J. 1989. Survey and ecology of Speyeria idalia (regal fritillary) on Block Island with notes on other species. Report #89-9. Lloyd Center for Environmental Studies, South Dartmouth, Mass., U.S.A. Submitted to The Nature Conservancy, Rhode Island Field Office.

Moranz, R.A., S.D. Fuhlendorf, and D.M. Engle. 2014. Making sense of a prairie butterfly paradox: the effects of grazing, time since fire, and sampling period on regal fritillary abundance. *Biol. Conserv.*, **173**:32–41.

Nagel, H.G., T. Nightengale, and N. Dankert. 1991. Regal fritillary butterfly population estimation and natural history on Rowe Sanctuary, Nebraska. *Prairie Naturalist*, **23**:145–152.

NatureServe. 2005. NatureServe Explorer: An Online Encyclopedia of Life [web application]. Version 7.0. NatureServe, Arlington, Virg., U.S.A. Available online at: http://www.natureserve.org/explorer. Accessed 25 Feb 2020.

Nowicki, P., S. Bonelli, F. Barbero, and E. Balletto. 2009. Relative importance of density- dependent regulation and environmental stochasticity for butterfly population dynamics. *Oecologia,* **161**:227–239.

Odendaal, F.J., P. Turchin, and F.R. Stermitz. 1989. Influence of host-plant density and male harassment on the distribution of female *Euphydryas anicia* (Nymphalidae). *Oecologia*, **78**:283–288.

Owensby, C. 2010. Managing Kansas Flint Hills grasslands. Symphony in the Flint Hills field journal. https://newprairiepress.org/cgi/viewcontent.cgi?article=1009&context=sfh.

Panzer, R. 2002. Compatibility of prescribed burning with the conservation of insects in small, isolated prairie reserves. *Conserv. Biol.*, **16**:1296–1307.

Pipoly, I., V. Bókony, M. Kirkpatrick, P.F. Donald, T. Székely, and A. Liker. 2015. The genetic sex-determination system predicts adult sex ratios in tetrapods. *Nature*, **527**:91–94.

Pollard, E. and T.J. Yates. 1993. Monitoring butterflies for ecology and conservation: the British butterfly monitoring scheme. Chapman and Hall, London, UK.

Pollick, S. 2004. Karner blue. *Toledo Magazine*, Toledo, p. 6.

Powell, A.F.L.A., W.H. Busby, and K. Kindscher. 2007. Status of the regal fritillary (*Speyeria idalia*) and effects of fire management on its abundance in northeastern Kansas, USA. *J. Insect. Conserv.,* **11**:299–308.

R Development Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ratajczak, Z., J.M. Briggs, D.G. Goodin, L. Luo, R.L. Mohler, J.B. Nippert, and B. Obermeyer. 2016. Assessing the potential for transitions from tallgrass prairie to woodlands: are we operating beyond critical fire thresholds? *Rangeland Ecol. Manag.*, **69**:280–287.

Reichman, O.J. 1987. Konza Prairie: a tallgrass natural history. University of Kansas Press, Lawrence, Kan., U.S.A.

Ries, L. and D.M. Debinski. 2001. Butterfly responses to habitat edges in the highly fragmented prairies of central Iowa. *J. Anim. Ecol.*, **70**:840–852.

Royer, R.A. and G.M. Marrone. 1992. Conservation status of the regal fritillary (*Speyeria idalia*) in North and South Dakota. U.S. Fish and Wildlife Service, Denver, Col., U.S.A.

Royle, J.A., D.K. Dawson, and S. Bates. 2004. Modeling abundance effects in distance sampling. *Ecology*, **85**:1591–1597.

Schtickzelle, N., M. Le Boulenge, and M. Baguette. 2002. Metapopulation dynamics of the bog fritillary butterfly: demographic processes in a patchy population. *Oikos,* **97**:349–360.

SCHULTZ, C.B. AND E.E. CRONE. 1998. Burning prairie to restore butterfly habitat: a modeling approach to management tradeoffs for the fender's blue. *Restor. Ecol.*, **6**:244–252.

SCRIBER, J.M., M.D. DEERING, L.N. FRANCKE, W.F. WEHLING, AND R.C. LEDERHOUSE. 1998. Notes on swallowtail population dynamics of three *Papilio* species in south-central Florida (Lepidoptera: Papilionidae). *Holarctic Lepid.*, **5**:53–62.

SCUDDER, S.H. 1889. The butterflies of the eastern United States and Canada with special reference to New England. S.H. Scudder, Cambridge, Mass., U.S.A.

SELBY, G.L. 2007. Regal fritillary (*Speyeria idalia* Drury): a technical conservation assessment. USDA Forest Service, Rocky Mountain Region. Available online at http://www.fs.fed.us/r2/projects/scp/assessments/regalfritillary.pdf. Accessed 25 Feb 2020.

SIMS, S.R. 2017. *Speyeria* (Lepidoptera: Nymphalidae) conservation. *Insects*, **8**:45.

SWENGEL, A.B. 1996. Effects of fire and hay management on abundance of prairie butterflies. *Biol. Conserv.*, **76**:73–85.

———. 1998. Effects of management on butterfly abundance in tallgrass prairie and pine barrens. *Biol. Conserv.*, **83**:77–89.

——— AND S.R. SWENGEL. 2001. A 10-year study to monitor populations of the regal fritillary, *Speyeria idalia*, (Lepidoptera: Nymphalidae) in Wisconsin, USA. *Great Lakes Entomol.*, **34**:97–115.

———. 2005. Influences of timing and population density on the sex ratio of the regal fritillary, *Speyeria idalia* (Lepidoptera: Nymphalidae). *Holarctic Lepid.*, **10**:17–30.

SWENGEL, S.R., D. SCHLICHT, F. OLSEN, AND A.B. SWENGEL. 2011. Declines of prairie butterflies in the Midwestern USA. *J. Insect. Conserv.*, **15**:327–339.

SZÉKELY, T., F.J. WEISSING, AND J. KOMDEUR. 2014. Adult sex ratio variation: implications for breeding system evolution. *J. Evol. Biol.*, **27**:1500–1512.

TABASHNIK, B.E. 1980. Population structure of pierid butterflies. *Oecologia,* **47**:175–183.

TOWNE, E.G., D.C. HARTNETT, AND R.C. COCHRAN. 2005. Vegetation trends in tallgrass prairie from bison and cattle grazing. *Ecol. Appl.*, **15**:1550–1559.

TROCHET, A., D. LEGRAND, N. LARRANAGE, S. DUCATEZ, O. CALVEZ, J. COTE, J. CLOBERT, AND M. BAGUETTE. 2013. Population sex ratio and dispersal in experimental two-patch metapopulations of butterflies. *J. Anim. Ecol.*, **82**:946–955.

UNDERWOOD, D.L.A. AND A.M. SHAPIRO. 1999. A male-biased primary sex ratio and larval mortality in *Eucheira socialis* (Lepidoptera: Pieridae). *Evol. Ecol. Res.*, **1**:703–717.

USFWS (UNITED STATES FISH AND WILDLIFE SERVICE). 2015. Status review for four Midwestern species. www.fws.gov/midwest/es/soc/Batch90DaySept2015.html. Accessed 16 March 2020.

VLAŠÁNEK, P., D. HAUCK, AND M. KONVICKA. 2009. Adult sex ratio in the *Parnassius mnemosyne* butterfly: effects of survival, migration, and weather. *Isr. J. Ecol. Evol.*, **55**:233–252.

VOGEL, J.A., D.M. DEBINSKI, R.R. KOFORD, AND J.R. MILLER. 2007. Butterfly responses to prairie restoration through fire and grazing. *Biol. Conserv.,* **140**:78–90.

———, R.R. KOFORD, AND D.M. DEBINSKI. 2010. Direct and indirect responses of tallgrass prairie butterflies to prescribed burning. *J. Insect. Conserv.*, 1**4**:663–677.

WAGNER, D.L., M.S. WALLACE, G.H. BOETTNER, AND J.S. ELKINTON. 1997. Status update and life history studies on the regal fritillary (Lepidoptera: Nymphalidae). p. 261–275. *In*: P.D. Vickery and P.W. Dunwiddie (eds.). Grasslands of northeastern North America ecology and conservation of native and agricultural landscapes. Massachusetts Audubon, Lincoln, Mass., U.S.A.

WEDEKIND, C. 2002. Manipulating sex ratios for conservation: short-term risks and long-term benefits. *Anim. Conserv.*, **5**:13–20.

ZERCHER, D., P. MCELHENNY, AND P. MOORESIDE. 2002. Habitat management plan for the regal fritillary (*Speyeria idalia*) at the Fort Indiantown Gap National Guard Training Center, Annville, Pennsylvania. 5-year implementation period: 2002-2006. Final plan prepared for the Pennsylvania Department of Military and Veteran Affairs, Fort Indiantown Gap, Environmental Section, Annville, Penn., U.S.A.

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