SPECIES STATUS ASSESSMENT
FOR
REGAL FRITILLARY (*Argynnis (Speyeria) idalia*):
EASTERN SUBSPECIES (*Argynnis idalia idalia*)
AND
WESTERN SUBSPECIES (*A. i. occidentalis*)

Eastern subspecies, Pennsylvania
Photo credit: Fort Indiantown Gap Wildlife Staff.

Western subspecies, North Dakota.
Photo credit: Kelly Krabbenhoft.

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Writers and Contributors:

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Version History:

- On June 16, 2024, we made minor editorial corrections and updates, primarily to citations, throughout the report, including adding FITG and Service (2024, entire), the completed candidate conservation agreement (CCA) for the eastern regal fritillary at the Fort Indiantown Gap National Guard Training Center.

EXECUTIVE SUMMARY

This species status assessment (SSA) report documents the results of our comprehensive, scientific review of the life history, ecology, threats, and viability of the eastern and western subspecies of the regal fritillary (*Argynnis (Speyeria) idalia*). There are two recognized subspecies of regal fritillary: (1) *A. i. idalia* (the eastern subspecies) and (2) *A. i. occidentalis* (the western subspecies) (Williams 2001b, p. 146; Pelham 2021, entire; Pelham 2023, entire). The eastern subspecies is currently found as a single population located on the Fort Indiantown Gap (FTIG) National Guard Training Center in Pennsylvania. The western subspecies currently occupies portions of 14 states, from Indiana to Colorado and from North Dakota to Oklahoma. The eastern and western subspecies are the subjects of this SSA report, and these two subspecies effectively comprise the entire species. For this assessment, we use the term "species" to refer to the regal fritillary and information presented for the species applies to both subspecies, unless specified otherwise. This SSA report provides the best available biological information to inform the U.S. Fish and Wildlife Service's (Service's) decisions for the regal fritillary under the Endangered Species Act (Act), including classification determinations for the subspecies, and any other actions, as needed. The SSA will be updated as needed and this version incorporates the most recent data available.

We used the three-part SSA framework (Service 2016, entire; Smith et al. 2018, entire) to guide our biological risk assessment for the eastern and western subspecies of regal fritillary. An SSA begins with a compilation of the best available biological information on a species, including its taxonomy, life history, and habitat, and its ecological needs at the individual, population, and species levels, based on how environmental factors are understood to act on the species and its habitat (Service 2016, p. 6). Next, an SSA describes the current condition of the species' habitat and demographics, and the probable explanations for past and ongoing changes in abundance and distribution within the species' ecological settings, such as areas representative of the geographic, genetic, or life history variation across the species' range (Service 2016, p. 6). Lastly, an SSA forecasts the species' response to probable future scenarios of environmental conditions and conservation efforts (Service 2016, p. 6). As a result, the SSA characterizes the species' viability, or its ability to sustain populations in the wild over time, based on the best scientific understanding of current and future abundance and distribution within the species' ecological settings.

Throughout the assessment, the SSA uses the conservation biology principles of resiliency, redundancy, and representation, collectively known as the "3Rs," as a lens to evaluate the current and future condition of the subspecies (Service 2016, p. 6). Resiliency is the ability for populations to sustain in the face of environmental and demographic stochastic events, or for populations to recover from years with low reproduction or reduced survival, and is associated with population size, growth rate, connectivity, and the quality and quantity of habitats. Redundancy is the ability for a species to withstand catastrophic events, for which adaptation is unlikely, and is associated with the number and distribution of populations. Representation is the ability of a species to adapt to changes in the environment and is associated with its diversity, whether ecological, genetic, behavioral, or morphological.

In the first stage of our SSA analysis, we identified what the eastern and western subspecies of regal fritillary need, first in terms of the habitat factors needed by individuals to breed, feed, and shelter, then in terms of the demographic factors that populations, or analytical units (AUs) for this assessment, need to be resilient, and finally, what the eastern and western subspecies need for redundancy and representation (Chapter 3). In the second stage of our analysis, we evaluated the stressors and conservation efforts that influence the needs (Chapter 4), and then we evaluated the current condition of those needs in terms of the 3Rs (Chapter 5). In the third and final phase of our analysis, we projected the future condition of the needs, again in terms of the 3Rs, using future scenarios to capture uncertainty associated with the future to year 2075 (Chapter 6). For the purpose of this assessment, we define viability as the ability of the eastern and western subspecies to sustain populations in natural ecosystems over a biologically meaningful timeframe, in this case, to 2075. The 2075 timeframe for this assessment is a period that captures over 50 annual generations of the regal fritillary and is also the approximate timeframe during which the eastern subspecies experienced a historical decline. This timeframe is also consistent with the time scale for which we have data available for the eastern and western subspecies and for which we can reasonably project future stressors and the future conditions of habitats and demographics.

ES.1 Summary of Life History, Ecology, and Distribution

The regal fritillary is a large butterfly found in the native grasslands of the central and northern Great Plains, portions of the Midwest, and a single location in eastern Pennsylvania. The species has one annual generation. Egg-laying occurs in late summer and fall when individual females may lay hundreds to thousands of eggs in native grassland habitats. The species overwinters as first instar larvae in grassland vegetation, emerging in spring to search for violets (*Viola spp*.), their only larval food. After five molts (six instars), the larvae pupate within the grasslands and emerge as adult butterflies beginning in late May through mid-July depending on their regional location. Adult males emerge approximately 1 to 2 weeks prior to females and are shorter-lived than females, surviving approximately 4 to 6 weeks, compared to the 8 to 12 weeks of females. Most females mate upon emergence and exhibit reproductive diapause, or delaying egg development and egg-laying until late summer and into early fall.

The regal fritillary is a landscape-level species, dependent on a shifting mosaic of suitable habitat resulting from periodic grassland disturbances, such as fire, grazing, and haying. These periodic disturbances sustain the species' habitat, but they can also cause individual- or population-level harm to regal fritillaries, particularly during the sedentary, early life stages. Adult females can move significant distances during their several-month long lifespan to access suitable habitats on the landscape.

The regal fritillary is also a "boom-and-bust" species, which means that when environmental conditions and habitat characteristics are favorable, significant increases in annual population abundance and distribution may occur. When conditions are unfavorable, individuals become scarce, and local extirpations may occur in areas that could be recolonized when conditions improve. The loss and fragmentation of suitable habitat may isolate local populations and contribute to local extirpations. This boom-and-bust nature may allow the subspecies to withstand environmental and demographic stochasticity, catastrophes, and environmental change. However, the specific habitat or demographic thresholds at which local extirpations may occur are difficult to define.

Historical records of regal fritillaries exist from 32 states (plus the District of Columbia) and 5 Canadian Provinces, although Canada has never been known to support permanent populations. Both subspecies historically occurred in the eastern U.S., but population collapses in that part of the range from the 1940s through the 1990s significantly reduced the overall species' range. Reasons for this collapse are not clearly understood, but multiple influences likely contributed, particularly habitat loss and degradation from conversion of grasslands to agriculture and other development activities. Figure ES.1 depicts counties within the species' range where observations were recorded currently, 2010 or later (blue), and historically, pre-2010 (orange).

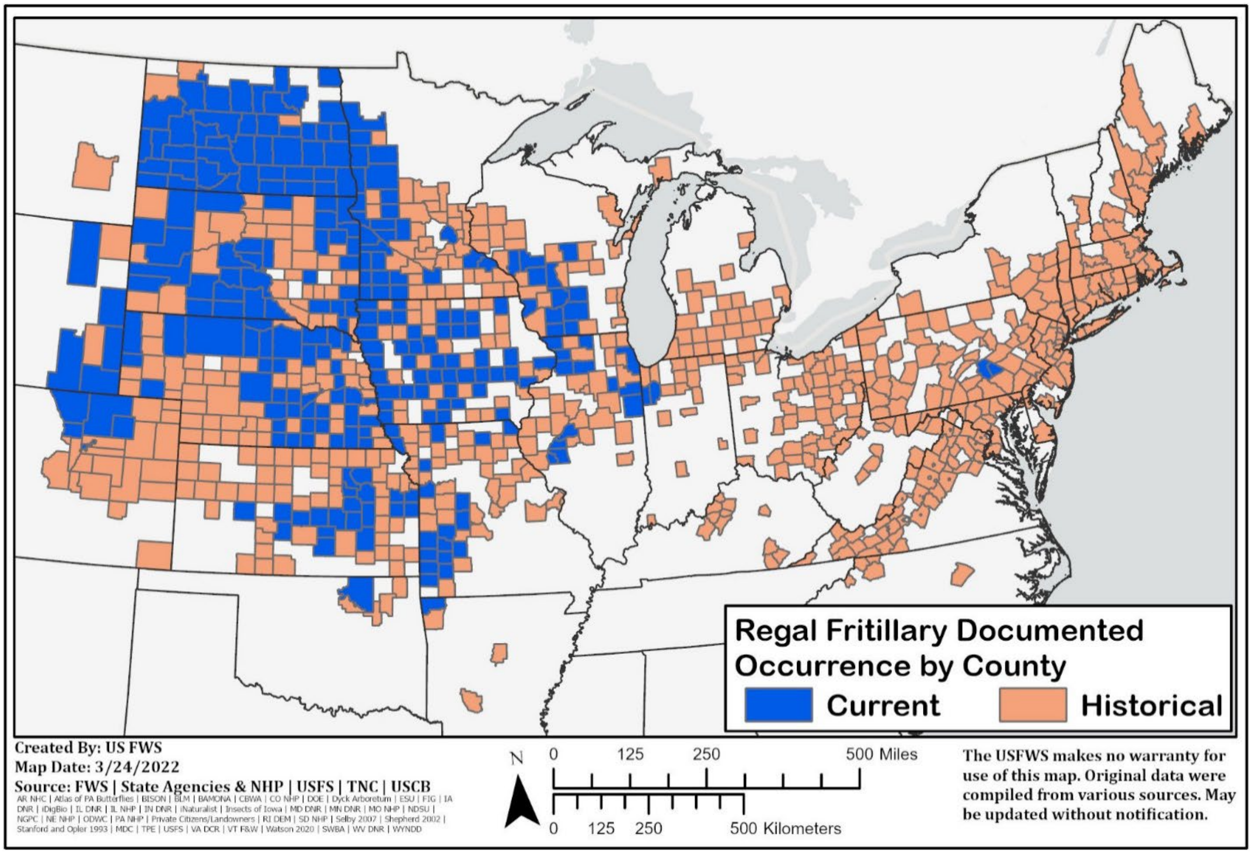

Figure ES.1. Map of U.S. counties with current (2010 or later [blue]) and historical (pre-2010 [orange]) records of regal fritillaries.

ES.2 Summary of Subspecies Needs

For this SSA, we identified the subspecies needs at three levels: individuals; populations, called analytical units (AUs) for this SSA; and the subspecies. We developed a conceptual model (Figure ES.2) illustrating the interactions of these components as they relate to the needed resiliency, redundancy, and representation of the subspecies. This model represents the overall viability of both subspecies in terms of the resiliency of populations (AUs), and the redundancy and representation of the subspecies.

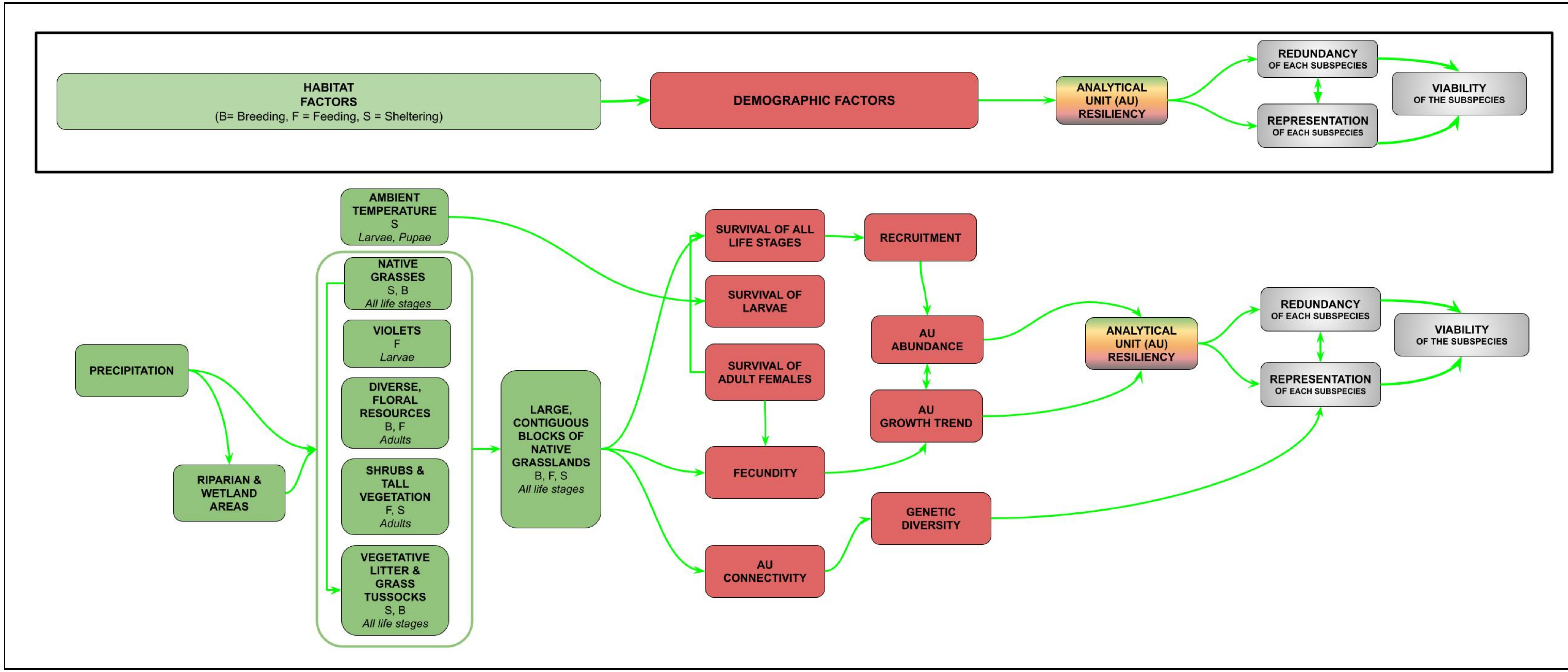

Figure ES.2. Conceptual model for the needs of the regal fritillary, eastern and western subspecies in terms of habitat factors (green boxes) needed by individuals to breed (B), feed (F), and shelter (S), and demographic factors (red boxes) that populations of the regal fritillary (analytical units, or AUs for this SSA) need to be resilient. Green arrows represent positive relationships between nodes. The core conceptual model for resiliency at the top of the model is included for reference.

In the conceptual model, green (habitat) boxes represent the needs of individual regal fritillaries to breed, feed, and shelter. These include violets to support larval growth, nectar sources that sustain breeding females into the fall, and native grasslands with tall vegetation that provide shelter for all life stages. The red (demographic) boxes in the conceptual model represent demographic factors associated with populations, such as large (e.g., more than 3.86 square miles (1,000 hectares)), native grassland patches that provide the individual needs. Additionally, these large patches of native grasslands need to be connected to other similar, large patches to ensure adequate population demographics such as survival rates, growth trends, and recruitment rates.

Viability of the subspecies requires an adequate number and distribution of sufficiently connected, large populations within AUs to ensure survival from stochastic (resiliency) or catastrophic events that may impact a broader area (redundancy). Such populations require adaptive capacity (genetic and/or environmental) to withstand future biological and physical changes to their environments (representation). Table ES.1 summarizes the subspecies needs.

Table ES.1. Summary of subspecies needs in terms of the 3Rs: resiliency, redundancy, and representation.

THE THREE Rs	NEEDS FOR VIABILITY	DESCRIPTION
RESILIENCY The ability of populations (AUs) of the subspecies to withstand environmental stochasticity, periodic disturbances within the normal range of variation, and demographic stochasticity	High population abundance	Overall high numbers of individuals present; exponential population growth occurs when conditions are favorable; numbers may drop, but remain relatively stable at high levels when conditions are less favorable; and able to withstand order-of-magnitude population fluctuations
	Large tracts of suitable habitats or complexes of multiple patches of proximal suitable habitat patches	Contiguous grasslands dominated by native species with required resources (i.e., adequate violets, nectar sources, grassland structure, and adequate environmental conditions); patch sizes that reach thousand(s) of hectares in size represent highest resiliency, albeit hundreds of hectares may support (less) viable populations
	Connectivity among suitable habitats	Distances among habitat patches are not prohibitive and the matrix facilitates movements (e.g., riparian corridors, grasslands) that allow access to necessary resources on a landscape scale and relatively frequent genetic exchanges and recolonizations
REDUNDANCY The ability of the subspecies to withstand catastrophic events that could lead to population collapse regardless of population health and for which adaptation is unlikely	Numerous resilient occupied areas distributed broadly across the species' range	Adequate numbers and distribution preclude catastrophic losses occurring via regional scale events (e.g., drought), allowing for species persistence in unimpacted areas
REPRESENTATION The ability of the subspecies to adapt to both near-term and long-term changes in its physical and biological environments (i.e., adaptive capacity)	Genetic diversity	High genetic diversity allows higher potential for adaptive capacity and regular genetic exchanges preclude problems such as inbreeding depression or genetic bottlenecks
	Ecological diversity	Diverse ecological settings allow for local adaptations that may buffer against stochastic or catastrophic events

ES.3 Summary of Cause-and-Effects: Stressors and Conservation Efforts

We evaluated sources, stressors, and other activities that can positively (conservation actions) or negatively (stressors) affect the regal fritillary at the individual, AU, or subspecies levels, either currently or into the future (Chapter 4). We also evaluated the potential cumulative effects of stressors that may act together in concert to influence AU resiliency and viability of the subspecies. A stressor is a change in a habitat or demographic resource, such as a decrease in violets, the primary larval food source, or decrease in abundance of AUs. Some stressors may directly influence the demographics of an AU through mortality of individuals resulting from actions or activities, such as agricultural conversion, while others, such as drought, may affect habitat factors that may indirectly affect individuals by influencing demographic factors. Some stressors may directly affect individuals and habitat factors at the same time, and stressors may act cumulatively. The stressors that we evaluated for the eastern and western subspecies of regal fritillary include:

- **Grassland conversion**, resulting from agricultural and urban development;
- **Pesticide** use and drift, including herbicide application;
- **Invasive plants**, including the encroachment of woody vegetation;
- **Drought;**
- **Climate change** and **local climate events;**
- **Periodic disturbances**, such as from fire, haying, and grazing;
- **Disease;**
- **Parasitism;**
- **Competition and hybridization with sympatric fritillaries;** and
- **Collection.**

Conservation efforts that may either reduce a stressor or improve the condition of habitat or demographics for the subspecies include:

- **Reintroduction programs;**
- **Land use management plans**, such as the Integrated Natural Resource Management Plan (INRMP) at FTIG; and
- **Voluntary conservation efforts.**

We developed a conceptual model to illustrate the relationships between the stressors, conservation effects, and their potential influence on AU resiliency (Figure ES.3). Then, we evaluated the potential effects of the stressors on AU resiliency, considering current and future conservation efforts (Chapter 4 and Appendix H). Conservation efforts (blue boxes) and stressors (orange boxes) may act upon the habitat and demographic factors and may influence the viability of the subspecies. While conservation measures occur locally in many areas to benefit the regal fritillary, most are voluntary in nature and may not be fully implemented or successful. Many stressors affect the subspecies, but a few such as agricultural conversion, periodic disturbances, and the effects of global climate change, particularly drought, appear to pose a relatively greater risk to the subspecies, particularly the western subspecies.

Our analysis of cause-and-effect revealed that grassland conversion, whether due to agriculture or development, historically contributed to the loss and fragmentation of the habitat needs of both subspecies and is likely to continue to reduce the availability of the subspecies' habitat needs in the future. Agriculture historically had the most severe impact to regal fritillaries, while numerous other forms of conversion have contributed to the loss and fragmentation of needed habitats. This stressor may result in the permanent loss of available habitats, increased fragmentation, and isolation that may reduce numbers of local populations and the overall abundance of AUs, resulting in potential declines in AU resiliency and overall decreases in the viability of the subspecies. As a result, we considered grassland conversion as a current and future driver of resiliency for AUs and potentially the viability of both subspecies.

Additionally, our cause-and-effect analysis determined that drivers for the western subspecies include grassland conversion, herbicide application, climate change factors, particularly drought, invasive grasses and woody encroachment (succession), and periodic disturbances from fire, haying, and grazing. For the eastern subspecies, grassland conversion and herbicide application are not considered risk factors on FTIG; future status is more reliant on management activities that ensure grassland habitat persists. Both subspecies are vulnerable to fragmentation and isolation that occurs with loss or degradation of habitat. Although disease, predation, parasitism, competition and hybridization with sympatric butterflies, and collection may affect individuals, we did not find that they may currently or into the future influence the viability of either subspecies. Finally, we found that the land management activities at FTIG have maintained habitats and the eastern subspecies, and if they continue as currently implemented, are likely to maintain habitats and the eastern subspecies into the future.

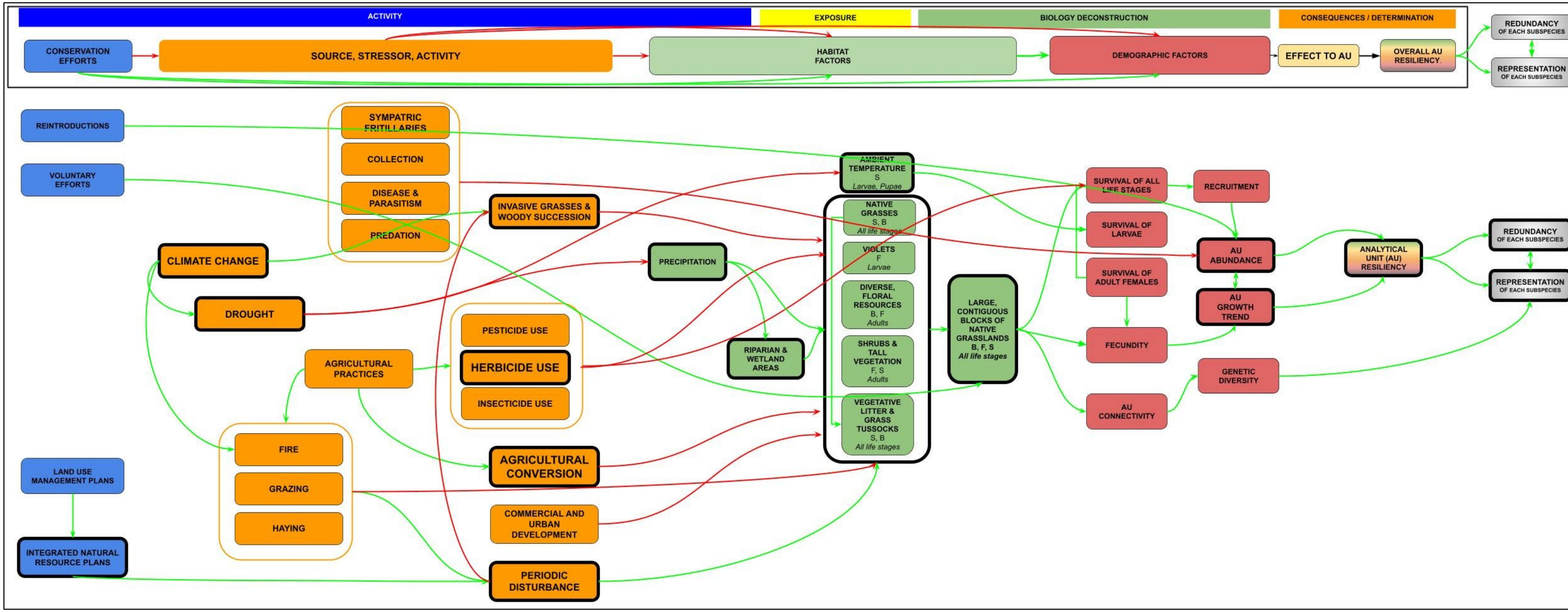

Figure ES.3. A conceptual model for the primary causes-and-effects (stressors and conservation efforts) that may influence the resiliency of analytical units (AUs) for the eastern and western subspecies of regal fritillary. The core conceptual model for resiliency at the top of the figure has been expanded to include activity and exposure pathways and is included for reference. Green arrows represent positive relationships between nodes and red arrows represent negative relationships between nodes. B = breeding; F = feeding; S = sheltering. The habitat and demographic factors that were measured to assess current and future resiliency are outlined in bold. The stressors and conservation efforts that we identified as drivers of resiliency are outlined in bold. Drivers of resiliency for the western subspecies include grassland conversion, herbicide application, climate change factors (particularly drought), invasive grasses/woody encroachment (succession), and periodic disturbances (fire/haying/grazing). For the eastern subspecies, grassland conversion and herbicide application are not considered risk factors on FTIG; future status is more reliant on management activities that ensure grassland habitat persists. Both subspecies are vulnerable to fragmentation and isolation that occurs with the loss or degradation of habitat, such that drought, invasive plants, and woody encroachment may influence the current and future resiliency of AUs, and potentially the viability of both subspecies. Although disease, predation, parasitism, competition and hybridization with sympatric butterflies, and collection may affect individuals, we did not find that they may currently or into the future influence the viability of either subspecies. Finally, we found that the land management activities at FTIG have maintained habitats and the eastern subspecies, and if they continue as currently implemented, are likely to maintain habitats and the eastern subspecies into the future.

ES.4 Summary of Current and Future Conditions

To evaluate resiliency, we adapted the U.S. Environmental Protection Agency's (EPA) Level III Ecoregions (EPA 2013, entire), modified to the extent of the regal fritillary's overall range, as analytical unit (AU) surrogates for regal fritillary populations. We also identified representation units based on genetic, habitat, climate, and phenology differences among groups of these units (Figure ES.3). The eastern subspecies has one AU across one representation unit, and the western subspecies has 21 AUs across three representation units (Figure ES. 3).

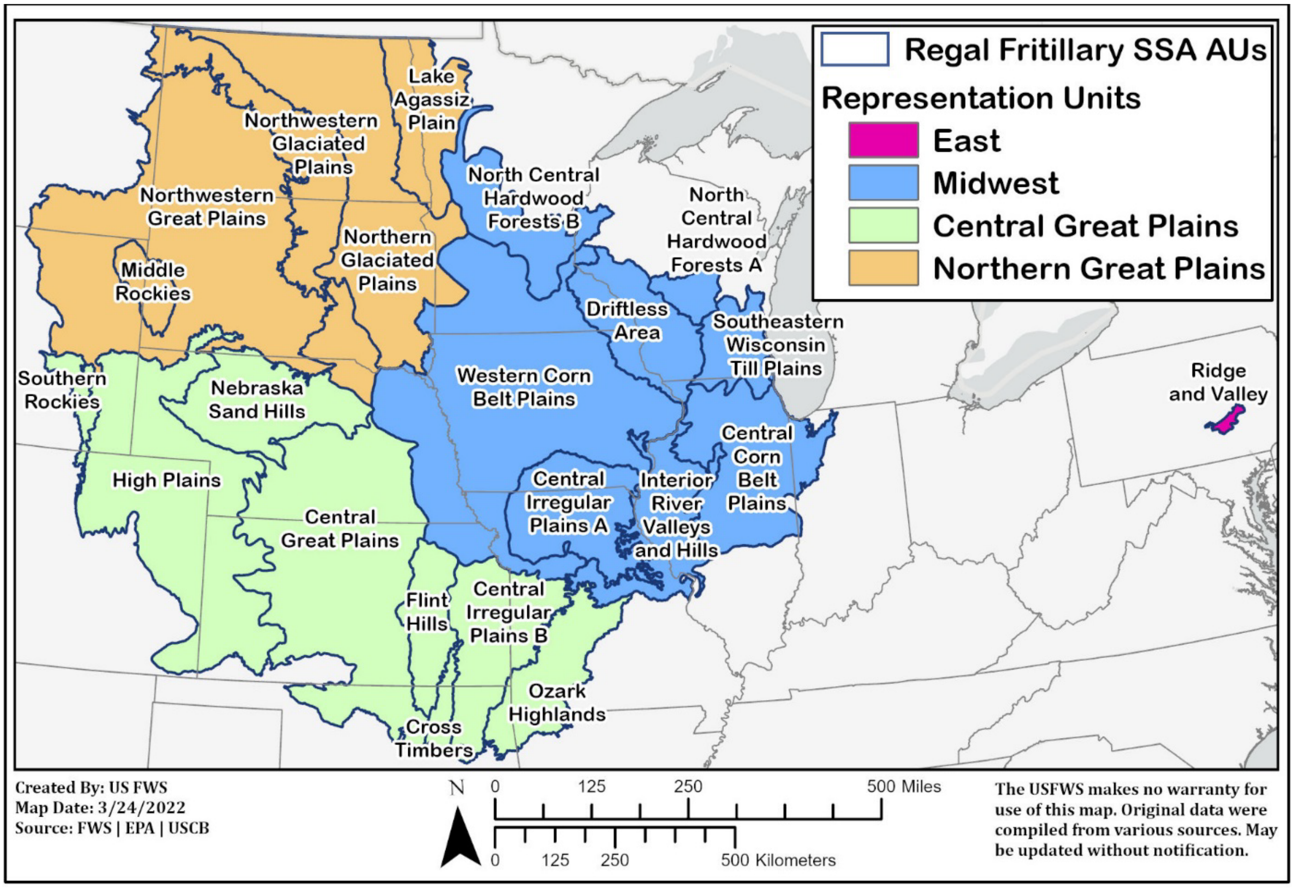

Figure ES.4. Map of the 22 analytical units (AUs) to evaluate resiliency and the 4 representative units.

After we identified the subspecies needs, we developed a condition category table to calibrate our evaluation of the resiliency of AUs. For this table, we selected a subset of the habitat and demographic factors that we identified as needs, such as those that we could measure, either quantitatively or qualitatively, consistently across the 22 AUs. Some of these factors were surrogates for more specific needs of the regal fritillary, such as the availability of violets or nectar sources), for which data were not readily available. The condition category table describes a range of conditions for five habitat and two demographic factors, from very high condition to extirpated condition, and is reproduced below in Table ES.2. We assigned each of these condition categories an associated point value, ranging from 0 for extirpated condition to 5 for very high condition.

Table ES.2. Condition category table (categorical model) used to evaluate the resiliency of AUs based on the current and future projected conditions of five demographic factors and two habitat factors. If any demographic factor is in extirpated condition, the AU has no resiliency, regardless of the condition of the habitat factors.

FACTORS	**HABITAT FACTORS**							**DEMOGRAPHIC FACTORS**	
	NATIVE GRASSLANDS (Quantity and Quality)		**RIPARIAN & WETLAND AREAS**	**AMBIENT TEMPERATURE**	**PRECIPITATION (Available Moisture)**	**LARGE, CONTIGUOUS BLOCKS OF NATIVE GRASSLANDS**		**ABUNDANCE**	**GROWTH TREND**
Summary of Evaluation Method (see Appendix I)	***Quantity:*** *Quantitative evaluation of the percent of AU that is native grasslands using geospatial data*	***Quality:*** *Qualitative evaluation of existing native grass types, violets, diverse floral resources, shrubby/tall vegetation, vegetative litter, and grass tussocks*	*Quantitative evaluation of the percent of AU that is potentially suitable habitat using geospatial data.*	*Quantitative evaluation of ambient temperatures*	*Quantitative evaluation of relative moisture supporting floral resources, individual health using climate data.*	*Quantitative evaluation of* ***patch size****, or percent of AU composed of patches sized 1,000+ ha (2,471 ac) using geospatial data.*	*Quantitative evaluation of* ***connectivity****: percent of AU comprised of grass patches using geospatial layers.*	*Qualitative evaluation of abundance using expert input.*	*Qualitative evaluation of abundance using expert input.*
VERY HIGH (5 points)	≥51% of AU is composed of native grasslands	75-100% of AU grasslands are high quality, native tallgrass dominant (75% or more), and diverse with a heterogenous mosaic of successional stages. Vegetative litter/tussocks always available within the majority of patches at ideal level (several years buildup). Violets are highly abundant (e.g., 5 or more plants per 1 m^2 (11 ft^2 [50,000+ per 1 ha or 2.5 ac]) throughout AU. Diverse floral resources are always abundant and available. Shrubs/tall vegetation is available (without woody encroachment concerns) in nearly all patches.	Potentially suitable habitat within riparian and wetland 100-m (328-ft) buffer represents 16.1% or more of AU	<1% of area of AU exceeds 41 °C (105 °F) for 2+ days during spring and summer	Spring precipitation ≥254 mm (10 in); summer precipitation ≥254 mm (10 in); <0.5 droughts/decade	≥81% of AU is composed of habitat patches sized1000+ ha (2471+ ac)	≥81% of AU is composed of connected habitat patches (within 3–5 km [1.9-3.1 mi])	Adults are abundant in nearly all populations throughout the AU in most years; approximately 25 per 1 ha (2.5 ac) or more and nearly ubiquitous throughout habitat; the AU is a consistent source for satellite areas within the AU or adjacent AUs	All populations in AU consistently exhibit exponential growth in good years and stable trend during poor years.
HIGH (4 points)	26–50%	50-74% of AU grasslands are high quality (limited degradation), native, tallgrass dominant (~50-74%), and heterogenous with a mosaic of successional stages. Vegetative litter/tussocks are available in most habitats at ideal levels (several years buildup). Violets are generally plentiful (2-4.9 plants/m^2) in most areas. Diverse floral resources are abundant and available annually. Shrubs/tall vegetation is available (without woody encroachment concerns) in most patches.	8.1–16%	1–20%	Spring precipitation 216–254 mm (8.50–9.99 in); summer precipitation 216–254 mm (8.50-9.99 in); 0.6-0.9 droughts per decade	61–80%	61–80%	Adults are abundant in most populations throughout the AU in most years; ubiquitous with ~10–24 individuals per 2.5 ac (1 ha) in good years; more patchily distributed and less common (5–10 individuals/ha) in poor years; the AU is a consistent source for satellite areas	Most populations in AU exhibit exponential growth in good years, and stable trend in poor years. Some smaller areas may be extirpated in poor years, but repopulation and growth happen quickly.
MEDIUM (3 points)	11–25%	25-49% of grasslands in the AU are native, diverse, and high-quality mixed grass (25-49% tallgrass composition). On average grasslands are of moderate quality, generally a mix of heterogenous native grasslands and homogenous nonnative grasslands or with woody encroachment. Vegetative litter/tussocks may or may not be available in most patches - about as likely to be present as not (buildup may be limited in many habitats with less than 2 years buildup or excessive with a decade or more of no disturbance). Violets are available, but at relatively lower densities (1-1.9 plants/m^2) in most areas. Diverse floral resources may be widely available some years but limited in others. Shrubs/tall vegetation may/may not be available or woody encroachment (succession) may be occurring in a few areas to the detriment of native grasses and floral resources.	4.1-8%	21–40%	Spring precipitation 152–216 mm (6.0-8.49 in); summer precipitation 152–216 mm (6.0-8.49 in); 1.0–1.9 droughts per decade	41–60%	41–60%	Adults are common to locally abundant in populations across some areas of the AU but absent in other areas most years; ~5–10 individuals per 1 ha (2.5 ac) in good years, 1–5 individuals per 1 ha (2.5 ac) or less in poor years - typically not a source for satellite areas	Populations in AU exhibit exponential growth infrequently. May provide some refugia and act as a source, but repopulation and growth of satellite areas is relatively slow.
LOW (2 points)	6–10%	5-24% of grasslands in the AU are diverse, native, and high quality. Overall, grasslands are low quality, often homogenous, non-native, shortgrass dominant (5-24% tallgrass). Vegetative litter/tussocks are usually not adequate or available in most habitats (e.g., denuded or overgrown and rarely ideal condition). Violet density is low (0.5-0.9 plants/m^2) in most areas. Floral resources are not diverse or abundant and are a limiting factor most years. Shrubs/tall vegetation may either not be available, or woody encroachment (succession) may become dominant over grasslands in some areas.	2.1-4%	41–60%	Spring precipitation 114-152 mm (4.50-5.99 in); summer precipitation 114-152 mm (4.50-5.99 in); 2.0-2.9 droughts per decade	21–40%	21–40%	Adults occur in low numbers within populations throughout the AU in most years; very few locally common/abundant populations exist; 1–4 individuals per 1 ha (2.5 ac) in good years, less than 1 individual 1 ha (2.5 ac) or absent in poor years; not a source for adjacent AUs, many areas may be sinks	Populations in AU typically do not exhibit exponential growth, even in good years. The AU does not act as refugia or source – repopulations are reliant on dispersers from adjacent AUs
VERY LOW (1 point)	1–5%	< 4% of grasslands in the AU are native, diverse, and high quality; the AU is dominated by homogenous nonnative or low-quality habitats; <4% tallgrass composition or shortgrass dominant. Vegetative litter/tussocks are extremely limiting; almost never available at appropriate level (denuded or overgrown). Violet densities are very limiting; 0.9 plants/m^2 or less) in most areas. Floral resources are a limiting factor nearly every year. Shrubs/tall vegetation are either not available or woody encroachment (succession) dominates in many/most areas.	1.1-2%	61–80%	Spring precipitation 76-114 mm (3.0-4.49 in); summer precipitation 76-114 (3.0-4.49 in); 3.0-3.9 droughts per decade	1–20%	1–20%	Adults typically occur in very low numbers within populations; uncommon to rare throughout the AU in most years; no locally abundant populations; less than 1 individual per 1 ha (2.5 ac) in good years, no individuals detected in poor years; most areas act as sinks	Populations in AU consistently exhibit little to no growth in most years, many are extirpated in poor years. Repopulation may take years if it occurs at all. AU may be a population sink or may only harbor dispersing adults occasionally with few to no populations most years.
EXTIRPATED (0 points)	< 1%	No good quality grasslands present	≤1.0%	81–100%	Spring precipitation <76 mm (3 in); summer precipitation <76 mm (3 in); ≥4.0 droughts per decade	<1%	<1%	Absent	Absent

To assess resiliency of all 22 AUs, we systematically evaluated the current condition of each of the habitat and demographic factors for each of the AUs and assigned conditions for each factor as defined in our condition category table. We then calculated a straight average across all seven factors to summarize the resiliency of each AU, ranging from very high to extirpated. AUs with higher levels of resiliency are more likely to withstand environmental and demographic stochasticity, or persist, than those with lower levels of resiliency, as calibrated with the condition category table. With resiliency evaluated for each AU, we could also assess redundancy and representation for both subspecies.

Following our assessment of current conditions in terms of the 3Rs for both subspecies, we projected a range of plausible future scenarios for the regal fritillary to year 2075. This was determined to be a biologically meaningful timeframe for our analysis, as it captures over 50 annual generations of the regal fritillary and is also the approximate timeframe during which the eastern subspecies experienced a historical decline. In order to capture the range of uncertainty associated with the future, we used scenario planning and developed three, plausible future scenarios based on projected changes in climate and the primary stressors to the subspecies. The three future scenarios that we used to evaluate future conditions for the subspecies are:

- **Scenario 1: Continuation of Existing Conditions/Trends.** Under this scenario, current stressors affecting the subspecies do change somewhat over time but continue generally per current trends.

- **Scenario 2: Moderately Worsening Conditions/Trends.** Current stressors become more problematic over time, showing an increase of the stressors and trends currently observed.

- **Scenario 3: Significantly Worsening Conditions/Trends.** This is the most severe scenario in which new stressors arise and/or current stressors are exacerbated significantly above current levels and trends.

To evaluate future conditions, we repeated our assessment of condition for the two demographic factors and five habitat factors using the same condition category table, but this time under each of the three future scenarios, considering plausible changes to 22 AUs under each scenario to year 2075. We again calculated a straight average for the resiliency for each AU. Table ES.3 and Figure ES.5 summarize our analysis of the current and future resiliency, redundancy, and representation (viability) for the eastern and western subspecies of regal fritillary.

Table ES.3. Current and future conditions for the eastern and western subspecies. These condition categories, ranging from extirpated (X) to very high resiliency, provide a calibrated, relative ranking system based on criteria developed to describe various conditions of habitat and demographic needs, now and into the future.

CURRENT AND FUTURE CONDITIONS - EASTERN AND WESTERN SUBSPECIES						
SUBSPECIES	**REPRESENTATION UNIT NAME**	**ANALYTICAL UNIT NAME**	**CURRENT RESILIENCY**	**FUTURE RESILIENCY SCENARIO 1**	**FUTURE RESILIENCY SCENARIO 2**	**FUTURE RESILIENCY SCENARIO 3**
EASTERN	East	Ridge and Valley	LOW	LOW	VERY LOW	X
WESTERN	MIDWEST	Central Corn Belt Plains	LOW	LOW	VERY LOW	X
		Central Irregular Plains - A	LOW	LOW	LOW	VERY LOW
		Driftless Area	LOW	LOW	LOW	X
		Interior River Valleys and Hills	LOW	LOW	VERY LOW	X
		North Central Hardwood Forests - A	LOW	LOW	LOW	VERY LOW
		North Central Hardwood Forests - B	LOW	LOW	LOW	X
		Southeastern Wisconsin Till Plains	LOW	LOW	LOW	X
		Western Corn Belt Plains	LOW	LOW	LOW	VERY LOW
	NORTHERN GREAT PLAINS	Lake Agassiz Plains	LOW	LOW	VERY LOW	VERY LOW
		Middle Rockies	MEDIUM	LOW	LOW	X
		Northern Glaciated Plains	MEDIUM	MEDIUM	LOW	VERY LOW
		Northwestern Glaciated Plains	MEDIUM	MEDIUM	MEDIUM	LOW
		Northwestern Great Plains	HIGH	MEDIUM	MEDIUM	MEDIUM
	CENTRAL GREAT PLAINS	Central Great Plains	MEDIUM	MEDIUM	LOW	VERY LOW
		Central Irregular Plains - B	MEDIUM	MEDIUM	LOW	VERY LOW
		Cross Timbers	X	X	X	X
		Flint Hills	HIGH	HIGH	MEDIUM	MEDIUM
		High Plains	MEDIUM	LOW	LOW	X
		Nebraska Sand Hills	HIGH	HIGH	HIGH	MEDIUM
		Ozark Highlands	LOW	LOW	LOW	X
		Southern Rockies	MEDIUM	MEDIUM	MEDIUM	X

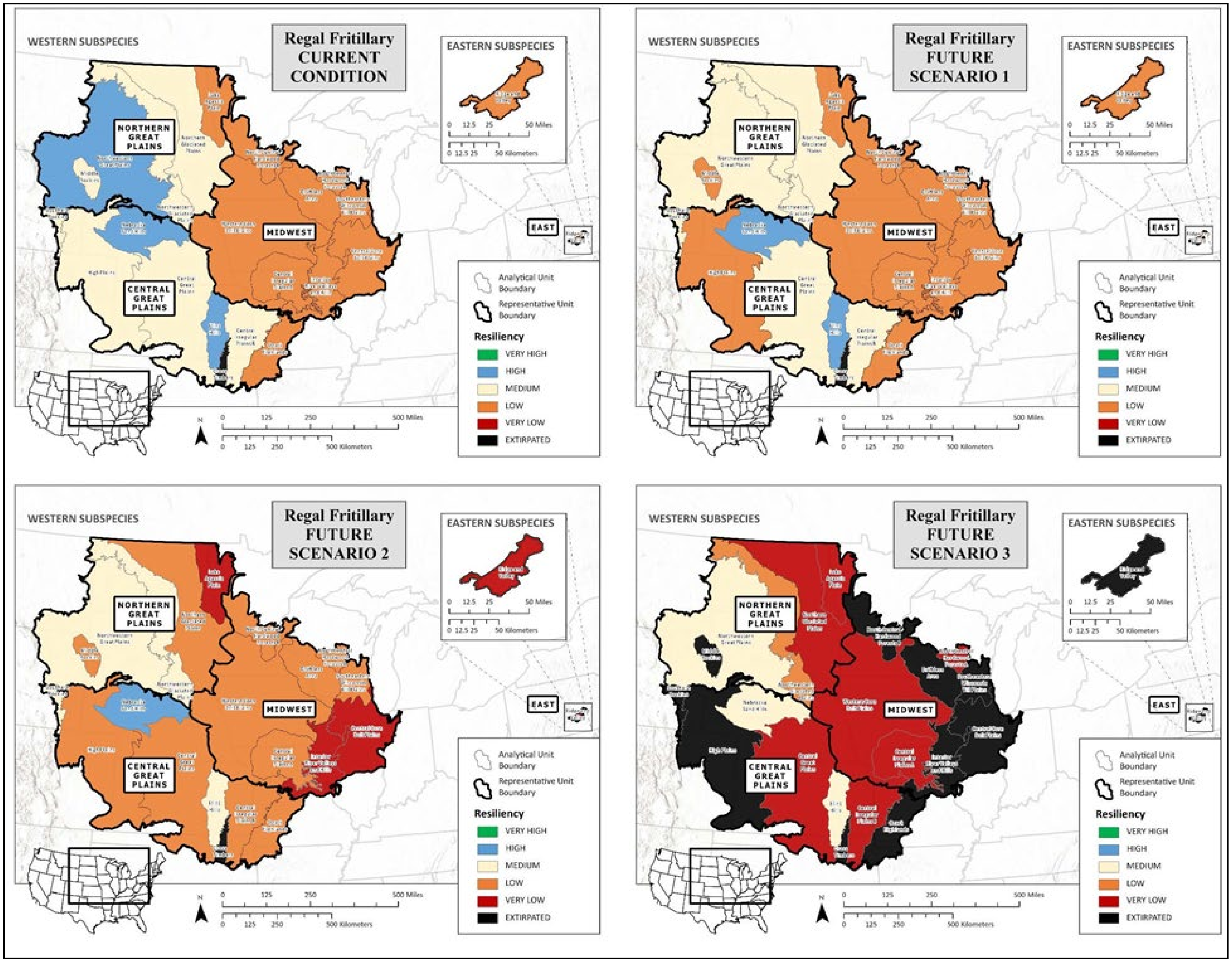

Figure ES.5. Maps of the current and future resiliency, redundancy, and representation of the eastern and western subspecies of regal fritillary.

Based on our evaluation of current and future resiliency across all 22 AUs, we then also evaluated the current and future redundancy for both subspecies, as summarized below in Table ES.4 and illustrated in the maps in Figure ES.4, above.

Table ES.4. Current and future redundancy of the regal fritillary: the number of AUs assigned each condition category as they exist currently and as projected under three future scenarios, for the eastern and western subspecies.

CURRENT AND FUTURE REDUNDANCY - EASTERN AND WESTERN SUBSPECIES								
RESILIENCY	EASTERN SUBSPECIES				WESTERN SUBSPECIES			
	CURRENT RESILIENCY	FUTURE RESILIENCY SCENARIO 1	FUTURE RESILIENCY SCENARIO 2	FUTURE RESILIENCY SCENARIO 3	CURRENT RESILIENCY	FUTURE RESILIENCY SCENARIO 1	FUTURE RESILIENCY SCENARIO 2	FUTURE RESILIENCY SCENARIO 3
Very High								
High					3	2	1	
Medium					7	6	4	3
Low	1	1			10	12	12	1
Very Low			1				3	7
Extirpated (X)				1	1	1	1	10

Representation for each subspecies within each of the four representation units and under the three future scenarios are provided below, along with current conditions (Table ES.5). Representation also includes consideration of the varying habitat conditions and genetic differentiation known to exist among the four representative units.

Table ES.5. Current and future representation of the regal fritillary by representation unit, eastern and western subspecies: the number of analytical units assigned each condition category as they exist currently and as projected under three future scenarios, for the eastern and western subspecies, in each of the four representation units

CURRENT AND FUTURE REDUNDANCY - EASTERN AND WESTERN SUBSPECIES																
RESILIENCY	**EASTERN SUBSPECIES**				**WESTERN SUBSPECIES**											
	EAST				**MIDWEST**				**NORTHERN GREAT PLAINS**				**CENTRAL GREAT PLAINS**			
	CURRENT RESILIENCY	FUTURE RESILIENCY SCENARIO 1	FUTURE RESILIENCY SCENARIO 2	FUTURE RESILIENCY SCENARIO 3	CURRENT RESILIENCY	FUTURE RESILIENCY SCENARIO 1	FUTURE RESILIENCY SCENARIO 2	FUTURE RESILIENCY SCENARIO 3	CURRENT RESILIENCY	FUTURE RESILIENCY SCENARIO 1	FUTURE RESILIENCY SCENARIO 2	FUTURE RESILIENCY SCENARIO 3	CURRENT RESILIENCY	FUTURE RESILIENCY SCENARIO 1	FUTURE RESILIENCY SCENARIO 2	FUTURE RESILIENCY SCENARIO 3
Very High																
High									1				2	2	1	
Medium									3	3	2	1	4	3	2	2
Low	1	1			8	8	6		1	2	2	1	1	2	4	
Very Low			1				2	3			1	2				2
Extirpated (X)				1				5				1	1	1	1	4

ES.5 Summary of Viability for the Eastern Subspecies

Currently, the eastern subspecies has one AU, the Ridge and Valley AU in Pennsylvania, with low resiliency. Although this AU currently has some very high and high conditions for a few habitat factors, its small size limits the conditions of the other habitat factors, which also contributes to the very low conditions for abundance and population trend. The primary stressor in this AU is woody encroachment, and the AUs resiliency has been maintained by suitable land disturbance activities and conservation actions that have reduced this stressor. Redundancy for the eastern subspecies is described as the one AU, with low resiliency, found as distributed on FTIG. Current representation for the eastern subspecies is similarly limited to the single, isolated population at FTIG. The east representative unit provides a different, more mesic, ecological type than the AUs for the western subspecies.

Given its currently low resiliency, and its redundancy and representation across one AU, the eastern subspecies is more at risk to stochastic events, catastrophic events, and environmental change than the western subspecies. The eastern subspecies is small, isolated, has unique habitat and genetic characteristics and stressors, and is the only known remnant of a subspecies that was historically more broadly distributed. However, resiliency is currently low, not very low or extirpated, and the subspecies has demonstrated the capacity to withstand stochastic and catastrophic events. Although ecological and genetic diversity are low, the adaptive capacity of the subspecies is less clear, although the eastern subspecies could share the adaptive capacity of the western subspecies as demonstrated across its three representative units. Ongoing activities and conservation actions for the eastern subspecies continue to reduce habitat loss and fragmentation associated with woody encroachment, which has helped maintain low resiliency in its single AU. As a result, the current viability for the eastern subspecies may depend largely on these beneficial actions and activities to control woody encroachment and improve habitats.

Under Future Scenario 1, stressors and conservation efforts remain at their current rates to year 2075, which represent the least amount of projected risk to the subspecies. Under this scenario, the habitat and demographic conditions, stressors and conservation efforts are expected to continue at current levels. Under this scenario, resiliency for the eastern subspecies' AU remains low, and there are no reductions in the habitat and demographic factors. Redundancy and representation also remain the same under this scenario. As a result, risk to the subspecies remains the same under Future Scenario 1, and the viability of the eastern subspecies remains the same.

Under Future Scenario 2, stressors increase moderately, so risk to the subspecies increases moderately. Under this scenario, the resiliency for the eastern subspecies' AU declines from low to very low. Redundancy and representation remain unchanged, although risk to the subspecies increases under this scenario due to the decline in resiliency for the eastern subspecies' one AU.

Future Scenario 3 represents the maximum projected increase in stressors, or the most amount of risk expected for the subspecies. Under Future Scenario 3 the eastern subspecies drops from low resiliency to extirpated with no resiliency. This future projection represents a complete loss of resiliency, redundancy, and representation for the eastern subspecies. As a result, Future Scenario 3 projects a complete loss of viability for the eastern subspecies.

Our biological risk assessment for the eastern subspecies concludes that the eastern subspecies is inherently more at risk from stochastic events, catastrophic events, and environmental change, given its small size and distribution across one AU. Its viability is tied to the condition of its remaining habitats and the reduction of its primary stressor, woody encroachment, through management and other activities. We projected that risk to the eastern subspecies' either stays the same or increases into the future. Therefore, viability of the eastern subspecies may remain the same, decrease, or decrease such that the subspecies no longer be viable under the most extreme scenario.

ES.6 Summary of Viability for the Western Subspecies

Currently, the western subspecies has 21 AUs distributed across 3 representation units, which features a diversity of climates, habitats, and genetics. Of the 21 AUs, one in the Northern Great Plains and two in the Central Great Plains, currently have high resiliency, 7 have medium resiliency, 10 have low resiliency, and one AU, the Cross Timbers AU, is currently extirpated with no resiliency. The Cross Timbers AU currently supports habitats and has recent observations, but lacks abundance, so is currently extirpated with no resiliency. For the western subspecies, no AUs currently have very high or very low resiliency. All the AUs in the Midwest representative units currently have low resiliency, largely as a result of habitat conditions following grassland conversion.

Genetically, the regal fritillaries in these three representative units share western haplotypes, which is the physical grouping of genomic variants that tend to be inherited together, but populations in the Midwestern unit exhibit relatively less genetic diversity than those in the Northern Great Plains or Central Great Plains – an indication of fragmentation and isolation. All western regal fritillary populations exhibit more genetic diversity than within the one population of the eastern subspecies in Pennsylvania.

Under Future Scenario 1 for the western subspecies, stressors and conservation efforts remain at their current rates to year 2075, which represent the least amount of projected risk to the subspecies. As with the eastern subspecies, conditions for the western subspecies decline under Future Scenario 1, with some declines projected in resiliency, although the declines are the least of all the future scenarios. Under this scenario, conditions of climate and stressors generally continue as they are now, so only three AUs decline in resiliency. All of the AUs that are currently resilient remain so under this future scenario, and one AU declines from high to medium resiliency. No AUs decline to very low resiliency under this scenario. Redundancy and representation for the western subspecies do not change in the future under this scenario. As a result, risk to the western subspecies remains largely unchanged under this scenario.

Under Future Scenario 2, stressors increase moderately, so risk to the subspecies increases moderately. As a result, several AUs of the western subspecies decline from low to very low resiliency. These AUs with projected very low resiliency are from the Midwest and Northern Great Plains representative units. The Cross-Timbers AU remains in extirpated condition with no resiliency under this scenario, and all other AUs have some resiliency. However, as stressors worsen, resiliency declines by one category for approximately half of the 21 AUs of the western subspecies. Redundancy and representation remain the same under this scenario, although the projected reduction in resiliency for 11 AUs under this scenario represents an increase in risk to the western subspecies. Therefore, under Future Scenario 2, viability of the western subspecies declines from current conditions, with more of a decline than projected under Future Scenario 1.

Future Scenario 3 represents the maximum projected increase in stressors. As a result, under Future Scenario 3, 10 of the 21 AUs of the western subspecies decline to extirpated condition with no resiliency. The projections under Future Scenario 3 may be somewhat pessimistic, as the average resiliency scores for these 10 AUs were actually within the very low range; however, the AUs were assigned an extirpated condition due to our rule that no AU could be considered resilient if its abundance or growth trend factors are in extirpated condition. In other words, although 10 AUs decline to extirpated under this scenario, the habitat factors may still be present. Although all three representative units remain under this scenario, the majority of the AUs within each representation unit have very low or no resiliency, which may represent a reduction in adaptive capacity under this scenario. Under this scenario, the resiliency, redundancy, and representation of the western subspecies declines, so this projection represents an extreme future reduction of the subspecies' viability.

Our biological risk assessment for the western subspecies concludes that risk to the subspecies increases into the future to year 2075. The viability of the western subspecies remains largely the same under two out of the three future scenarios, although we project reductions in resiliency across some AUs under both Future Scenarios 1 and 2. Viability declines the most under one future scenario (Future Scenario 3), when stressors are projected to be the most extreme. Therefore, in the future, the viability of the western subspecies may remain largely the same, with reductions in resiliency but not redundancy and representation if stressors increase at their current rates, or decrease more substantially if conditions reach their most extreme projected states, with substantial declines in resiliency, redundancy, and representation.

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Chapter 1 – Introduction and Analytical Framework

This species status assessment (SSA) report summarizes the biology, life history, ecology, and stressors (threats) for the eastern (*A. i. idalia*) and western (*A. i. occidentalis*) subspecies of regal fritillary (*Argynnis (Speyeria) idalia)*. This report also summarizes the results of a biological risk assessment for the eastern and western subspecies, using the SSA framework (Service 2016, entire; Smith *el al*. 2018, entire). Throughout this report, unless specified otherwise, information presented for the species applies to both the eastern and western subspecies.

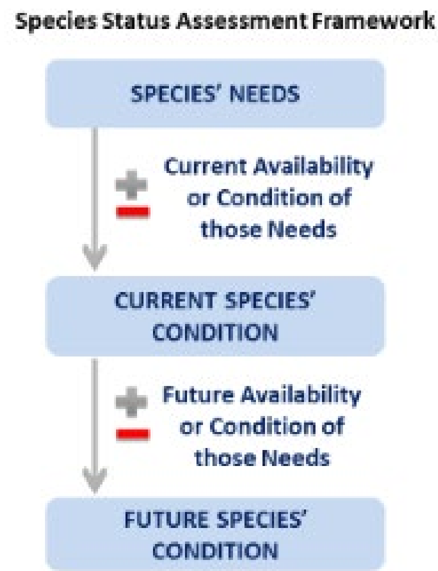

Figure 1. The SSA framework's three basic stages (Service 2016, p. 6).

The SSA framework is an analytical approach to deliver foundational science to help inform the U.S. Fish and Wildlife Service's (Service's) decisions under the Act (Service 2016, p. 4). This SSA report is intended to provide a clear, in-depth characterization of the subspecies' biology and ecology; the influence of environmental stressors and conservation management actions on the subspecies' viability; the current biological status, also called "current condition" for each subspecies; and the projected, plausible future biological status for each subspecies, also called "future condition," under a range of future scenarios. Viability describes the ability of a subspecies to sustain populations in the wild over time (Service 2016, p. 9). This SSA report for the eastern and western subspecies is not meant to accumulate all information regarding the regal fritillary but provides foundational scientific information to help inform the Service's responsibilities under the Act, including listing determinations and other actions, as needed. This SSA report is a living document and can be easily updated as new scientific information becomes available in order to best support all functions of our Endangered Species program.

Importantly, this SSA report does not make any decisions by the Service, such as whether a species or subspecies should be listed under the Act. It is not a decision document constituting a final agency action. Instead, this SSA report provides a review of the best available scientific and commercial information regarding the biological status, or condition, of the eastern and western subspecies of regal fritillary. Thus, this SSA report is a stand-alone, science-based document produced independently from the Service's application of policy or regulation, and it provides a review of the available information strictly related to the life-history, ecology, stressors, and viability of the eastern and western subspecies of regal fritillary. Any decisions under the Act, such as listing determinations, will be made by the Service after reviewing this document and all relevant laws, regulations, and policies, and the results of any decisions will be announced in the *Federal Register*, with opportunities for public input, if appropriate.

The SSA framework has three, iterative assessment stages, as summarized below and illustrated above in Figure 1.

- **Stage I:** Subspecies' Needs – An SSA begins by describing the ecological needs of the species at the individual, population, and species levels based on how environmental factors act on the species and its habitat.
- **Stage II:** Current Subspecies' Condition – Next, an SSA describes the current condition of the species' habitat and demographic needs, and the probable explanations for past and ongoing changes in the abundance of populations the distribution and diversity of the species.
- **Stage III:** Future Subspecies' Condition – Lastly, an SSA projects the species' response to probable future scenarios of environmental conditions and conservation efforts.

As a result, the SSA characterizes the subspecies' viability, or its ability to sustain populations in the wild over time, based on the best scientific understanding of its current and future abundance, distribution, and diversity (Service 2016, p. 6; Smith *et al.* 2018, pp. 305–306).

Throughout this report, we describe the needs and viability of the eastern and western subspecies in terms of the conservation biology principles of resiliency, redundancy, and representation, collectively known as the 3Rs (Shaffer and Stein 2000, pp. 307–310; Wolf *et al.* 2015, entire; Service 2016, pp. 12–13, 21; Smith *et al.* 2018, entire). The 3Rs are defined as follows:

- **Resiliency** is the ability for populations to persist in the face of stochastic events, or for populations to recover from years with low reproduction or reduced survival, and is associated with population size, growth rate, and the quality and quantity of habitats. Resiliency is positively related to abundance (population size) and growth rate and may be influenced by connectivity between populations. Populations need an abundance of individuals within habitat patches of adequate quantity and quality to survive and reproduce despite disturbance (Service 2016, p. 12). Demographic factors, including abundance, population growth rate, survival, genetic health, connectivity, and the quantity and quality habitats influence the resiliency of regal fritillary populations. We evaluated resiliency at the scale of analytical units (AUs), which we developed as population-like units for the subspecies.

- **Redundancy** is the ability for the subspecies to withstand catastrophic events, such as a rare destructive natural event or episode involving many populations for which adaptation is unlikely, and is associated with the number and distribution of populations. Catastrophes are stochastic events that lead to population collapse regardless of population health and for which adaptation is unlikely (Mangal and Tier 1993, p. 1083). Redundancy is about spreading risk among multiple populations to minimize potential loss of the species from catastrophic events and is characterized by having multiple, resilient populations distributed within the species' ecological settings and across the species' range. Redundancy can be measured by the number of populations (Aus), their spatial extent, and degree of connectivity. The analysis entails assessing the cumulative risk of catastrophes occurring over time.

- **Representation** is the ability of the subspecies to adapt to changes in the environment over time and is associated with its diversity, whether ecological, genetic, behavioral, or morphological. It is characterized by the breadth of genetic and environmental diversity within and among populations and measures of representation may include the number of varied occupied niches, genetic diversity, heterozygosity, alleles per locus, or other geographic, genetic, or life history variation of the subspecies.

In general, species risk will decrease, or at least does not increase, with increases in resiliency, redundancy, and representation. In other words, the more redundant and representative the species is, and the more resilient its populations, the more likely the species is to sustain populations over time, even under changing environmental conditions. A species with a high degree of resiliency, representation, and redundancy (the 3Rs) is better able to adapt to novel changes and tolerate environmental stochasticity and catastrophes. As a result, species viability will increase with increases in resiliency, redundancy, and representation (Smith *et al.* 2018, p. 306).

Throughout this report, we use the 3Rs together to characterize the current and projected future viability for the eastern and western subspecies. For the purpose of this assessment, we define viability as the ability of the subspecies to sustain multiple populations across diverse representative units in natural ecosystems over a biologically meaningful timeframe, in this case, by approximately 2075, or approximately 50 years into the future. Viability is not a specific state, but rather a continuous measure of the likelihood that the subspecies will sustain populations over time (Service 2016, p. 9). Therefore, exploring and describing the relationships of what influences the 3Rs given the subspecies' unique life history does not result in a conclusion on whether the subspecies is viable, but instead sets out foundational relationships used to explore potential changes from the species' current condition to its projected future conditions (Service 2016, p. 13). In addition, the term viability denotes a trajectory opposite to extinction and a focus on species conservation (Service 2016, p. 9). The 2075, 50-year, timeframe for this assessment is a period that accounts for approximately 50 annual generations of regal fritillary and allows adequate time for AUs to respond to stressors and conservation efforts. It is also the time-period to which we can reasonably project climate conditions based on the best available climate models across the range of the subspecies. Additionally, the timeframe is biologically meaningful because it also represents the approximate timeframe during which the historical range of the eastern subspecies contracted to its current distribution in one AU.

The objectives of the SSA report are as follows:

- Summarize regal fritillary biology, including its taxonomy, distribution, habitat, life history, and life cycle (Chapter 2, with additional information in Appendix B (taxonomy), life history (Appendix C), and Appendix D (species distribution model));
- Describe the ecological needs at the individual, population (AU), and subspecies levels in terms of resiliency, redundancy, and representation (Chapter 3);
- Identify known stressors (threats) that negatively influence viability and the conservation actions that positively influence viability (Chapter 4);
- Describe the current condition in terms of the resiliency of populations (AUs), and the redundancy and representation for the eastern and western subspecies (Chapter 5);
- Project the response of the eastern and western subspecies to plausible future scenarios of environmental conditions and conservation efforts (Chapter 6); and

- Synthesize viability for the eastern and western subspecies by summarizing their projected future conditions with the current condition in terms of risk (Chapter 7).

The Service's decisions under the Act are based on an assessment of a species' risk of extinction. This SSA report is intended to inform an assessment of extinction risk by describing the eastern and western subspecies' current biological status (Chapter 5) and assessing how this status may change in the future under a range of plausible future scenarios (Chapter 6). We evaluate the current biological status of the eastern and western subspecies by assessing the factors that positively and negatively affect the subspecies (Chapter 4) and describe the current condition of the subspecies in terms of the 3Rs (Chapter 5). We then evaluate the future biological status by describing a range of plausible future scenarios representing a range of conditions for the primary factors affecting the subspecies and forecasting the future condition for each scenario in terms of the 3Rs (Chapter 6). Chapter 7 summarizes the current and future conditions to characterize the viability of the eastern and western subspecies of regal fritillary.

Regulatory History

For informational purposes, we provide a summary of the regulatory history for the regal fritillary in Appendix A. This summary includes a summary of regulatory activities by both Federal and state entities.

Core Conceptual Model of Viability for the SSA

For our assessment of viability, we relied on the SSA framework's core conceptual model for resiliency to describe the current and future viability of the eastern and western subspecies of regal fritillary, in terms of all 3Rs (Service 2016, p. 10; Smith *et al.* 2018, entire) (Figure 2). This conceptual model illustrates the relationship between habitat factors that are important to individuals, demographic factors that are important to populations, the resiliency of these populations, and the redundancy and representation at the subspecies level. As described in more detail below, for this SSA, we refer to populations of regal fritillaries in terms of analytical units (AUs) as surrogates for populations. Habitat factors are those resources needed by individual regal fritillaries to breed, feed, and shelter in order to survive from one stage in its life cycle to the next and allow successful dispersal of some individuals. Demographic factors include abundance and trends that AUs need to be resilient to withstand stochastic events. In general, the subspecies need a certain number and distribution of resilient populations in order to withstand catastrophes (redundancy) and diversity to adapt to novel, environmental change (representation).

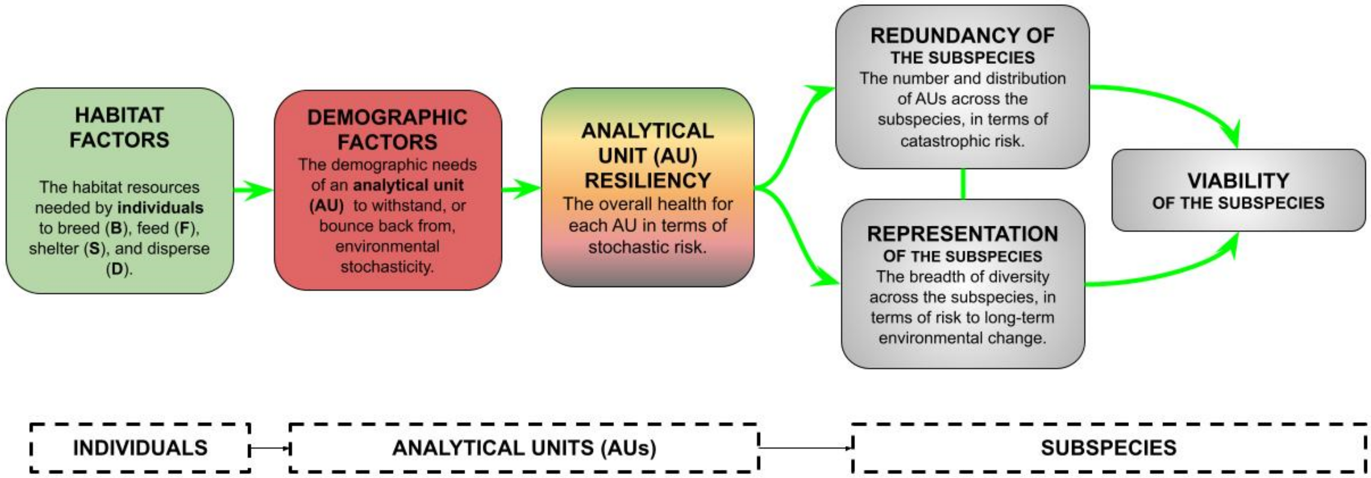

Figure 2. Core conceptual model used for our analysis of viability for the eastern and western subspecies of regal fritillary in terms of the 3Rs of conservation biology: resiliency, redundancy, and representation. Throughout this report, habitat factors are illustrated in green and demographic factors in red. Model based on the core conceptual model for the species status assessment (SSA) framework (Service 2016, p. 10). Throughout our assessment, analytical units (AUs) are surrogates for populations and are the scale at which we evaluated the 3Rs.

Analytical Units (AUs) to Evaluate the 3Rs

According to the SSA framework, at the population level, we describe the resources, circumstances, and demographics that most influence the resiliency of a population. These may vary if populations are distributed across different ecological settings. Species viability corresponds to the resiliency of its populations, and therefore, it is necessary to understand and determine for the analysis how populations should be defined for the subject entity of the SSA analysis. For some species or subspecies, identifying population structures or other delineations may be helpful and necessary in order to evaluate resiliency (Service 2016, p. 12).

For the regal fritillary, it is difficult to define the boundaries of their populations given their high mobility and boom-bust population cycles, with frequent local extirpations followed by recolonizations. Thus, for the purposes of our SSA analysis, we developed a species distribution model (SDM), as described in Appendix D, to help delineate analytical units (AUs) as surrogates for populations of the eastern and western subspecies. These AUs are based on the EPA's Level III Ecoregions (EPA 2013, entire), with additional refinement as described in Appendix E, and are shown in Figure 3. In brief, Ecoregions are ecosystems with areas of similarities among: type, quality and quantity of biotic, abiotic, terrestrial and aquatic resources, identified via patterns and composition observed in geology, landforms, soils, vegetation, climate, land use, wildlife and hydrology. They are appropriate AUs for the regal fritillary based on their alignment with known conditions on the landscape and known or potential occupation by regal fritillaries. Further, we selected these ecoregions as an appropriate scale at which to measure resiliency for both subspecies because they:

- Capture similarities within habitats at which individuals could move, travel, and disperse within a metapopulation structure;

- Are an appropriate scale to capture an approximately 160-kilometer (km) (100-mile (mi)) dispersal distance of individual adults (P. Hammond, personal communication, 2021);
- Encompass the large, intact landscapes needed by the species;
- Encompass potential refugia areas (shown by the next finer ecoregion level, Level IV) within them while including adjacent areas for dispersals; and
- Largely coincide with agriculturally dominated areas (unsuitable sites for the species) as well as suitable habitats that are less likely to be converted based on physical features, which is helpful in analyzing both current and future conditions.

To improve our analysis, we modified the boundaries for several of the AUs, largely those at the southern periphery of the overall range and the Ridge and Valley AU in Pennsylvania, so that they more accurately reflect the current or projected distribution of the subspecies. Appendix E explains these modifications in greater detail.

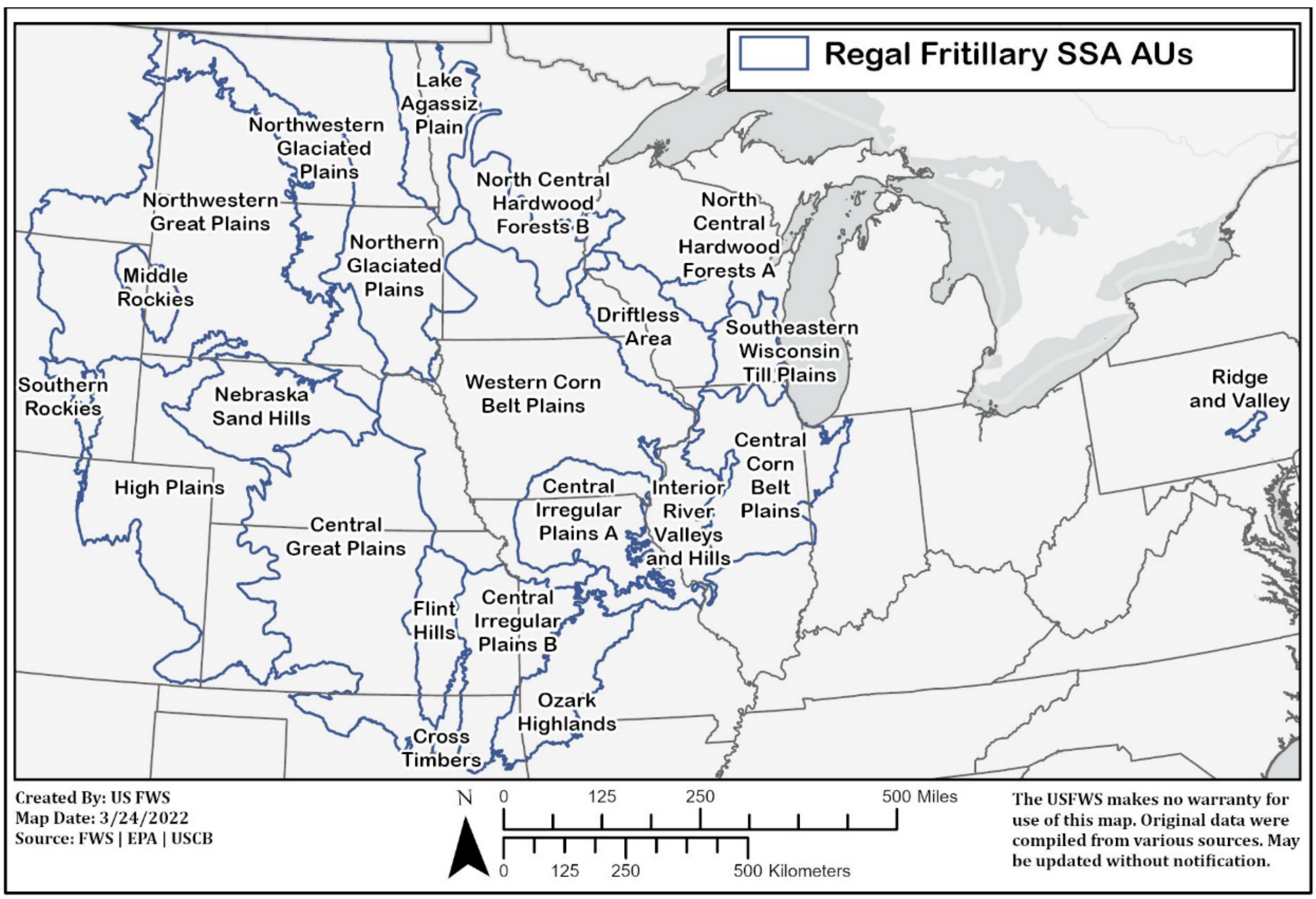

Figure 3. Map of regal fritillary analytical units (AUs), based on modified EPA Level III Ecoregions (EPA 2013, entire), listed in Table 1, with additional detail regarding their development in Appendix E.

For this SSA, we evaluated 22 AUs distributed across portions of 16 states. We note that despite historical records and recent observations (D. Debinski, personal communication, 2023), Montana is currently considered to be unoccupied by the western subspecies. The eastern subspecies has one AU (Ridge and Valley) in Pennsylvania, and the western subspecies has 21

AUs. The AUs are distributed across four representation units, which capture ecological and genetic diversity across the subspecies (Table 1). We discuss these representative units in more detail below, with additional detail in Appendix E.

Table 1. The 22 AUs and 4 representation units for the regal fritillary. The eastern subspecies has one AU in one representative unit and the western subspecies has 21 AUs across three representative units.

SUBSPECIES	REPRESENTATION UNIT NAME	ANALYTICAL UNIT (AU) NAME
EASTERN	EAST	Ridge and Valley
WESTERN	MIDWEST	Central Corn Belt Plains
		Central Irregular Plains - A
		Driftless Area
		Interior River Valleys and Hills
		North Central Hardwood Forests - A
		North Central Hardwood Forests - B
		Southeastern Wisconsin Till Plains
		Western Corn Belt Plains
	NORTHERN GREAT PLAINS	Lake Agassiz Plain
		Middle Rockies
		Northern Glaciated Plains
		Northwestern Glaciated Plains
		Northwestern Great Plains
	CENTRAL GREAT PLAINS	Central Great Plains
		Central Irregular Plains - B
		Cross Timbers
		Flint Hills
		High Plains
		Nebraska Sand Hills
		Ozark Highlands
		Southern Rockies

Representation Units

To evaluate representation, we identified 4 representation units across the 22 AUs. These four representation units are the:

- **East**: A single AU and one population composed of several colonies on FTIG military base in Pennsylvania;
- **Midwest:** Eight AUs across primarily northern Illinois, northeastern Indiana, Iowa, southern Minnesota, northern Missouri, and southern Wisconsin;
- **Northern Great Plains:** Five AUs across primarily eastern Montana (currently unoccupied), North Dakota, South Dakota, and northeastern Wyoming;
- **Central Great Plains:** Eight AUs across primarily northeastern Arkansas, northeastern Colorado, Kansas, southwestern Missouri, Nebraska, northeastern Oklahoma, and southeastern Wyoming.

The eastern subspecies occurs only in the East representation unit; the western subspecies occupies the Midwest, Northern Great Plains, and Central Great Plains representation units (Figure 4). In the list above we highlight the portions of the states within each unit as a general locality reference. Also, although only historical records and recent observations (D. Debinski, personal communication, 2023) of regal fritillary exist in Montana, but occupied ecoregions extend into the State, and potential exists for subspecies occurrence. Thus, the AU boundaries in Montana were trimmed, but not excluded. We briefly describe these representation units below.

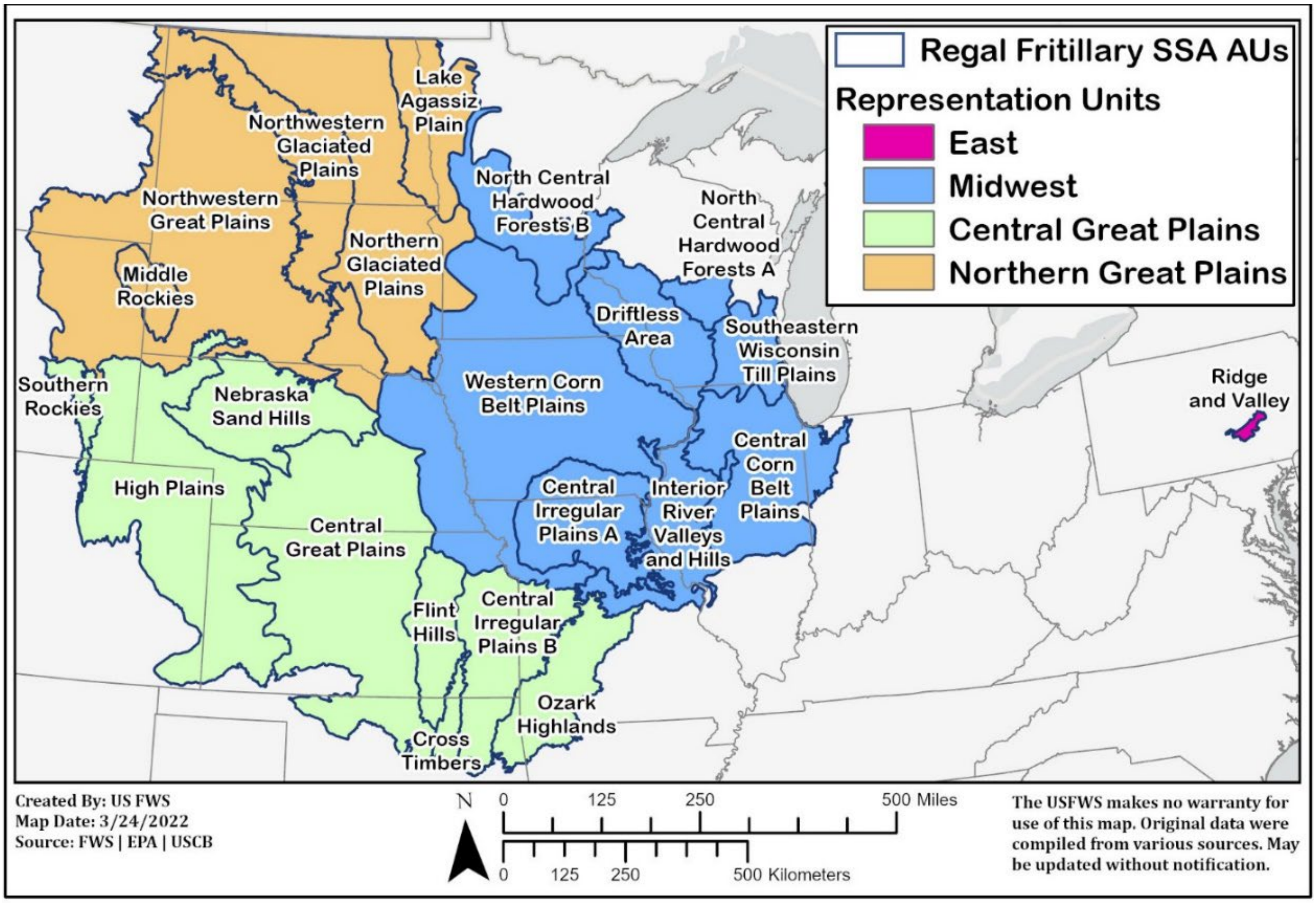

Figure 4. Regal fritillary representation units (as named in the legend) and the 22 AUs (labeled on the map) within them.

East Representation Unit

The East representation unit is composed of a portion of the EPA Level III Ridge and Valley Ecoregion. This is the only AU known to be occupied by the regal fritillary east of the state of Indiana. Based on occurrence data and information from species experts, the regal fritillary only occurs in grassland sites within FTIG (Figure 5). As a result, we modified the boundary of this AU slightly, as described in Appendix E. The FTIG population of regal fritillaries in the East unit exhibits distinct haplotypes that are not present in any other known extant regal fritillary population (Williams et al. 2001b, p. 146). The nearest population of the western subspecies is about 869 km (540 mi) to the west of the eastern subspecies population; thus, the potential for natural genetic exchange between the two subspecies is currently highly unlikely.

Figure 5. Regal fritillary habitat (foreground) at Fort Indiantown Gap National Guard Training Center (FTIG), Pennsylvania. Photo credit: FTIG Wildlife Staff.

Midwest Representation Unit

The Midwest representation unit is composed of the following eight AUs: North Central Hardwood Forests, Driftless Area, Western Corn Belt Plains, Southeastern Wisconsin Till Plains, Central Corn Belt Plains, Central Irregular Plains, and Interior River Valleys and Hills AUs. Much of this area was historically dominated by vast tallgrass prairies but today is an agriculturally dominated landscape with prairie remnants existing primarily as small, isolated patches, many of which are under protective conservation status. There are relatively large, restored tallgrass prairies in the Midwest, including the Neal Smith National Wildlife Refuge in Iowa and the Nachusa Grasslands in Illinois.

Native grasslands historically and currently occupied by regal fritillaries in much of the Midwest have been described as "wet tallgrass prairie", particularly in the eastern portions of this region (e.g., northern Indiana, Illinois, eastern Iowa, southern Michigan and Wisconsin, and northeastern Missouri), with regular summer rainfall that favors many mesic-adapted plants and insects (Hammond 1995, p. 3). As noted previously, mesic areas are identified as important habitats for the regal fritillary, but relatively drier sites are also noted as suitable. Dry upland prairie hillsides, dry sand prairie, wetland complexes associated with river terraces, marsh areas along stream margins, regrown old fields that had been previously drained and plowed, mixed-grass and tallgrass prairies, high-quality tallgrass prairie, and prairie meadows are some of the terms used to describe regal fritillary habitats in this region (Figure 6) (Selby 2007, p. 27). Areas occupied in the Midwest today are nearly always identified as small, isolated native prairie remnants; tallgrass prairie ecosystems have been converted almost completely to agriculture. Large size of habitats and connectivity among them are generally lacking in the Midwest representation unit.

Figure 6. Midwest regal fritillary habitat at Nachusa Grasslands (The Nature Conservancy) in Illinois. Photo Credit: Wayne Schennum

Northern Great Plains Representation Unit

The Northern Great Plains representation unit is composed of the Northwestern Great Plains, Northwestern Glaciated Plains, Northern Glaciated Plains, Lake Agassiz Plains, and Middle Rockies AUs. The majority of these are grassland dominated ecoregions, with exception of the Middle Rockies (generally known as the Black Hills of South Dakota), which is a forest-dominated landscape, but contains grasslands and supports regal fritillary populations. Habitats occupied by the regal fritillary in the Northern Great Plains representation unit, particularly North and South Dakota, which make up the bulk of this unit, are described as virgin prairie (Royer and Marrone 1992, p. 4). In South Dakota (Figure 7), habitats are tallgrass prairie near marshes in the northeast, and undisturbed mixed-grass prairie along such areas as the Missouri River breaks and Fort Pierre National Grassland (Marrone 2002, p. 210).

Figure 7. Tallgrass prairie/marsh habitat in South Dakota. Photo Credit: Mick Zerr.

The tallgrasses in the Northern Great Plains are relatively drier than Midwest tallgrass habitats and become progressively drier in the mixed-grass and short grass prairies further west within this representation unit.

The western side of the unit also typically includes relatively large intact grasslands as rangeland becomes more prevalent than row-crops. Along the western boundary of the species' range, shortgrass prairies dominate and are among the driest habitats occupied by the species. While regal fritillary observations occur there, these areas seem to represent marginal sites that do not support large or persistent regal fritillary populations (Selby 2007, pp. 23, 24). There are very few records in Wyoming and adults may not be observed until late in the season (August) (see Crawford and Tronstad 2020). Similarly, many records in northeastern Colorado may be of wandering adults, not necessarily indicative of breeding populations in the state (Selby 2007, p. 24); these (in addition to a lone historical Montana observation) are often considered "strays" (Selby 2007, pp. 10, 23–24). Recent observations in the State of Montana (D. Debinski, personal communication, 2023) may suggest that there are more individuals in Montana than just strays, but the State is currently considered unoccupied. During drought in the Great Plains, both violets and nectar sources may become very scarce, particularly in shortgrass areas, but during relatively wet years, these resources become more available again, and some adults may disperse westward into shortgrass habitats (P. Hammond, personal communication, 2021). Mesic habitats within such drier sites (e.g., wetlands or sub-irrigated meadows; Selby 2007, p. 27) and riparian areas (Figure 8) maintain populations during drought, providing required nectar sources and violets (Kral-Obrien et al. 2019, p. 306).

Figure 8. Riparian regal fritillary habitat in Wyoming. Photo credit: Katrina Cook.

Much of the eastern side of the Northern Great Plains has been converted to agriculture, with some pockets of prairie remaining in areas not typically suitable for cropping due to topography or rocky terrain. However, the level of conversion is not currently known to result in genetic population structure for regal fritillaries in the Great Plains as has been noted in the Midwest and East due to fragmentation and isolation (Williams et al. 2003, pp. 16–17).

A primary factor setting the Northern Great Plains apart from the Central Great Plains is climate. The northern limits of the species range are in the Northern Great Plains, and the species' presence may be dictated by temperature or moisture-related requirements (not completely understood), and a short growing season. The regal fritillary's phenology in this part of the range is delayed in comparison with southern areas by up to a month. Temperatures in this portion of the Great Plains can be extremely cold in the winter and often include season-long snow cover not observed in much of the Central Great Plains representation unit. Larval emergence from diapause may occur as late as May, with adult emergence from the chrysalis occurring in late June to mid-late July and fall freezing conditions occurring earlier than in the Central Great Plains.

Central Great Plains Representation Unit

The Central Great Plains representation unit is composed of the Southern Rockies, High Plains, Nebraska Sandhills, Central Great Plains, Flint Hills, Cross Timbers, Central Irregular Plains, and Ozark Highlands AUs. Like the Northern Great Plains, the majority of AUs here are grassland dominated ecoregions, primarily mixed-grass prairie, with shortgrass becoming more dominant along its western edge, and some tallgrass areas in the eastern portion of the unit.

Regal fritillary habitats in the Central Great Plains are described as non-degraded mixed-grass prairie, wet meadows, sub-irrigated meadows associated with stream drainages, non-degraded prairie near marshes, (possibly) moist areas associated with irrigation projects, foothills zone mixed-grass prairie, northern sandhill prairies and tallgrass prairie with well-drained soils and facultative upland plants (Selby 2007, p. 27–28; Caven et al. 2017, p. 198) (Figure 9).

Figure 9. Regal fritillary habitat at the Niobrara Prairie Preserve in Nebraska. Photo credit: Chris Helzer, The Nature Conservancy.

As in the Northern Great Plains, the shortgrass prairies on the western edge of the Central Great Plains are among the driest habitats occupied by the species, representing marginal sites that may not support large or persistent regal fritillary populations. In such areas, groundwater-fed streams or springs may provide adequate moisture for violets and nectar sources to support some regal fritillaries, but much of the shortgrass is not suitable, as it lacks required resources. Colorado records in the Rocky Mountain foothills represent the westernmost portion of the regal fritillary's range, and records are relatively few there. Western occurrences today are thought to be occupied opportunistically by dispersing individuals observed late in the flight season (P. Opler, personal communication, 2021; Fritz 1997 *in* Selby 2007, p. 27; Crawford and Tronstad 2020, p. 6). Drought in the Central Great Plains, as in the Northern Great Plains, can significantly reduce violet and nectar sources, and in turn, regal fritillary populations.

Also similar to the Northern Great Plains, much of the Central Great Plains has been converted to agriculture, particularly in the eastern portions of this unit. However, there are some areas, such as the Flint Hills in Kansas, that exhibit considerably large, connected patches of tallgrass prairie. As noted above, the Northern Great Plains and the Central Great Plains are currently considered a single population from a genetic standpoint, as fragmentation and isolation are not currently at a level resulting in genetic population structure (Williams et al. 2003, p. 14).

As described in the previous section, a primary factor in decoupling the Central Great Plains from the Northern Great Plains is the overall climate of each. The environmental conditions can be significantly different in each representation unit. Overwintering conditions (warmer, less or no snow cover, shorter season length), spring conditions (warmer, earlier onset), summer conditions (warmer, earlier onset, longer season length), and fall conditions (warmer, longer season length) have the potential to affect the regal fritillary differently than in northern areas of the Great Plains. Regal fritillaries may emerge from winter diapause as early as March in this representation unit, and male butterflies can be observed in May (as much as a month prior to observations of adults in the Northern Great Plains).

Analytical Methodology for this SSA

We followed the three-stage SSA framework for our biological risk assessment of the eastern and western subspecies of regal fritillary. The three stages of the analysis are identifying the needs at the individual, population (AU), and subspecies levels, then evaluating the current condition and future condition of those needs (Service 2016, p. 6; Smith *et al.* 2018, entire). Specifically, the SSA-framework begins with an assessment of the subspecies needs, followed by an assessment of the current condition of those needs, considering positive and negative factors that influence resiliency, and ending with an evaluation of the projected future condition of those same needs (Service 2016, p. 6). Throughout our analysis for this SSA, AUs are synonymous with populations and are the scale at which we measured the 3Rs.

Figure 10 shows the complete conceptual model for our SSA analysis of the eastern and western subspecies, broken into the three phases of the SSA's framework, ultimately characterizing viability in terms of the 3Rs. As summarized in this report, we first reviewed the life history, ecology, historical and current range and distribution, life stages, and life cycle for the regal fritillary (Chapter 2). Next, based on our review of the life history and ecology, we identified the habitat factors needed by individuals, the demographic factors needed for an AU, and the redundancy and representation needed by the subspecies (Chapter 3). Then we evaluated stressors and conservation actions that affect resiliency, either positively or negatively, by directly influencing demographic factors and indirectly by influencing habitat factors (Chapter 4). We then evaluated the current condition for each of these habitat and demographic needs for the 22 extant AUs, and then summarized current condition for the subspecies in terms of the 3Rs (Chapter 5). Finally, we developed future scenarios to capture that range of uncertainty regarding future stressors and repeated the evaluation of condition for all 22 AUs, under each future scenario, using the same methodology that we used to evaluate current condition (Chapter 6). We then summarized the change in conditions from current to future to summarize changes in the 3Rs to describe viability and risk to the subspecies (Chapter 7).

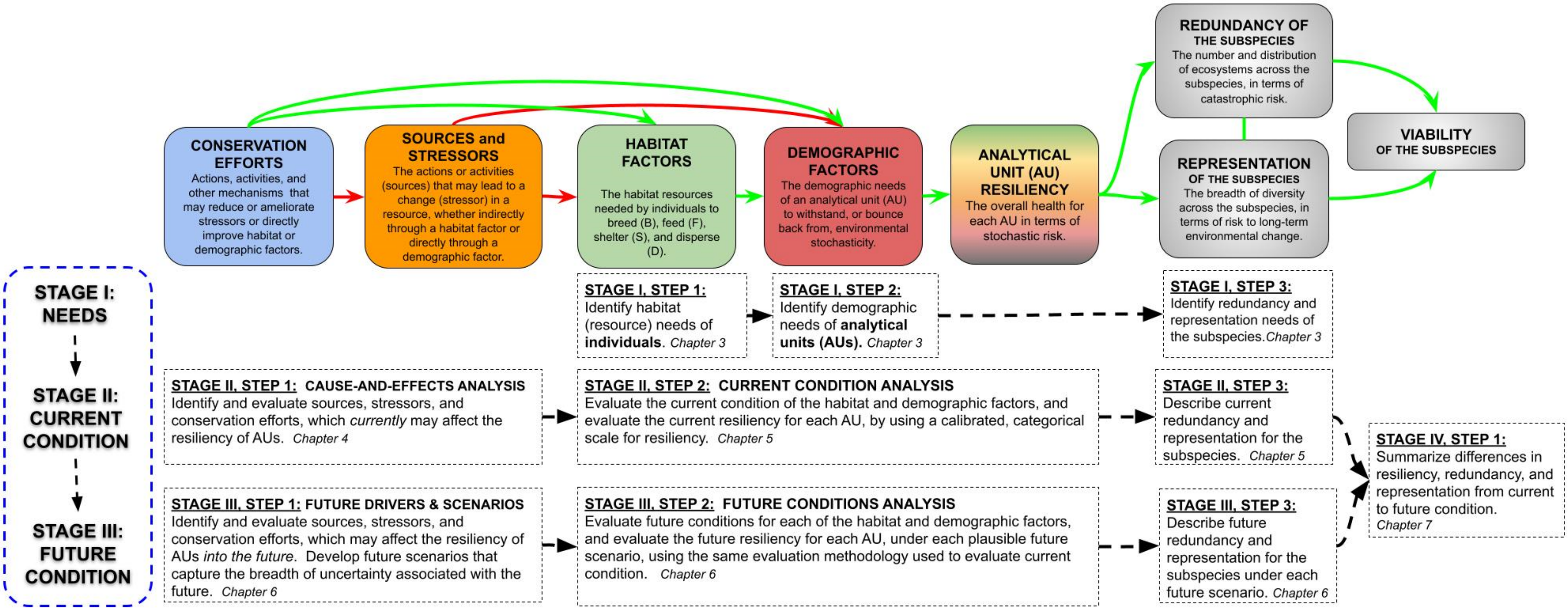

Figure 10. A conceptual model of our analytical framework for the SSA that we used to evaluate current and future condition for the eastern and western subspecies of regal fritillary. The three-stage SSA framework (species needs, current condition, and future condition) and the core conceptual model for viability guided our analysis (Service 2016, p. 6; Smith et al. 2018, entire). Green arrows represent positive relationships between nodes and red arrows represent negative relationships between nodes. Dashed boxes and arrows represent the steps of our analysis. Throughout our SSA, AUs are synonymous with populations. We note that the core conceptual model's inclusion of habitat factors, which indirectly influence resiliency, and demographic factors, which directly influence resiliency, facilitates a broad, inclusive, and cumulative evaluation of AU resiliency starting with individuals, and considers the conservation efforts, sources, and stressors that may directly and indirectly influence them.

Summary of Major Uncertainties and Assumptions

Although the regal fritillary is a large, charismatic butterfly that has received considerable attention by lepidopterists, many factors related to the species' habitat and population dynamics may be unknown, locally and rangewide. For example, the number of current extant regal fritillary populations cannot be determined with accuracy; extirpations and recolonizations occur annually, and there are no systematic surveys throughout the species' range. As a result of the inability to quantify many habitat and demographic needs and factors affecting the subspecies, we used surrogates or other sources of the best available scientific information to help inform our SSA analyses. Given the lack of consistent, rangewide demographic monitoring, we relied on expert opinions to provide the best available information. Below, we summarize the major uncertainties and assumptions that we encountered during our analysis:

- Local Occupancy may be unknown or difficult to project based on available habitats. The presence of suitable habitat does not necessarily equate to occupancy by regal fritillaries, as other factors, which may be unknown, may influence local occupancy. Occupancy can change quickly within and between years.

- Fluctuations in regal fritillary population abundance cannot be predicted with accuracy. Population dynamics are observable, based on adult presence, yet are difficult to evaluate, measure, and predict. One exception is drought; a year lag time typically reveals a drop in numbers.

- Exact mechanisms of population fluctuations are often undetermined. Some conditions, such as drought or wildfires, may cause population contractions, but many times, conditions that influence abundance are not well known. Potential factors include: resource competition, pesticide impacts, overwintering conditions, and disease. Seemingly suitable habitats may or may not be occupied, perhaps due to these or other unknown factors.

- Many demographics cannot be quantitatively determined. The species is generally known to be highly fecund, and adults are highly mobile, but metrics of wild populations such as survival rates, emigration and immigration rates, and hatching success are not known. Captive rearing information is available, but it is not clear if the information is precisely applicable to wild populations. Early life stages in particular are small and cryptic and highly difficult to find, quantify, and monitor. Demographics are also variable among years and between sites.

- Availability of individual needs cannot be determined or predicted with accuracy. Factors including violets, nectar sources, bunchgrasses, litter, and shrubby/tall vegetation are necessary for regal fritillary presence; however, these are highly variable habitat factors that shift with local climatic conditions and/or disturbance regimes among sites within and between years. The amount and availability of these resources cannot be accurately obtained on a rangewide scale.

- Native prairie is a surrogate for individual needs. If native prairie exists and has an appropriate disturbance regime, individual resource needs are presumed to be present, but this may not be the case in all areas. For example, aerial application of herbicides to control, minimize, or eliminate weeds to enhance forage or hay quality is an ongoing practice in some areas that eliminates forbs, making these sites unsuitable for regal fritillaries.

- National Land Cover Database (NLCD) Grassland/Herbaceous landcover is a surrogate for native prairie. The western subspecies in particular is considered to be a native grassland specialist, but native prairie has not yet been digitized throughout the range; NLCD (Dewitz 2021, entire) data is currently the best available information rangewide, but this data does not discriminate precise vegetative cover (i.e., whether grasslands are dominated by native species versus invasive species or are replanted grasslands that may not contain necessary resources) and overestimates actual suitable habitat for the species.

- Distance thresholds for connectivity among habitat patches are not well defined. The minimum distance/connectivity needed among patches to facilitate persistence of healthy populations is not precisely known. Not all adults disperse. Conversely, some adults can roam far beyond study area boundaries, limiting researchers' ability to collect specific parameters of dispersal capabilities. Selection against dispersal exists with severe fragmentation in a hostile matrix of agriculture. For the purposes of this SSA, 3.1 mi. (5 km) is considered a distance that at least some regal fritillaries are able and likely to disperse to nearby suitable habitats on an annual basis.

- Minimum patch/population size is not well defined. Suggested estimates are provided in the literature, however, numerous synergistic effects related to habitat loss, as well as the lack of distance thresholds for connectivity mentioned above, render this a difficult parameter to accurately define. For the purposes of this SSA, 100-hectare (ha), or 247-acre (ac.) patches may be considered a minimum size to support small populations, while 1000 ha (2471 ac.) or more is the size identified as supporting healthy, large populations.

To help reduce uncertainty, we reached out to scientific experts on the subspecies, their habitats, and stressors. The experts helped provide the qualitative assessments of the current conditions for the habitat and demographic factors, for each AU. Based on expert feedback, we continued to gather quantitative data to measure the current conditions, replacing the experts' qualitative ranking when possible and retaining expert qualitative rankings, as needed, if other data or information were not available. The experts also helped review our assessment of current and future conditions as we developed the analysis.

Chapter 2 – Description, Ecology, Distribution, and Trends

In this chapter, we describe the regal fritillary, its taxonomy, life history, and historical and current distribution and trends. Throughout this chapter, unless specified otherwise, information presented for the species applies to both the eastern and western subspecies. The review provides scientific background on important aspects of the regal fritillary's life history and ecology in advance of our identification of ecological needs at the individual, population (analytical unit, or AU), and subspecies levels in Chapter 3. It also provides background on distribution and trends in advance of our current condition analysis in Chapter 4. This chapter is not meant to be a comprehensive compilation of all information known about the species, and instead summarizes important aspects of its biology to inform our viability analyses. We provide additional detail regarding the taxonomy of the species in Appendix B and its life stages in Appendix C.

Species Description

The regal fritillary is a large, non-migratory butterfly with dorsal orange forewings and dark hindwings that feature black bars, fine white markings, and two rows of large spots at the base (Figure 11). The contrast of predominantly orange forewings and dark hindwings is a useful aid in field identification as it is easily observable during flight, distinguishing the regal fritillary from other large butterfly species. Adults are similar in size to the monarch butterfly (*Danaus plexippus*), with wingspans ranging from approximately 6.8 to 10.5 centimeters (cm) (2.67 to 4.13 inches (in)) (Selby 2007, p.14). Females are slightly larger than males (Royer and Marrone 1992, p. 3) and the sexes are distinguished by the two rows of large spots on the hindwings (Figure 12). Adult females are often more reddish orange than males. Larger adult specimens generally occur in more southern parts of the overall range, presumably due to warmer and longer spring and summer seasons that allow for better development (P. Hammond, personal communication, 2021). Body size may also be related to the size of available habitats (Öckinger et al. 2010, entire; König and Krauss 2019, entire)

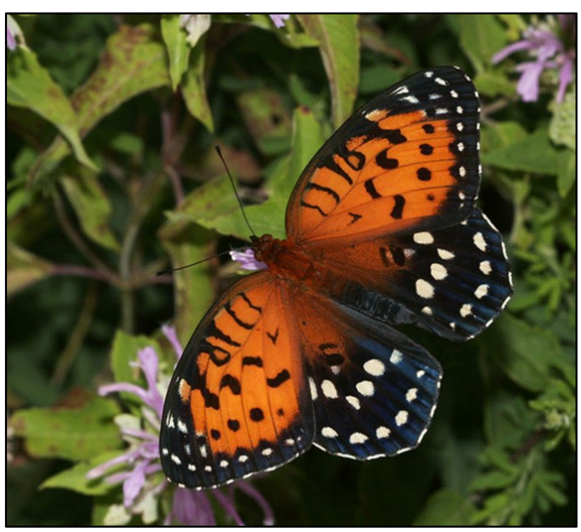

Figure 11. An adult female regal fritillary.

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Figure 12. Adult female regal fritillary (left) from Nebraska, and adult male (right) from North Dakota. Females are slightly larger than males. Coloration and pattern are mostly similar between females and males, but they are easily distinguished by the two hindwing rows of large spots: on females, both bands of spots are white (left photo); on males the peripheral band is orange (right photo). Photo credit: Jim P. Brock.

Taxonomy

The regal fritillary is a member of the brush-footed, or four-footed family of butterflies, a group named for their small, often hairy forelegs resembling brushes, which are not used for walking and may be entirely sensory (Royer and Marrone 1992, p. 22). All New World species of *Argynnis* (formerly *Speyeria*) occur in North America from central Mexico to central Canada, and the group includes 16 species (Dunford 2009, p. 1; Pelham 2021, entire; Pelham 2023, entire). Table 2 summarizes the current taxonomic nomenclature of the regal fritillary and its two subspecies.

Table 2. Regal fritillary taxonomy.

REGAL FRITILLARY TAXONOMY	
Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta (insects)
Order:	Lepidoptera (butterflies and moths)
Superfamily:	Papilionoidea (true Butterflies)
Family:	Nymphalidae (brush-footed butterflies)
Subfamily:	Heliconiinae (heliconians and fritillaries)
Tribe:	Argynnini
Genus:	*Argynnis*
Subgenus:	*Speyeria*
Species:	*idalia*
Subspecies:	*idalia* (Drury 1793) (eastern) *occidentalis* (Williams 2001b) (western)

Appendix B provides additional detail regarding the taxonomy of the species and the scientific support and recognition for the eastern and western subspecies. The best available scientific information indicates that there are two valid subspecies of regal fritillary: *A. i. occidentalis*, the western subspecies, and *A. i. idalia*, the eastern subspecies. Currently, FTIG in Pennsylvania supports the only known remaining population of the eastern subspecies, which is isolated from the nearest known extant occurrence of the western subspecies by approximately 869 km (540 mi). The two subspecies, which effectively comprise the entire species, are the subjects of our SSA analysis and this SSA report.

Life Stages and Life Cycle

The regal fritillary exhibits four life stages, which are typical of all butterfly species:

1. Eggs;
2. Larvae;
3. Pupae; and
4. Adults.

The regal fritillary's life cycle across these four life stages is illustrated in Figure 13. The life cycle diagram also highlights important resource needs for the life stages. For example, larvae (caterpillars), the longest of the life stages, feed exclusively on the leaves of violets (*Viola* spp.). Adults feed on a variety of nectar sources. We summarize life history characteristics for each of these life stages below, with additional detail provided in Appendix C. Additionally, Table 3 summarizes the annual phenology of the regal fritillary's four life stages.

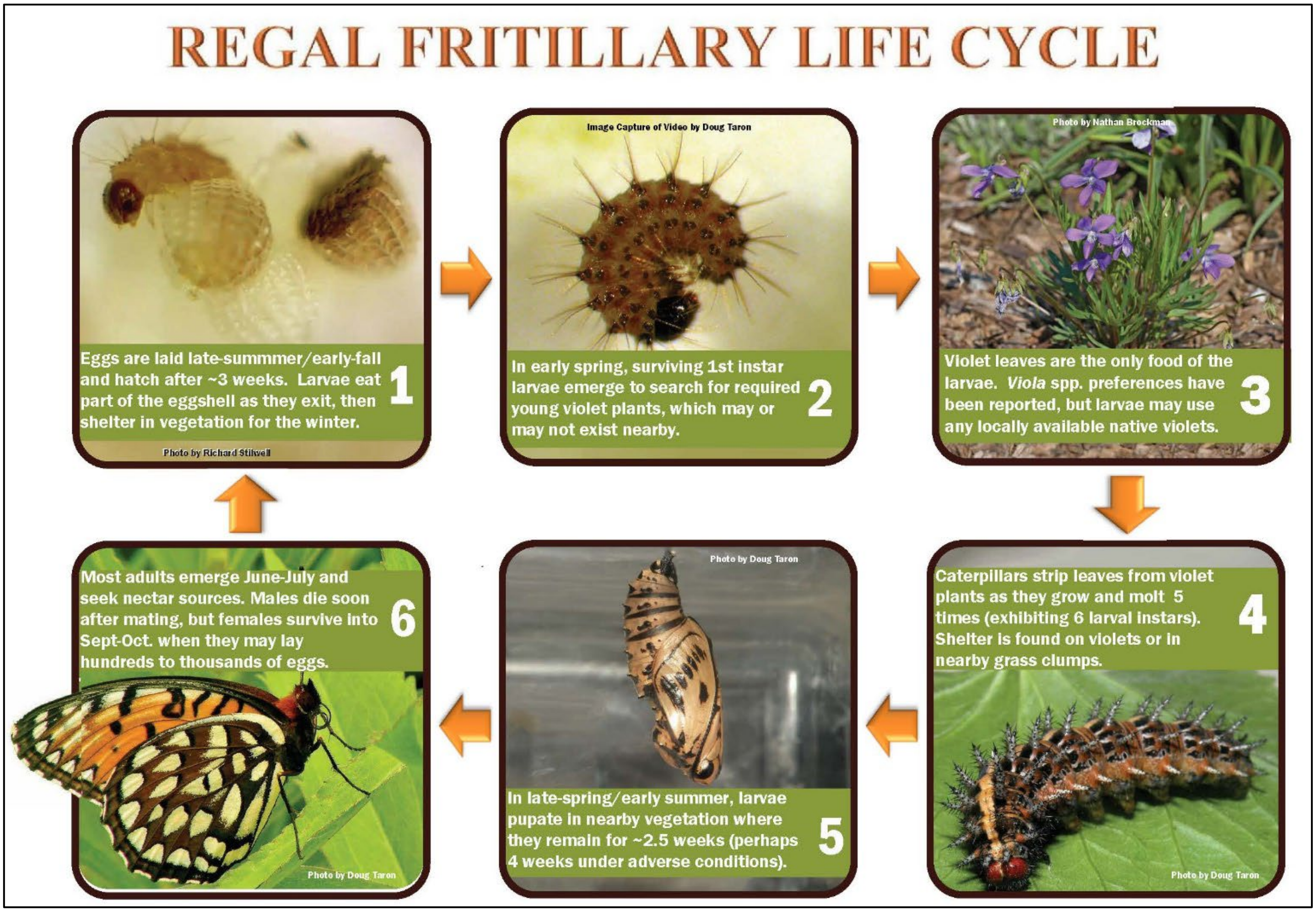

Figure 13. Regal fritillary life cycle and general ecology. First instar hatch in the fall, consume their eggshells, undergo winter diapause in the grasslands, and emerge in the spring to search for violets required for survival and growth. Last instar larvae must consume the foliage of numerous violet plants in order to complete development, and may move considerable distances in search of food depending on the density and spacing of individual violet plants within a habitat area. Mature larvae pupate in vegetation near the ground, emerge as adults, and while most males die soon after mating, the females undergo reproductive diapause, aestivating until egg-development and laying begins in late summer/early fall. Most of the critical nutrition (sugars, amino acids and small proteins) needed for successful egg production is acquired from flower nectar at this time. Senesced violets provide olfactory stimuli that induces oviposition near where the plants will grow the following spring. Diagram originally developed by Reiman Gardens, Ames, Iowa; modifications made by the Service with permission.

Table 3. Regal fritillary life stage phenology. Timeframes are approximate and encompass dates from northern and southern parts of the species' range. Phenology varies annually and by locality, as factors such as local weather, available resources, and latitude affect both the initiation of each life stage and the length of time spent within each stage. The first larval instar (life stage after hatching but before undergoing a molt) is distinguished due to its relatively long length compared to subsequent instars (life stages between molts).

REGAL FRITILLARY PHENOLOGY

LIFE STAGE (duration)	MONTHS OF OCCURRENCE[1]											
	J	F	M	A	M	J	J	A	S	O	N	D
Egg[2] (about 3 weeks)								■ (2nd ½)	■	■		
Larva: 1st Instar (6-7 months, diapause)	■	■	■	■	■ (1st ½)				■ (2nd ½)	■	■	■
Larva[3]: 2nd-6th Instars (1.5 - 3 months)				■	■	■						
Pupa[4] (about 2.5 weeks)					■	■	■					
Adult Male Flight[5] (1-3 months)					■ (2nd ½)	■	■	■				
Adult Female Flight[6] (2-4 months)						■	■	■	■	■		

[1] Months of Occurrence are divided to one-half month level and represent the timeframes during which each life stage has been documented or is suspected to occur based on observations of timing of the life stage itself or the timing of previous or subsequent life stages.

[2] While averaging about 3-4 weeks (NatureServe 2021), in a laboratory setting the egg life stage has been reported as short as 10 days (Wagner 1995, p. 2) and as long as 53 days (E. McKinney, personal communication, 2021).

[3] First larval instar may molt after only 4-5 days with remaining instar development lasting about 5-6 weeks longer (laboratory setting; Wagner et al. 1997, p. 270). Alternatively, this stage has been documented to last 23 days before the first molt with the remaining spring development of instars 2-6 extending 11 more weeks (setting unknown; Edwards 1879, p. 217). Females take longer to reach larval maturity than males (Mattoon et al. 1971, p.247).

[4] Females emerge from the chrysalis 1-3 weeks later than males (Mattoon et al. 1971, p. 247; Nagel et al. 1991, p. 149); females spend more time than males in pupal stage (M. Swartz, personal communication, 2020); pupal development may last a month (Wagner et al. 1997, p. 262).

[5] Males may die shortly after mating after living only a few weeks and become rare in August, but few may survive into September.

[6] Females die within about 10 days of completing egg-laying (Wagner et al. 1997, p. 266) and have been documented in the wild to survive 90 days (Barton 1993 *in* Wagner et al. 1997, p. 262), but can survive in captivity months longer (i.e., into December; P. Hammond, personal communication, 2021).

Regal fritillaries begin their life cycle as eggs, which are oviposited (laid) singly by adult females in grassland landscapes, usually in shaded microsites, on the underside of senesced vegetation, on rocks, or on the ground from late August to October (Wagner et al. 1997, pp. 262, 266; Kopper et al. 2000, p. 657; McCullough et al. 2017 p. 149). Females do not typically lay their eggs on live vegetation and some authors have observed this does not necessarily occur on, or near, violets (*Viola* spp.), the larval host plant (Kopper et al. 2000, p. 663). However, recent observations note the dead remains of dried violet stems and leaves provide olfactory stimuli to females that induces oviposition in the general vicinity where violet plants will grow during the following spring (P. Hammond, personal communication, 2023). Eggs may incubate from 10 days to 53 days (Wagner 1995, p. 2; E. McKinney, personal communication, 2021), likely depending on environmental conditions. Hatch rates of eggs in the wild are unknown, but average 64.3 percent in the laboratory (Wagner et al. 1997, p. 269).

As eggs hatch into larvae, the larvae eat the eggshell before overwintering in nearby vegetation. In early spring, surviving larvae emerge and search for young violets, their primary source of food. While some local food preferences by regal fritillary larvae in various parts of the range have been noted, most local native violet species would likely suffice (Royer and Marrone 1992, p. 21). Larval behavior or strategies to find these plants are unknown and the tiny larvae must navigate many obstacles on the grassland floor in their search for violets (Kopper et al. 2000, pp. 661, 663). Larvae may shelter from predators and unfavorable weather in leaf curls of young violets or at the base of violet plants, in the folds of leaf litter, and in warm season grass tussocks (Kopper et al. 2001, p. 96; Ferster and Vulinec 2010, p. 39). The larval stage may end as soon as early May in the southern part of the range (R. Moranz, personal communication, 2020), while late May and into June are more typical timeframes for central parts of the range (Wagner et al. 1997, p. 262) and later still in mid to late July in northern, cooler regions (Royer and Marrone 1992, p. 25).

From late spring to early summer, the larvae pupate in nearby vegetation for approximately 2.5 weeks or longer if conditions are unfavorable (Wagner et al. 1997, p. 262). Mature larvae pupate in the leaf litter of warm season grasses (Selby 2007, p. 32; Ferster and Vulinec 2010, p. 7), which provides shade, cover, and potentially camouflage, as the tan coloration resembles senesced vegetation. Pupation may occur in early May in southern parts of the species' range, but more often occurs in late May or June, and this stage may extend into July (Edwards 1879, p. 219).

Regal fritillary adults are first observed in June and become more common in July as more adults emerge and numbers of both sexes peak (Wagner et al. 1997, p. 262; Kopper et al. 2001b, p. 174; Caven et al. 2017, p. 188). Regal fritillaries are protandrous, which means that the males mature and emerge as adults earlier than females, by about 2 to 3 weeks (Nagel et al. 1991, p. 149; Wagner et al. 1997, p. 262). Males may appear as early as the end of May in southern areas (Powell et al. 2007, p. 300; R. Moranz, personal communication, 2020; K. McCullough, personal communication, 2020). After emerging from the chrysalis, males tend to stay close to the natal area, near the still-pupating females, which allows for mating to begin almost immediately after adult females appear (Nagel et al. 1991, p. 149). Males spend much of their time "patrolling" in search of females (i.e., flying fast, steady, and close to the grass) (Kopper et al. 2001b, p. 174; Selby 2007, p. 29) and begin to die off in mid-July, becoming relatively scarce in late July and August, although some worn individual males may survive into September (Kopper et al. 2001b, pp. 174–175; K. McCullough, personal communication, 2020).

Some females may disperse after mating or eventually exhibit a summer aestivation (dormancy) period, becoming more reclusive in August, spending their time feeding and sheltering in vegetation clumps as well as small trees and shrubs (Kopper et al. 2001a, p. 428; Kopper et al. 2001b, p. 175; Ferster and Vulinec 2010, p. 40). They become more active in late August, approximately one week prior to oviposition (egg-laying) (Kopper et al. 2001a, pp. 428–429), and some survive into late October before expiring, usually within 10 days after laying their last egg (Wagner et al. 1997, p. 266). The adult flight period typically spans several months from late spring to mid-autumn, varying annually, by location and by sex. The adult stage is relatively long, with some individuals surviving up to 90 days in the wild (Barton 1993 in Wagner et al. 1997, p. 262).

During this period, adults rely on nectar sources for food and may also require moisture and nutrients from soils (Wagner et al. 1997, p 268; Selby 2007, p. 33). Regal fritillaries may sip water and obtain minerals from trails (Schennum 2017, p. 6). Adults feed on a variety of plants and abundant high-quality resources may improve female fecundity and fertility, increasing individual reproductive output (Wagner et al. 1997, p. 266). Females must acquire adequate nutritional sources to survive into September, which is when they lay most of their eggs. Local nectar sources change as seasons progress and greater mobility allows access to these resources.

Additionally, while dispersal during egg-laying has been documented (Schweitzer 1989, p. 135), an initial post-mating dispersal phase has also been observed (Kopper et al. 2001b, p. 174; P. Hammond, personal communication, 2021). As daily temperatures increase (generally approaching 90 degrees), adults appear to fly less and females generally become more sedentary, tending to hide in the shade of clumps of grass or shrubby vegetation during the heat of the day (summer aestivation) (Kopper 2001a, p. 428; Kopper et al. 2001b, p. 174; P. Hammond, personal communication, 2021). Then in late August, stimulated by a rise in hormone levels coinciding with egg development about a week before laying eggs, females disperse again in search of areas to oviposit (Kopper et al. 2001b, pp. 174, 176).

Regal fritillaries are not migratory, but are capable of strong and rapid flight, and individuals may move long distances from breeding colonies (Selby 2007, p. 26). The species is considered “very dispersive”; individuals move widely in their search for nectaring sites (Schweitzer 1989, p. 135). Individuals, particularly long-lived adult females, may be capable of moving more than 161 km (100 mi) (P. Hammond, personal communication, 2021). Despite this documented ability to move significant distances, shorter movements within or between habitat patches may be more normal (e.g., Nagel et al. 1991; p. 148; Ferster and Vulinec 2010, p. 38; Selby 2007, p. 26). Additionally, nectar availability, habitat isolation, and varying levels of permeability of habitat edge types may play a role in dispersal.

Females are longer-lived and known to be more prone to dispersal than males, particularly when ovipositing (Schweitzer 1989, p. 135; Schweitzer 1992a, p. 8). Males may move long distances, perhaps when nectar availability changes or when the habitat patches are expansive with fewer barriers (Schweitzer 1992a, p. 20). Males may also move among suitable habitat patches, often up to 5.5 km (3.4 mi.) between suitable habitat patches (Marschalek 2020, p. 894). Generally, males tend to adhere more to the natal patch, patrolling back and forth to find newly emerged females with which to mate (Nagel et al. 1991, p. 149; Selby 2007, pp. 25–26). Males also do not live as long as females, resulting in less opportunity to move over time. In contrast to the low patrolling flight patterns of males, female regal fritillaries may often be observed flying high and are capable of cruising multiple kilometers a day.

In short, two dispersal periods and longevity of approximately 3 months allows females to move significant distances. This dispersal ability allows for recolonization of sites that may become extirpated when conditions impacting required resources, such as drought that reduces violet or nectar availability. Recolonization can occur immediately if source populations exist adjacent to extirpated sites, or it may take years if populations contract significantly and the species is reduced to survive only in small suitable habitat patches at low densities (P. Hammond, personal communication, 2021).

Reproduction occurs once per year (univoltine). The regal fritillary reproductive strategy is characterized as a "sweepstakes" method (Wagner 1995, p. 3), whereby the female lays a very high number of eggs across the landscape, expending no post-laying maternal effort, resulting in a high number of hatched larvae on the ground to face the poor odds of surviving the winter and finding young violets in the spring. Females exhibit reproductive diapause; while mating occurs early in adult life, egg-development in females is delayed for 6 to 8 weeks, through mid-to-late summer, generally without males present (Kopper et al. 2001a, p. 430). This may be an adaptation to the phenology of the *Viola* host plants; hot summers, sometimes with drought, result in violet senescence in late summer so these plants are not available to larvae (Kopper et al. 2001a, pp. 429–430). Reproductive diapause, combined with larval diapause in winter, results in availability of young violet leaves to young larvae in the spring (Kopper et al. 2001a, p. 431). Delay of oviposition until late summer and early fall can also reduce egg and larval exposure to desiccating heat, parasitoids and predators (Kopper et al. 2001a, p. 431).

Habitat

The regal fritillary is considered an indicator of the health of native prairie (Royer and Marrone 1992, p. 4) and a "specialist" species (Swengel 1996, p. 76). At its most basic level, regal fritillary habitat is composed of grasslands with necessary components of native violets (*Viola* spp.) for larvae to eat and nectar sources for adults. Warm season native bunchgrasses are also important, providing shelter for individuals in all life stages (Ferster and Vulinec 2010, p. 39; Caven et al. 2017, p. 199). Moisture levels are mesic (moderately moist) in the East and more xeric (dry) in the Midwest and Great Plains. The habitat must be relatively non-degraded; the species cannot survive in altered landscapes such as row crops, non-native pastures, or developed areas that surround prairie remnants (Selby 2007, p. 3), and forested habitats are not suitable.

Less than pristine habitats may also support the species. In Wisconsin, habitats such as old fields, once impacted by agriculture but reverted to grasslands containing the basic vegetative components described in the previous paragraph, have supported populations (Swengel 2001, pp. 4–5). In Nebraska, some "degraded" native prairies with considerable thatch buildup and few nectar sources, may contain numerous native violet plants and can produce more regal fritillaries early in the season than more floristically diverse (and recently burned) sites nearby; the diverse floral sites then attract regal fritillary adults to the nectar sources later in the season (Helzer 2012, p. 9). The occurrence of larval food sources in one patch and adult food sources in another is not ideal, particularly if the patches are not adjacent. Females may be lured away from abundant violet patches and lay eggs in sites with more diverse nectar sources, but fewer violets (Helzer 2012, p. 9).

As a result, care must be taken when determining habitat suitability based solely on observations of adults. Regal fritillary adults may be able to move across the landscape in response to changing conditions in order to locate resources, while as larvae, they are generally limited to the area in which they hatched (McCullough et al. 2017, p. 148). The latter is more difficult to detect; few studies document specific details regarding regal fritillary larvae habitats (but see Kopper et al. 2001c; Ferster and Vulinec 2010; McCullough et al. 2017). Williams (1999, pp. 6–7) described an Illinois prairie less than 2.59 square kilometers (1 square mile) in size that supported hundreds of adult regal fritillaries annually but searches for violets revealed only three plants. Immediately after emerging from the chrysalis, adults may linger in the natal area with violets, but later in the season, adults may be more randomly distributed within habitat patches irrespective of violet distribution (Nagel et al. 1991, p. 149). Adults may also be observed in unsuitable habitats (e.g., non-native grasslands or backyard flower gardens that present nectar sources, but they may not contain violets) and they may only be transient in these areas, or alternatively, such sites may act as sinks if females lay eggs there (Kelly and Debinski 1998, p. 274, Swartz et al. 2015, p. 814). Regal fritillary counts can be highly variable, with annual abundance linked to climatic variation, among other factors (Swengel 2004, p. 3) making correlation of regal fritillary populations to violet or nectar availability more challenging to predict.

The eastern and western subspecies share many of the same habitat needs, including violets, warm season bunchgrasses, and nectar sources. However, the eastern subspecies has a few unique habitat conditions and factors that influence the quality and quantity of its habitats. Historically occupied eastern subspecies habitats are marshes, swamp edges, wet meadows, fields, grasslands, heathlands, coastal pasture haylands, and native grasslands (Selby 2007, p. 27). Many of these were described as "unnatural" (some dominated by native grasses, others by exotics), referring to pastures and haylands cleared for agriculture that may have peaked during colonial times and since been lost to reforestation (Wagner et al. 1997, p. 271). The immediately adjacent forests at FTIG represent unsuitable habitats and are likely barriers to dispersal for regal fritillaries there. Forested habitats are much less prevalent (or absent) in habitats occupied by the western subspecies. An evolutionary adaptation by the eastern subspecies to relatively more mesic (than xeric) conditions has been suggested (Williams 1999, p.5), but it is not clear whether this is valid as the species is reported to occupy mesic and xeric habitats within the same geographic area (Mason 2001, p. 20). More specifically, the regal fritillaries at FTIG occur in grasslands in an old field successional stage dominated by broomsedge (*Andropogon virginicus*), little bluestem (*Schizachyrium scoparium*), and a variety of other warmseason and coolseason grasses with healthy populations of arrowleaf violet (*Viola sagittata*) and a variety of nectar sources (Zercher et al. 2002, p. 13).

Open habitats for the eastern subspecies at FTIG were originally and are currently maintained via ongoing military exercises at the military base, while regal fritillary special consideration areas (SCAs) managed specifically for the regal fritillary are maintained with fire, mowing, and tree cutting (Ferster and Vulinec 2010, pp. 39, 40; M. Swartz, personal communication, 2021). The tussocks formed by the warm season bunchgrasses, particularly little bluestem, are important habitat components used by all life stages by the eastern subspecies (Ferster and Vulince 2010, p. 40; Swartz et al. 2015, p. 814). Milkweed and thistles are favored nectar sources at the site; these include common milkweed (*A. syriaca*), butterfly milkweed (*A. tuberosa*), swamp milkweed (*A. incarnata*), pasture thistle (*C. pumilum*), field thistle (*C. discolor*), plus wild bergamot (*M. fistulosa*) (Ferster and Vulinec 2010, pp. 39–40). The primary *Viola* species used is the arrowleaf violet. At FTIG, the *Viola* spp. density averages 1.79 plants per 11 feet squared (ft^2), or per 1 meter squared (m^2), (Ferster and Vulinec 2010, p. 39). Shrubby components of the grasslands used for shelter include young trees (less than 5 ft) such as scrub oak (*Quercus berberidifolia)*, Virginia pine (*Pinus virginiana*), black cherry (*Prunus serotina*); low bushes such as blueberry and huckleberry (*Vaccinium*); some non-natives such as hawthorn (*Crataegus*), honeysuckle (*Lonicera*) and autumn-olive (*Elaeagnus umbellata*); thickets of nectar plants like goldenrod (*Solidago*) are also used (M. Swartz, personal communication, 2021).

Population Structure

Population structure of the regal fritillary includes localized colonies, populations, and metapopulations, which we summarize below and illustrate in Figure 14. The AUs used in this analysis approximate metapopulations, and as described above, our suitable units of analysis for our evaluation of the 3Rs.

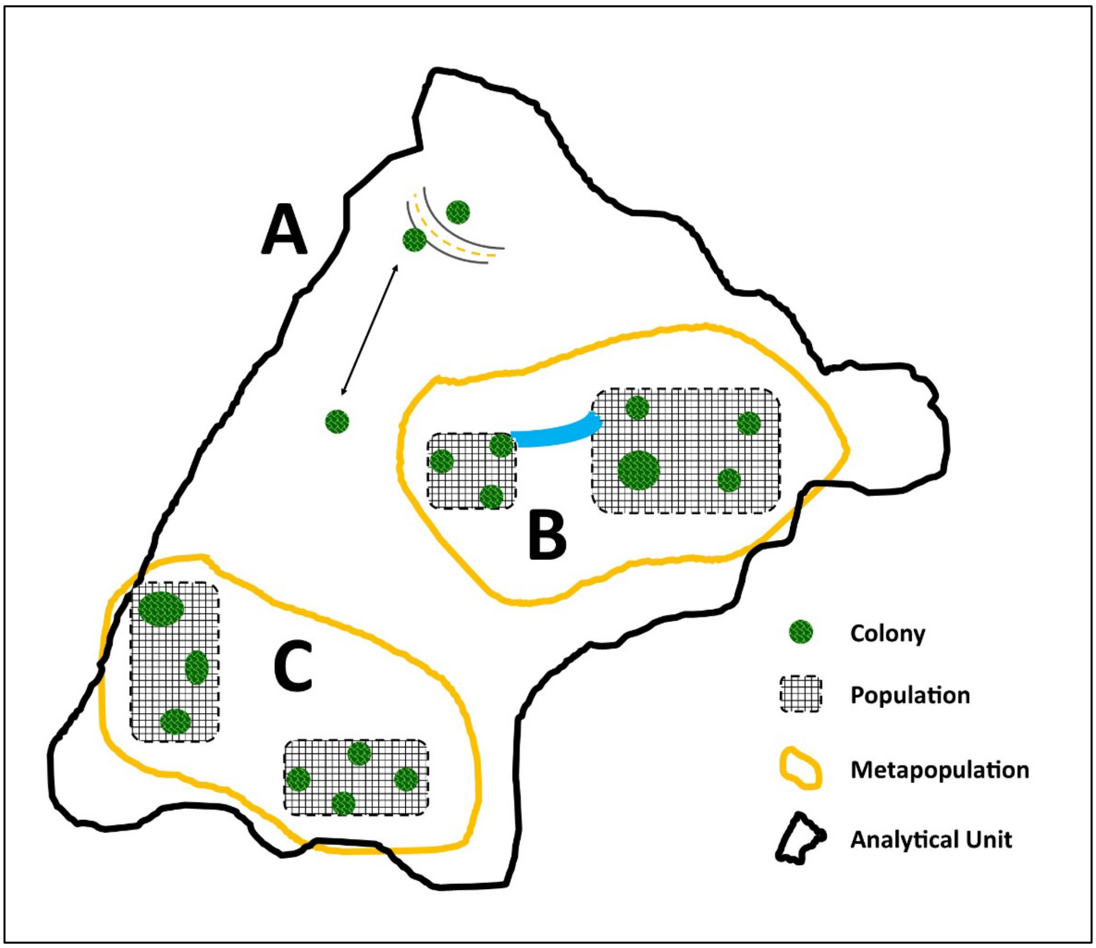

*Figure 14. Population Structure Diagram for the regal fritillary. "**Colony**" is a collection of individuals within a relatively small, occupied area of suitable habitat that may be separated from other occupied areas by unsuitable habitats or barriers that render dispersal or genetic exchange between other suitable habitat patches less likely or unlikely to occur. "**Population**" is (a) an aggregate of three or more colonies in suitable habitats with varying diversity to support regal fritillary life-history needs over time that are located in proximity to each other (e.g. 5-8 km (3-5 mi)) with an adequately permeable surrounding matrix and/or corridors facilitating inter-patch movements, or (b) occupied relatively large, contiguous suitable habitat patch with adequate heterogeneity supporting individuals, either throughout the area or as connected colonies within, separated from the nearest other occupied area by perhaps 16-24 km (10-15 mi) making inter-population exchanges less likely. "**Metapopulation**" is a set (three or more) of regal fritillary populations, separated by perhaps 32-160 km (20-100 mi) linked by infrequent dispersal by adults, spread*

over multiple habitats/breeding sites, with some local areas remaining occupied despite losses of individual populations and colonies, providing reliable refugia during adverse conditions and resulting in a reliable source for recolonizations in favorable conditions. ***"Analytical Unit"*** *is the unit of analysis (EPA Level III Ecoregions) used to evaluate current and future resiliency. The diagram characterizes the following three population structures:*

A. *These three discrete colonies would not be considered a population because they are either (a) too far away from each other or (b) unsuitable habitat between them is a barrier. The black arrow shows a distance that renders dispersal or genetic exchange between the two patches less likely or unlikely to occur; therefore these are two distinct colonies. The highway symbol shows unsuitable habitat making dispersal or genetic exchange between these two colonies less likely or unlikely to occur; therefore, these are two distinct colonies, otherwise, they would be close enough to be one colony.*

B. *The riparian corridor symbolized by the thick blue stripe facilitates occasional dispersal by adult between populations, therefore this represents a metapopulation.*

C. *The three colonies on the left are separated enough to be considered discrete colonies but are proximal and the habitat between them is permeable, therefore they comprise a population. The four colonies on the right are separated enough to be considered discrete colonies but are proximal and the habitat between them is permeable, therefore they comprise a population. The habitat matrix between the two populations facilitates occasional adult dispersal between the two populations, therefore this is a metapopulation.*

Colonies

A "colony" may be considered any area occupied by regal fritillaries. We consider a colony to be a collection of individuals within a relatively small, occupied area of suitable habitat that may be separated from other occupied areas composed of unsuitable habitats or barriers that render dispersal or genetic exchange between patches less likely or unlikely to occur.

Populations

Populations of regal fritillaries may be considered as either:

- Aggregates of three or more colonies in suitable habitats with varying diversity to support regal fritillary life-history needs over time that are located in proximity to each other (e.g., 5-8 km [3–5 mi.]) with an adequately permeable surrounding matrix or corridors facilitating inter-patch movements; or

- Occupied relatively large, contiguous suitable habitat patches with adequate heterogeneity supporting individuals, either throughout the area or as connected colonies within, separated from the nearest other occupied area by perhaps 16 to 24 km (10–15 mi) making inter-population exchanges less likely.

Populations are more resilient than small individual colonies, but depending on their size and level of isolation, some regal fritillary populations may also represent "islands." Persistence may vary from a relatively short time to many years, but isolated populations of butterflies rarely last more than 100 years unless sufficient numbers of individuals (500 to multiple thousands) exist within them (Mason 2001, p. 5). Even relatively large populations of regal fritillaries consisting of hundreds of individuals (or even thousands in some years) are not immune to rapid decline and extirpation from stochastic events as has been observed in Iowa after severe drought beginning in 2012 (P. Hammond, personal communication, 2021). The largest populations, typically occupying large, diverse, contiguous habitats on a landscape-level scale, may function as "mainlands" that support regal fritillaries even when conditions become unfavorable and annual numbers are reduced. These sites may act as a source for recolonization of nearby areas when better conditions return.

Metapopulations

Regal fritillary demography may function primarily at the metapopulation level (Schweitzer 1989, p. 135). Generally, metapopulations are aggregates of populations that collectively support regal fritillaries annually and have some inter-population exchanges that occur very infrequently due to long distances and unsuitable habitats between occupied areas. However, the existence of metapopulations today may partly be a function of habitat fragmentation or other factors that have reduced or eliminated populations. In the historical Midwest and Great Plains regions where habitat was relatively contiguous, the species may have functioned primarily at a patchy population scale, whereby individuals could occupy locations within the grasslands that exhibited necessary resources and were able to move freely throughout vast grasslands to access suitable patches as needed, resulting in significant and broad gene flow over large areas (Royer and Marrone 1992, p. 26). Metapopulations may have been more prevalent in eastern habitats, which were limited by the isolating presence of unsuitable forested areas in between occupied grasslands. Today, fragmentation of the prairies, particularly in the Midwest, has resulted in smaller, more widely separated populations with genetic exchanges occurring at reduced rates from historical levels.

We consider metapopulations as sets of 3 or more regal fritillary populations, separated by approximately 32 to 160 km (20 to 100 mi) linked by infrequent dispersal by adults, spread over multiple habitats and breeding sites, with some local areas remaining occupied despite losses of individual populations and colonies, providing reliable refugia during adverse conditions and serving as source for recolonizations in favorable conditions. As explained above, our AUs approximate this metapopulation structure, and capture similarities within habitats at which individuals could move, travel, and disperse within a metapopulation structure.

Distribution and Trends

Sometimes described as "a former landscape level species now reduced to scattered, sometimes isolated, remnant colonies," the regal fritillary was "probably about as characteristic of tallgrass prairie as bison and far more abundant" (NatureServe 2021, entire). It was considered common among prairie and grassland butterflies in the U.S., particularly in tallgrass prairie habitats (Hammond and McCorkle 1983(84), p. 219), but its range has contracted substantially, most severely in the East and Midwest, generally in a north to south and east to west manner (Wagner et al. 1997, p. 261; Selby 2007, p. 17).

Historical Distribution

The regal fritillary's historical range extended from southeastern Montana to Maine across the northern U.S. and from eastern Colorado to northwestern North Carolina across the southern U.S.; this included 32 occupied states in the United States (U.S.), plus the District of Columbia (Selby 2007, pp. 10, 14). Scattered historical records also occurred in four southern Canadian provinces (Manitoba, New Brunswick, Ontario, and Saskatchewan) on the U.S. border (Selby 2007, p. 10), and as recently as 2015, a single stray individual was documented in a fifth province: Alberta (Pohl et al. 2015, pp. 7–8). We note that Nova Scotia is often listed in the literature as being within the range of this species; however, recent efforts to locate valid regal fritillary records from the province were unsuccessful (J. Calhoun, personal communication, 2023). Additionally, relevant sources (Layberry et al. 1998; Pohl et al. 2018) lack any records of the species in Nova Scotia; thus the province is not included herein as part the regal fritillary's historical range. Records in Canada are not considered representative of permanent populations (Selby 2007, p. 10). Similarly, while potential regal fritillary habitat exists in eastern Montana in limited areas such as north-facing/low ravines with bluestem grasses like habitats further east (P. Hammond, personal communication, 2021), the species has not been tracked in the State, breeding populations are not known there, and the single historical Montana regal fritillary record is considered a "stray" (Selby 2007, pp. 10, 20). The species has been observed recently in Montana (D. Debinski, personal communication, 2023), but the State is considered unoccupied. Additionally, the assumption that a specimen of the regal fritillary that is figured in an historical drawing (c.1810) was collected in Georgia is likely erroneous (Calhoun 2007, entire).

The New England portion of the range may have experienced population losses perhaps beginning as early as the 1930s as noted in Massachusetts. Declines were observed in the 1940s in Connecticut (Wagner et al. 1997, p. 262). The species had disappeared entirely from Maine by 1941 - the extreme northeastern part of its range where it may never have been very common (P. deMaynadier, personal communication, 2020). Vermont's last specimen was also observed in 1941 (Zahendra 2010, p. 123). Declines became precipitous in the 1950s, accelerated particularly in the 1970s, and some occupied areas were extirpated (Schweitzer 1989, p. 134; Williams 1999, p. 3). The species was believed to be extirpated from Connecticut by 1971 (Wagner et al. 1997, pp. 261–262). By 1989, Schweitzer (1989, p. 134) advised that "records more than 2 years old be regarded as historic in the Northeast due to the rapidity of the decline there." The species was not observed in Massachusetts after 1990. The species occupied several eastern coastal Islands. The last of those, Rhode Island's Block Island, was believed to have been extirpated in 1991 (Wagner et al. 1997, p. 264).

From the 1970s into the 1990s many other eastern states recognized the loss of regal fritillaries. Examples of last known records include: Maryland - 1993 (J. Selfridge, personal communication, 2021); Kentucky - 1973 (S. Fulton, personal communication, 2021); New York - 1988 (New York State Department of Environmental Conservation 2013); and Michigan - 1989 (D. Cuthrell, personal communication, 2021). At least 40 known historically occupied areas in Pennsylvania were extirpated between 1930 and 1992 (Barton 1995, p. 1). After 2009, the date of the last known regal fritillary observation in Virginia (Chazal 2014, p. 2), FTIG in Pennsylvania became the sole remaining site in the east still harboring a known population of regal fritillaries. As noted in *Taxonomy*, above, this is the only known extant population of the eastern subspecies.

The rapid decline in the east is not clearly attributable to a single cause. Extinction and colonization occur naturally but rates may have become unbalanced toward extinction or decreased colonization, particularly due to land use changes, resulting in a collapse of metapopulation functions (NatureServe 2021; Schweitzer 1993, p. 9). Shrinking and fragmentation of habitats due to development and forest succession (lack of fire and grazing), adverse weather events (e.g., cold/damp springs), excessive collecting, pesticide use (e.g., spongy (gypsy) moth [*Lymantria dispar*] spraying), and even isolated events such as hurricane salt spray have been noted as possible factors in the decline (Williams 1999, p. 3; Schweitzer 1993, p. 9). In other areas westward, the loss of native prairie since the 1800s via conversion to agriculture likely had the most significant impact on the regal fritillary.

Current Distribution

Currently, the species occupies portions of 15 U.S. states across its overall range. Based on information collected from states within the historical range of the regal fritillary in 2020 and 2021 (from state agencies as well as various sources such as individual reports and web-based citizen science observations), we developed a county map identifying those with current (2010 or later) versus historical (pre-2010) records (Figure 15). Historically, there at least 760 counties known to be occupied by the species, including both the western and eastern subspecies (Selby 2007, p. 17). Our mapping exercise identified 878 U.S. counties with either current or historical records.

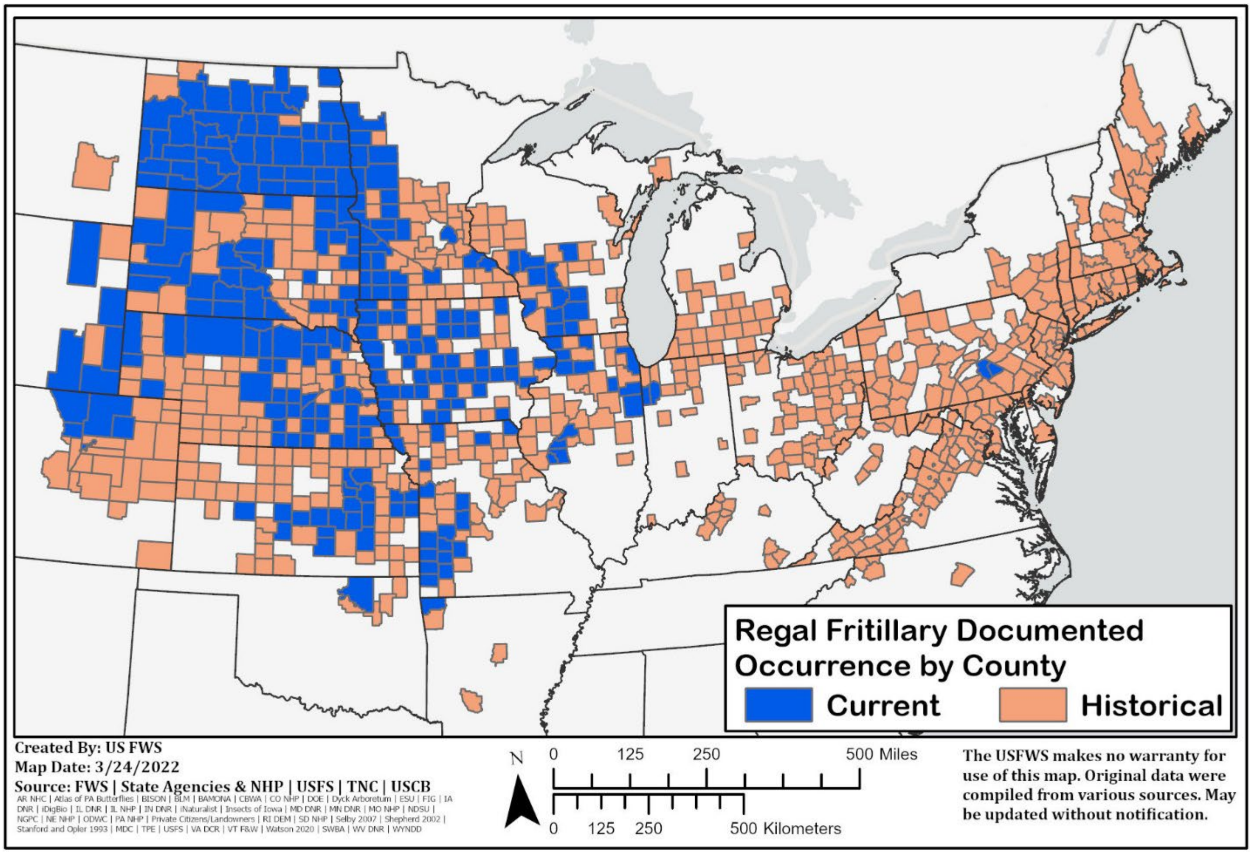

Figure 15. Map of known records of occurrence of regal fritillaries by county. Records are defined as current (in blue) if dated 2010 or later; all others are labeled historical (orange).

Since 2010, out of the 878 counties with historical or current regal fritillary records, the species has been documented in 269 (31 percent) counties in 15 states. Of these currently occupied states, four (Iowa, South Dakota, North Dakota, and Wyoming) have a higher proportion of counties with recent records than without; however, regal fritillary observations since 2010 do not necessarily depict the exact current occupied area of the subspecies. Only suitable grassland habitats within counties would be occupied, and a lack of recent observation records in a county does not necessarily indicate species absence; many states have not conducted monitoring specifically for the regal fritillary. Updates to county-level occurrences are anticipated over time with additional survey efforts and/or opportunistic observations. Table 4 identifies the number of counties in occupied states that meet the definitions of current and historical.

Table 4. The number of counties in each state within the extant range of the regal fritillary that have observation records dated 2010 or later (Current) and observation records dated prior to 2010 (Historical), with totals.

STATES WITH EXTANT POPULATIONS	NUMBER OF COUNTIES BY EXISTING STATUS		
	Current	Historical	TOTAL
Arkansas	1	3	4
Colorado	5	19	24
Iowa	46	28	74
Illinois	13	25	38
Indiana	2	16	18
Kansas	20	48	68
Minnesota	27	34	61
Missouri	18	37	55
North Dakota	43	3	46
Nebraska	41	50	91
Oklahoma	1	4	5
Pennsylvania	2	43	45
South Dakota	33	30	63
Wisconsin	12	25	37
Wyoming	5	2	7
TOTAL (and percentage)	269 (42%)	367 (58%)	636

North Dakota recently conducted statewide pollinator surveys with annual sampling in every county from 2017 to 2019 (Limb et al. 2019, entire; Limb et al. 2022, entire); regal fritillaries were detected in most of them. Notably, counties in the northern part of North Dakota have recent regal fritillary observations, but in past decades the species typically was not observed north of U.S. Interstate 94 which excluded approximately the northern two-thirds of the State (Royer and Marrone 1992, p. 5; P. Hammond, personal communication, 2021). This may represent a relatively recent northward shift in the regal fritillary's Great Plains range. Increases in temperature and a lengthened growing season have been documented in North Dakota, along with earlier flowering of many plant species, including violets (Dunnell and Travers 2011, p. 940). A similar northward shift has been suggested in Wisconsin; Swengel and Swengel (2017, p. 19) found evidence of potential northward population expansion, perhaps a response to changed climate.

Given the lack of standardized surveys across the range, the area of occupancy and number of functional regal fritillary colonies, populations, and metapopulations within the above counties cannot be determined with certainty. NatureServe (2021, entire) estimates the number of extant occurrences to be between 81 and 300, described generally as "a few hundred scattered remnant colonies or metapopulations with most occupying about 100 hectares or less"; about 100 occurrences may represent viable metapopulations (metapopulation parameters not defined), and of these, a range of 13–40 ("almost certainly more than 20") occurrences may be considered appropriately protected and managed which included compatible management activities (specifically appropriate fire and grazing) (NatureServe 2021, entire). Observation records also do not necessarily equate to an established population; dispersers may be observed, but their presence may or may not be indicative of long-term occupancy.

The regal fritillary was estimated to be "endangered, threatened, or extirpated" in approximately 40 percent of its range in the 1980s (Schweitzer 1989, p. 135). Estimates regarding the species' decline today are as high as 99.9 percent from historical levels based on habitat loss and range contraction (NatureServe 2021, entire). The shorter-term trend is suggested to be at a 30 to 70 percent rate of decline (NatureServe 2021). NatureServe (2021, entire) indicates the "most ominous trend for regal fritillary now may be the breakdown of metapopulation dynamics over most of the range," citing a combination of increased local extinction rate and/or decreased colonization rate that led to the nearly complete elimination of the species in the east. Detailed losses by state and region, including the almost complete disappearance of the species from the eastern portion of the range, are provided by Selby (2007, pp. 17–20). Below we summarize the distribution and trends for the eastern and western subspecies.

Eastern Subspecies Distribution

The current range of the eastern subspecies is well defined and is limited to one general area that is closely monitored and managed for the subspecies. All known eastern populations (many of which were of the eastern subspecies) of approximately 100 to 200 adults in the 1980s were extirpated by about 1991 (NatureServe 2021, entire) with exception of a single population at FTIG in Pennsylvania. The FTIG base, established in 1931, has been used continuously for military training exercises that periodically disturb the grounds and maintain grassland patches as an old field successional stage within ecoregions typically dominated by forests (Ferster et al. 2008, p. 142). The FTIG facility is about 8 km (5 mi) wide and about 18 km (11 mi) long, located in a valley spanning two counties, Dauphin and Lebanon, in south-central Pennsylvania. The eastern subspecies is isolated, and the nearest western subspecies population is located approximately 869 km (540 mi) to the west in Indiana.

The regal fritillary is considered endangered by the Pennsylvania Biological Survey (PABS); however, no Pennsylvania State agency has the legislative authority to enforce conservation regulations for terrestrial invertebrates (Zercher et al. 2002, p. 3). The U.S. Department of the Army seeks grants to proactively fund conservation of the regal fritillary eastern subspecies within its borders to help meet objectives in the Integrated Natural Resource Management Plan (INRMP; Pennsylvania Dept. of Military and Veterans Affairs 2021; see section 4.13.1.1). These conservation efforts are driven by the PABS ranking, the 2015–2025 Pennsylvania Wildlife Action Plan (PGC-PFBC [Pennsylvania Game Commission and Pennsylvania Fish & Boat Commission] 2015). Currently, there are no legal protections specifically for the eastern subspecies.

In 1999, FTIG set aside approximately 158 ac (64 ha) in three separate areas (Service 2023, pp. 1, 3) to further the study of the eastern regal fritillary, including determining its life history requirements, and to conduct management experiments (Hovis 2009, p. 6). By 2002, that acreage was expanded to 219 ac (89 ha) and included a fourth area (Service 2023, p. 3). Those areas, now called special consideration areas (SCAs), are former military training areas containing high quality grassland/old field habitat dominated by native warm-season grasses, violets, and nectar plants (Figure 16) (Hovis 2009, p. 6; Ferster and Vulinec 2010, p. 39). These areas were selected based on the highest density of eastern regal butterfly observations at the time from the population data (Zercher et al. 2002, p. M-15).

Over time, the eastern regal fritillary population grew and shifted throughout suitable open habitat adjacent to the original SCAs (Figure 16). These expanded areas, termed Regal Research Areas (RRAs), currently encompass approximately 457 ac (185 ha) (the original SCA 219 ac [89 ha] plus an additional 238 ac [96 ha] of expansion habitat) (Figure 16) (Service 2023, pp. 1–3). The RRAs represent the areas in which staff focus the bulk of their monitoring efforts because it currently holds the highest densities of the butterflies (Service 2023, p. 3).

The regal fritillary was able to expand into additional areas because of FTIG's work to manage the existing habitat and to better link the areas through habitat improvements (PADMVA 2022, p. F-15). The original SCAs were much more isolated and were managed on a much smaller scale and with a less frequent disturbance interval than happens now (Service 2023, p. 3). The current RRAs are now better connected because of FTIG's creation of military ranges and other training areas (Service 2023, p. 3). For example, the RRA Charlie 4 subpopulation numbers increased after a timber sale improved growing conditions for native grasses, violets, and nectar plants that allowed for better dispersal between RRA Charlie 4 and Range 23, and Range 36/Delta 3 (PADMVA 2022, p. F-15). Other land clearing efforts throughout the training areas in the eastern end of the military installation have also created opportunities for habitat improvement, but the grasslands in those areas have not responded as quickly, continuing to leave the Bravo 12 subpopulation somewhat isolated (PADMVA 2022, p. F-15). Conversely, the Range 36 subpopulation has colonized new areas and increased in size due to increases in fire frequency combined with timber clearing and construction of new ranges (PADMVA 2022, pp. F-15–F-16; Service 2023, p. 4).

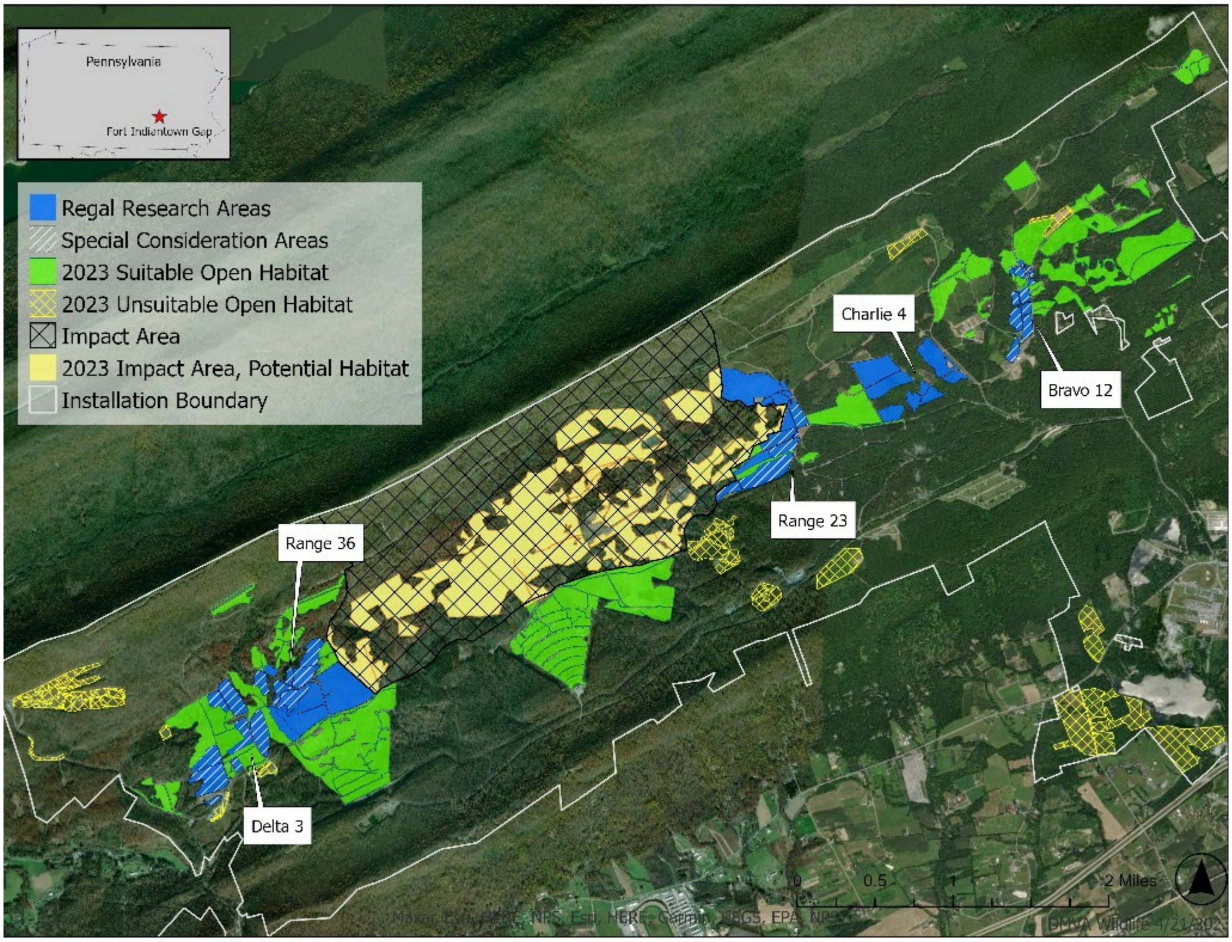

Figure 16. Map of the suitable and unsuitable open habitat areas, SCAs, RRAs, and impact area of FTIG's training corridor zone. Map courtesy of PADMVA.

The eastern regal fritillary is not systematically monitored or formally managed in the impact area, yet they are a functional part of this remnant eastern subspecies population, existing in an area that cannot currently be accurately quantified and has not been included in past estimates of the regal fritillary population size at FTIG (M. Swartz and V. Tilden, personal communication, 2021). There are approximately 722 ac (293 ha) of other potentially suitable habitat within the "impact area" of the installation (Figure 16, above); this area is adjacent to and between the Range 23 and Range 36 RRAs, and eastern regal fritillary individuals have been intermittently documented here (Zercher et al. 2002, p. M-11). The impact area is subject to more frequent disturbances, particularly burns resulting from training activities which occur every year (albeit in a patchy manner). Although survey data are lacking from the impact area due to the presence of unexploded ordnance, in 2020, FTIG Wildlife Staff conducted authorized basic roadside presence/absence surveys in the impact area, as well as in other open sites on FTIG. The intent of the presence/absence surveys was to evaluate whether the species may have shifted and could be using these alternate sites as much or more than the RRAs (Zercher et al. 2002, p. M-11; PADMVA 2022, p. F-11; Service 2021, p. 2). Few to no regal fritillaries were observed. While not a comprehensive survey, the resulting preliminary data suggests that the RRAs currently exhibit the highest densities of the butterflies at FTIG (V. Tilden, personal communication, 2021); other areas on FTIG may be used by regal fritillaries, but to a lesser degree.

Additionally, 930 ac (376 ha) of suitable open habitat (Figure 16, above, and Figure 17) could potentially be eastern regal fritillary habitat with little or no change in current management. The habitat is largely managed for fuel reduction or military training, or both, which has produced conditions which may, or does, support the butterfly in some capacity (Service 2023, p. 2).

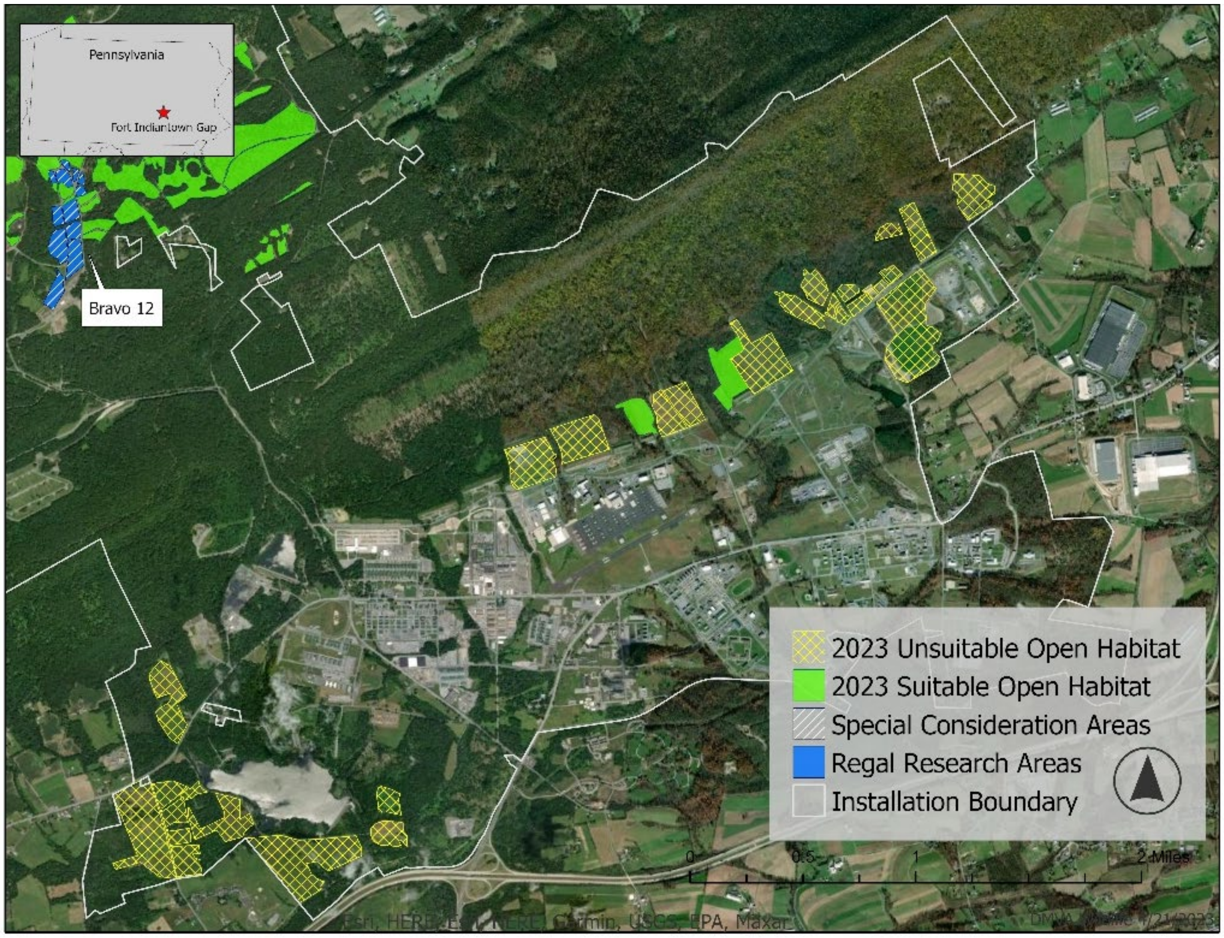

Figure 17. Map of the suitable and unsuitable open habitat areas of FTIG's cantonment zone. The upper left corner of the map also shows RRA Bravo 12 in the easternmost area of the corridor zone. Map courtesy of PADMVA.

While the eastern regal fritillary almost exclusively occupies areas within the training corridor (areas north of Blue Mountain), they have occasionally been documented in open habitats south of Blue Mountain in the cantonment (military camp) (Service 2023 p. 3) (see Figure 17, above). Blue Mountain is forested with no open habitat connecting the corridor and cantonment areas (Service 2023, p. 1). The cantonment contains numerous functional areas such as operations, educational and simulations, along with maintenance buildings and support facilities, classroom and educational facilities, dormitories, barracks and other lodging facilities, a medical clinic, chapel, conference center, shopping and dining options, recreational and athletic facilities (PADMVA 2022, p. 33). While some small patches in the cantonment are defined as suitable habitat, they are not necessarily equal in suitability compared to those habitats defined as suitable in the corridor. This is due primarily to small patch size and significant isolation from the main populations in the corridor. In addition, many areas that appear to be open in the cantonment are not documented as open habitat because of very high mow frequency, construction, other fluctuating uses, or a combination of these activities (Service 2023, p. 3).

Eastern Subspecies Trends

The eastern subspecies population is found only at FTIG in Pennsylvania. In 1992, monitoring began at this military base (Ferster 2005, p. 8). Five mark-release-recapture studies have been used to estimate population size at FTIG since 2001 (Table 5) and revealed population expansions and contractions, which are not unexpected for this species.

Table 5. Regal fritillary population estimates by year on regal fritillary research areas at the Fort Indiantown Gap National Guard Training Center (FTIG), based on mark-recapture studies conducted intermittently since 2001 (table courtesy of FTIG Wildlife Staff).

RRA	2001*	2005	2009	2014	2017
Bravo 12	NA	241.67	110.34	93.07	28.98
Charlie 4	NA	2	12	413.67	23.76
Range 23	NA	324.84	648.55	789.45	280.34
Range 36[1]	NA	452.42	1063.56[a]	4138.52[a]	479.77
TOTAL	**900**	**1020.93**	**1834.45**	**5434.71**	**812.85**

** only a single super-population number is presented here due to issues with Mark-Release-Recapture methodology and data collection during this sampling year; [a] Additional acreage surveyed; [1] The name of this RRA was changed in May 2023. Earlier documents and cited references may refer to this area as Range 36/Deltas, Range 36/Deltas 1 and 3, Deltas 1 and 3, or Delta.*

After peaking in 2014 at over 5,400 individuals with continued high numbers observed in 2015, the population decreased in 2016 and it has not rebounded to 2014–2015 numbers to date (M. Swartz, personal communication, 2021). The last regal fritillary population estimate (2017) was approximately 800 individuals; several population estimates have been near 1,000 individuals (M. Swartz, personal communication, 2021). Mark-recapture surveys did not include the impact area; a mark-recapture study would have occurred in 2020 but was canceled due to complications and logistics with the COVID-19 pandemic (M. Swartz, personal communication, 2021). FTIG staff plan to continue with occupancy surveys of the impact area with safety assistance from the Air National Guard (M. Swartz, personal communication, 2021). Estimated population size has fluctuated significantly at times, as is typical for the species. Recently, FTIG personnel have supplemented regal fritillary populations with captive reared larvae for reintroduction efforts, but it is not clear if these actions have increased the population size at FTIG. Despite the variability in numbers, the overall trend of the eastern subspecies at FTIG appears relatively stable over time, usually hovering near 1,000 individuals on the SCAs. The population size has both increased and decreased, but has not reached precipitously low numbers (i.e., less than 50; Hanski and Thomas 1994, p. 169) at any point over the past two decades of monitoring.

The FTIG population of regal fritillaries exhibit distinct mitochondrial haplotypes that are not present in any other known extant regal fritillary population (Williams 2002, p. 151). While it appears to have been limited primarily to mid-Atlantic and New England states, the eastern haplotype was also documented well outside of those regions in at least one location, Kentucky (Keyghobadi et al. 2013, p. 240); however, the single remaining eastern subspecies population today is in Pennsylvania. The nearest population of the western subspecies, *A. i. occidentalis* is about 869 km (540 mi) west of the *A. i. idalia* population; thus, the potential for natural genetic exchange between the subspecies is unlikely.

Additionally, the regal fritillary within FTIG exhibit signs of restricted gene flow; genetic structure has been identified within each occupied meadow at the military base using analysis of mitochondrial and microsatellite loci (Keyghobadi et al. 2006, p. 3). Among three occupied Pennsylvania meadows separated by distances ranging from 1.5 to 10 km (0.9 to 6.2 mi), genetic differences were detected at levels like those described by Williams et al. (2003) for highly fragmented Midwestern populations. Keygohbadi et al.'s (2006) results suggest that genetic differentiation due to fragmentation occurs in the FTIG population and probably reflects high rates of genetic drift in the small sub-populations. This reduction in dispersal with fragmentation may be due to direct behavioral change or natural selection against migrants (Keygobadi et al. 2006, p. 3). In contrast, more recent analysis of 198 individuals from the FTIG population determined that allelic diversity was similar among the occupied meadows (defined as "east" and "west" meadows within FTIG boundaries), with no evidence of population differentiation (Rutins et al. 2022, p. 4). Rutins et al. (2022, p. 4) note that while long-term monitoring has indicated a relatively stable population at FTIG (Ferster and Vulinec 2010), large fluctuations have been observed during annual surveys over the past 15 years (Zografou et al. 2017; Unpublished data *in* Rutins et al. 2022, p. 4). These recent fluctuations are potential bottlenecks, which have likely impacted genetic diversity by reducing the overall population size and increasing levels of inbreeding among the remaining individuals, leading to excess homozygosity (Rutins et al. 2022, p. 4).

Western Subspecies Distribution

The western subspecies occurs in 21 AUs and 3 representative units. Midwest populations of *A. i. occidentalis* that exist in AUs within Illinois, Indiana, Iowa, Minnesota, Missouri, Wisconsin, and Arkansas have been relegated mainly to small, isolated patches of prairie remnants (e.g., less than 40 ha [98.9 ac]; Robertson et al. 1997 *In* Panzer and Schwartz 2000, p. 363), scattered across a primarily agricultural landscape. Many of these are fire-managed conservation areas in contrast to working lands of pastures and hayfields that are more representative of occupied areas to the west and south (e.g., western Iowa and Missouri) (NatureServe 2021, entire).

The Northern and Central Great Plains are the stronghold for the subspecies; AUs within Kansas, Nebraska, North Dakota, and South Dakota have relatively larger, more numerous, and overall, less fragmented suitable habitat patches than the Midwestern states (Selby 2007, p. 20). A "few hundred" relatively widely distributed occupied sites were estimated to exist in a core area described as portions of Kansas, Missouri, and Nebraska with over 70 occupied sites estimated to exist in Kansas alone (NatureServe 2021, entire). The Missouri-Kansas border is identified as a location with many metapopulations that exist mostly in small active pastures (NatureServe 2021, entire). Most breeding populations outside of the Kansas-Missouri area may average approximately 100 to 200 individuals, described as "marginally viable" but with considerable annual changes, as commonly occurs with this species (NatureServe 2021, entire). An estimated

20 populations were noted to exist in Minnesota and/or South Dakota with no additional details provided (NatureServe 2021, entire).

Populations of the western regal fritillary at the western periphery of the Great Plains tend to be more isolated than the core area of portions Kansas, Missouri, and Nebraska (Selby 2007, p. 14). The dry shortgrass prairies of the west are limiting to the regal fritillary with scattered occurrences generally in riparian zones or other moist habitats where nectar sources and violets are available. States on the western and southern fringes of the regal fritillary's range (Wyoming, Colorado, Oklahoma, and Arkansas) are relatively sparsely occupied, with regal fritillaries occurring only in portions of those states, near their borders with adjacent occupied states. We also note that drought beginning in 2012 resulted in observed extirpations (e.g., many sites in Iowa that have yet to be recolonized as of 2021) (P. Hammond, personal communication, 2021), further demonstrating the difficulty in accurately quantifying western subspecies occupancy through time on a rangewide basis.

Approximately 84 percent of the western regal fritillary's gross, overall range (the outer boundary of all 21 AUs) is privately owned (Table 6). Approximately 7 percent of this gross, overall range is Tribal, 4 percent is State, 2 percent is managed by the Bureau of Land Management, 2 percent is managed by the U.S. Forest Service, and less than 1 percent each is managed by the Service, the Department of Defense, and the National Park Service (Table 6)

Table 6. Approximate landowner percentages across federal land management agencies and state agencies throughout the range of the western subspecies. Percentages calculated using the BLM Surface Management Agency Dataset (Bureau of Land Management, 2022).

Landowner Category	Approximate percent (%) ownership/management authority in the western subspecies overall, gross range
Bureau of Indian Affairs (BIA)	6.77%
States	3.52%
U.S. Forest Service (USFS)	2.52%
Bureau of Land Management (BLM)	1.56%
U.S. Fish and Wildlife Service (Service)	1.16%
Department of Defense (DOD)	0.27%
National Park Service (NPS)	0.16%
Private, Local Government, Unknown, or Undefined	84.04%

Western Subspecies Trends

The western subspecies historically occupied a much larger portion of the range than the eastern subspecies. Thus, while the eastern subspecies was nearly eliminated with the east-to-west retraction in the species' range, western subspecies populations remained. However, the western subspecies is generally considered to have a declining trend, largely a result of conversion of grasslands to agriculture and development. Habitat fragmentation generally decreases east to west across the western subspecies' range, and as the size and number of prairie remnants increases, there is a corresponding increase in size, number and long-term viability of populations (Selby 2007, p. 18).

Swengel and Swengel (2016) analyzed regal fritillary incidence and abundance via the North American Butterfly Association's 4th of July Butterfly Count (4JC) data between 1977–2014. The early days of the program had few established survey sites, but the number increased over time. Despite the addition of more survey sites, during the 1991–2014 timeframe, all measures of regal fritillary occurrence and abundance declined including: (1) the percent of counts with regal fritillaries reported; (2) the percent of counts in a year that documented either more than a 100 or more than 500 regal fritillaries; (3) the number of individuals per count; and (4) the highest regal fritillary total on a single count (Swengel and Swengel 2016, p. 4). While there are inherent issues with these data (e.g., sites and searches are not random, survey timing does not necessarily correspond with peaks in butterfly numbers, survey sites were few in the early days of the program), the 4JC is useful from the perspective of being a large, annual, consistently conducted, continent-wide documentation of butterflies. A recent study also used 4JC data and found that individuals used grassland locations with minimal forest cover (Post van der Burg et al 2023, entire).

Loss of native grassland habitat required by the regal fritillary that began with breaking of the prairie sod in the 1800s continues today. A study tracking cropland changes from 2008 to 2016 identified crop expansions at a rate of over 404,685 ha (1 million ac) per year with "pre-eminent hotspots" of this activity identified in portions of the regal fritillary's range including the Prairie Pothole region of North and South Dakota and the Dissected Till Plains of Iowa and Missouri (Lark et al. 2020, p. 3).

There are local exceptions to the declining trend as some populations exist on relatively large suitable habitat sites, of which some are actively managed for the regal fritillary or other pollinators. Eastern Kansas and western Missouri are areas where the species appears to be relatively stable (NatureServe 2021, entire), and there are other individual locales described similarly. An area identified as Buena Vista Grassland in Wisconsin supports a relatively stable population despite annual variability (Swengel and Swengel 2017, p. 18). Buena Vista Grassland is managed for prairie chickens (*Tympanuchus cupido*) and is described as "a complex of 8 sites (24–1,350 ha [59-3,336 ac]) aggregated into one of the largest grassland complexes east of the Mississippi River, with about 5,000 ha (12,355 ac) of public land and a large amount of surrounding private grassland." (Swengel and Swengel 2017, pp. 4, 18). Fort McCoy, also in Wisconsin, is a 24,000 ha (59,305 ac) military base with native habitats and disturbances via military activities that had been surveyed for butterfly species without detecting regal fritillaries for several years, but now has a significant population (Swengel and Swengel 2017, pp. 7, 16–17). Recently, North Dakota surveys have revealed a broader distribution of the species than was formerly known including more northerly counties than had been documented previously (Limb et al. 2019, entire). Kankakee Sands in Indiana is an approximately 3,440-ha (8,500-ac) site owned by The Nature Conservancy; significant restoration and management activities have resulted in a regal fritillary metapopulation that has resulting in satellite colonies in adjacent areas of Indiana and Illinois (Shuey et al. 2016, pp. 774, 778). Features common among these sites include large collective or individual patch sizes, connectivity among habitats, and ongoing management/disturbance regime.

Species Distribution Modeling (SDM) Effort

We developed a species distribution model (SDM) of potential regal fritillary habitat to help us better understand its current distribution and to help identify population-like analytical units (AUs) for our SSA analysis. The SDM helped identify EPA Level III Ecoregions (EPA 2013, entire) as suitable AUs for us to evaluate the current and future conditions for the subspecies in terms of the 3Rs, starting with resiliency of each AU. We describe this SDM effort in Appendix D and the results are illustrated in Figure 18.

We note that the SDM output does not necessarily reflect precise current occupancy by the regal fritillary. It is a predictive model, indicating where suitable habitat may exist, and occurrence is likely. The SDM may be useful to help determine future surveys and where conservation efforts could be focused within the current range of the species, as needed. A limitation of the model is the inherent bias in using opportunistically obtained regal fritillary observations (i.e., observations not obtained via randomized standard surveys conducted across the range). Also, local factors critical to species presence cannot be precisely represented or predicted rangewide at the resolution of this model. Local factors include the local disturbance regimes or stochastic events that may affect habitats and populations, or the presence of habitat patches on a smaller scale (e.g., wetland edges) not captured by the model.

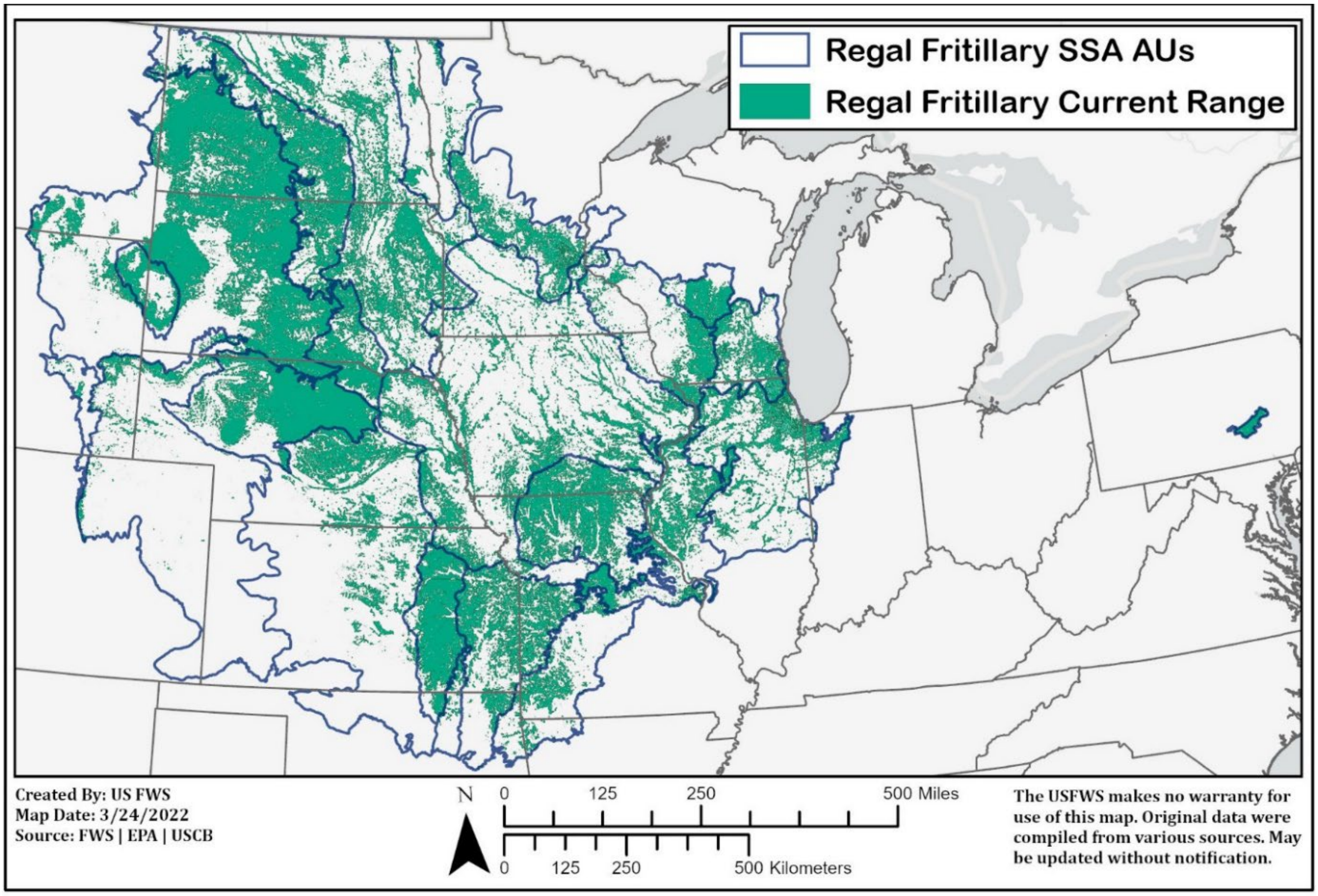

Figure 18. Regal fritillary analytical units (AUs) and approximate, predicted current range based on a species distribution model. We note that the species distribution model may over or under-predict potential current range in particular areas.

Chapter 3 – Needs of the Eastern and Western Subspecies

The needs of a subspecies can be evaluated hierarchically, starting at the lowest level with an individual's basic resource (habitat) needs for breeding, feeding, and sheltering. Then, needs can be described at the population and subspecies levels by describing resiliency needed for populations (AUs) to withstand stochastic events, redundancy to withstand catastrophic events, and representation to adapt to environmental change. An understanding of the subspecies' needs at the individual and population levels helps best inform our viability analysis. In this chapter we summarize the habitat and demographic needs of regal fritillary individuals, AUs, and the eastern and western subspecies of regal fritillary. Our understanding of the subspecies' needs was derived from our summary of life history and ecology in Chapter 2, with additional information provided in Appendix C. The hierarchal relationship between these scales allows for a broad and inclusive understanding of the viability needs of the subspecies in terms of resiliency, redundancy, and representation.

Individual Needs

We identified habitat (resource) needs for all four regal fritillary life stages (Table 7; Appendix F). The following habitat factors are needed by individuals of both subspecies to successfully complete all stages of their life cycle, whereby they may then contribute to the resiliency of an AU:

- **Native grasses**, either tallgrasses or mixed grasses, although the eastern subspecies may be more tolerant of nonnative grasses;
- **Violets,** as larval food;
- **Diverse floral resources,** as nectar and shelter sources for adults;
- **Shrubs and tall vegetation,** to provide shelter for adults;
- **Vegetative litter** and **grass tussocks**, as shelter for all life stages;
- **Ambient temperatures**, needed for larval and pupae development.
- **Moisture**, needed to prevent desiccation and support violets and nectar sources

We discuss these individual resource needs in more detail below and added them to our conceptual model in Figure 19. In general, regal fritillary individuals need an adequate abundance of violets and nectar sources, appropriate grassland conditions (litter, tall or shrubby cover, warm season bunchgrass tussocks), and adequate moisture and ambient temperatures must be available annually for regal fritillary populations to persist in any area. Such habitats are maintained by periodic disturbances and need to be sufficiently large and contiguous.

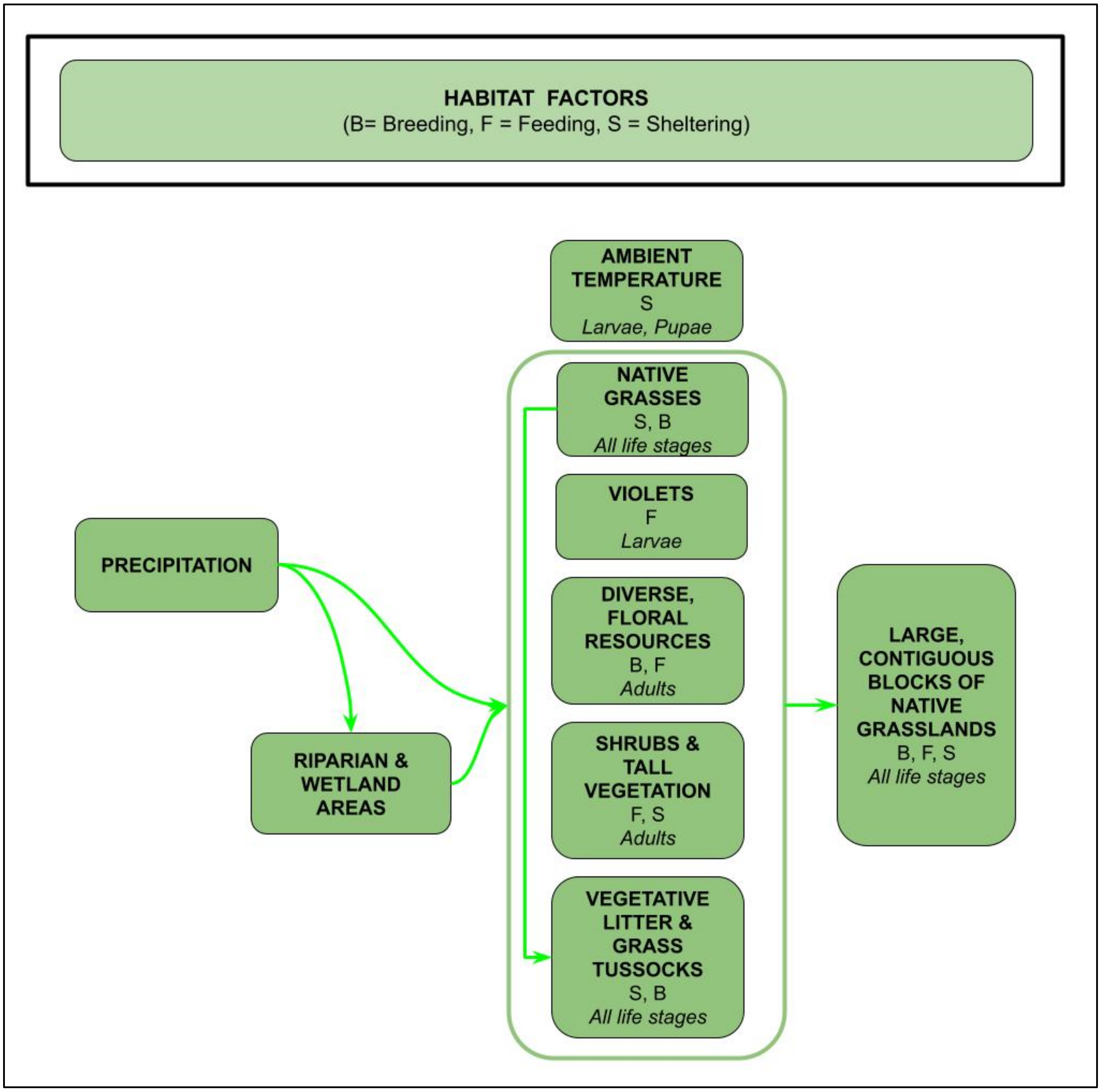

Figure 19. Conceptual model for regal fritillary habitat needs (resource needs), or the habitat factors (green boxes) needed by individuals to breed (B), feed (F), and shelter (S). Green arrows represent positive relationships between nodes.

Table 7. Table of individual resource needs of the regal fritillary at each life stage: egg, larva, pupa, and adult.

INDIVIDUAL RESOURCE NEED	**DESCRIPTION OF THE NEED**	**METRIC(S) TO DESCRIBE QUALITY OR QUANTITY**	**LIFE STAGE AND FUNCTION OF EACH RESOURCE NEED (B=Breeding, F=Feeding, S=Sheltering)**			
			EGG	**LARVA**	**PUPA**	**ADULT**
NATIVE GRASSES (Tallgrass or Mixed Grass) (S, B) (Note: historical eastern habitats were often, but not necessarily, native grasses)	Warm season bunchgrasses, such as big & little bluestem grasses, used as shelter by all life stages	Amount of bluestem in grassland. Big bluestem is a dominant grass in tallgrass prairie and a component of mixed-grass prairie. Little bluestem prefers drier microclimates	(S) Eggs are laid on (adhere to) vegetation, typically shaded microsites on underside of grasses, sometimes on the ground or on rocks	(S) Use bunchgrasses for shelter during winter diapause and during development	(S) Adhere to underside of warm season bunchgrass tussocks or other grassland vegetation in litter layer	(S) Shelter in shady tall bunchgrasses and shrubby vegetation (B) Mate almost immediately after female emergence in grassland natal area, before dispersal
VEGETATIVE LITTER AND GRASS TUSSOCKS (S, B)	Warm season grass tussocks and duff	Amount of senesced or downed vegetative cover. Recently burned, heavily grazed areas have little or no litter; undisturbed prairies have thicker litter layers associated with perennial grasses and moist microclimates; too much litter can suppress violets and nectar sources	(S) Eggs are laid near or on the ground, among vegetative litter and senesced grasses and sometimes bare ground.	(S) Vegetative litter affords shelter during winter diapause and spring development stages	(S) Adhere to underside of warm season bunchgrass tussocks or other grassland vegetation in litter layer	(B) Seek out sites with senesced vegetation on which to lay eggs
VIOLETS (F)	Native *Viola* spp. are sole larval host plants; varies throughout range. Examples: bog white, lance-leaf, or lance-leaved violet (*V. lanceolata*), Nuttall's violet (*V. nuttallii*), birdfoot (*V. pedata),* blue prairie violet (*V. pedatifida*), arrowleaf violet (*V. sagittata*), wood violet (*V. sororia*), and Johnny jumpup (*V. tricolor*)	Presence, density, and availability of violets. Violets do not always correlate with presence or density of regal fritillaries, but larvae will not survive without presence and adequate supply of violets	NA	(F) First instars need young violets, all instars feed exclusively on Viola spp.	NA	NA
AMBIENT TEMPERATURE (S)	"Normal" temperatures are ideal. Isotherms defining range: 7 °C (45 °F) in the north; January mean isotherm about 2 °C (36 °F) or lower in the south (NatureServe 2021). Sublethal effects may occur at 38 °C (100.4 °F); significant mortality between 40–42 °C (104–107.6 °F); 100% mortality at 44 °C (111.2 °F) (Nail 2016, pp. 4, 9, 13, 15).	Temperature deviations from normal, particularly if extreme or for extended time may impact individuals by slowing development, increase desiccation, causing mortality or affecting availability of required resources (e.g., violets)	Extreme heat or cold may slow hatching, increase risk of desiccation, prolong exposure to mortality factors or cause mortality directly	Extreme heat or cold may slow larval development, prolong exposure to mortality factors or cause mortality directly. Early spring warmth may rouse larvae before violets are available (F) cold can reduce or slow violet growth; heat can prematurely senesce violets	Extreme heat or cold can slow development, prolong exposure to mortality factors or cause mortality directly	(B) Cool temperatures can limit activity, hinder mating (S) Hot temperatures reduce flights, induce female aestivation
DIVERSE FLORAL RESOURCES (Nectar Sources) (F, S)	Adults require nectar sources throughout flight period, May-October. High quality nectar sources vary seasonally (typically, but not always pink or purple in color). Important sources include: milkweeds (*Asclepias*), thistles (*Cirsium*), coneflowers (*Echinacea*), blazing-stars (*Liatris*), bergamots (*Monarda*), goldenrods (*Solidago*), clovers (*Trifolium*), and ironweeds (*Vernonia*).	Abundant diverse nectar sources available May-October	NA	NA	NA	(F) Males require several weeks of nectaring to survive long enough to mate (die soon after); females require several months of nectaring to survive long enough to lay eggs (die soon after). (F,B) Good nutrition (high quality and abundant nectar sources and minerals) improves female fertility and fecundity
MOISTURE (F)	Vulnerable to conditions too wet (e.g., drowning) or too dry (desiccation or premature violet or nectar source senescence)	Exact individual moisture requirements unknown, may align with conditions for presence of violets or bluestem; species occurs in dry-mesic habitats	Unknown	(F) Unknown - likely obtain moisture from violets and dew or rain when available. (F/B) Drought can cause premature senescence of violets resulting in stunting or starvation, and potentially fewer females (which develop slower, need violets longer than males). High moisture can lead to fungal infections. Flooding can cause drowning	Largely unknown; high moisture may lead to fungal infections	(F) Adults use standing water for minerals, get most of the water they need from nectar. (B) Drought can cause premature senescence of nectar sources, requiring dispersal to find resources; survival or female fecundity and fertility may be reduced.
SHRUBS OR TALL VEGETATION (F, S)	Adults shelter (and nectar, if available) in shrubs (e.g., snowberry, bayberry) or shrub-like vegetation (e.g., tall thick grasses, woody forbs, weeds)	Some (unknown) level of shrub component, or vegetation that affords shrub-like shelter (relatively tall, thick)	Unknown - may help provide shaded microclimate	Unknown - may help provide shaded microclimate	Unknown - may help provide shaded microclimate, or substrate to which to adhere for pupation	(F/S/D) Additional nectar sources, sites to rest, refugia from adverse conditions (e.g., presence of heat, storms, aerial predators)

Below, we discuss each of these resource needs that we identified for the regal fritillary.

Native Grasses

All life stages need appropriate grass structure for shelter, and adult females lay their eggs on grasses. In general, individuals need a sufficient quality and quantity of native grasses. The regal fritillary is a native prairie indicator species (Hammond and McCorkle 1983(84), p. 218) and prairie specialist (Swengel 1996, p. 76), so native grasses are a clear need. However, the species can occur in degraded prairie, and the species' range historically included areas well east of the Midwestern and Great Plains prairies of today where it occurred "overwhelmingly in anthropogenic grasslands such as infrequently mowed hayfields and (apparently primarily) moist pastures, very often associated with streams" and in some areas, "largely dominated by exotic grasses" (Schweitzer 1992a, p. 4). In the eastern part of the species' historical range, including the potential mixing zone of eastern and western subspecies (i.e., Ohio, western Pennsylvania, Virginia, and West Virginia) (Keyghobadi et al. 2013, p. 239), the habitat has been described as meadows, native prairie, tallgrass prairie, mesic grasslands, pastures, abandoned fields, hay fields, mesic prairies near woodlands, and floodplain forest openings and edges (Selby 2007, p. 27). Wet areas are often emphasized, but dry mountain pastures have also been mentioned as regal fritillary habitat in the east (Opler and Krizek 1984, p. 109; Shuey et al. 1987, p. 5). Individuals may occupy areas other than pristine prairie, such as wooded areas with low, weedy growth, and some small trees, approximately 2.4 ha (6 ac) in size, and surrounded by deciduous forest (Calhoun 1981, p. 42).

Abundant common milkweed and yarrow may provide nectar sources for the regal fritillaries at some locations (J. Calhoun, personal communication, 2021). At most habitats in the Midwest and Great Plains, the regal fritillary is most often found in open native prairies. Regal fritillaries appear to be more associated with block habitats as opposed to linear (e.g., road ditches or railroad rights of way) habitats (Davis et al. 2007, p. 1351) except for linear riparian areas that may serve as dispersal corridors (Swengel and Swengel 2017, p. 16).

Higher percent grass cover is associated with increased presence of regal fritillaries (McCullough et al. 2019, pp. 7–8), particularly big bluestem (*Andropogon gerardi*) (Caven et al. 2017, p. 199) and little bluestem (*Schizachyrium scoparium*) (Ferster and Vulinec 2010, p. 39). The probability of regal fritillary presence has been shown to increase when percent cover by big bluestem reaches 50 to 55 percent (Caven et al. 2017, p. 198). At FTIG in Pennsylvania, occupied habitats are 20 to 45 percent bunchgrass cover and tussock formation, particularly of little bluestem and broomsedge (*Andropogon virginicus*) (Swartz et al. 2015, p. 823). It is not clear whether all bunchgrass species support regal fritillaries. Increased cover of switchgrass (*Panicum virgatum*) (a type of bunchgrass) above 8 percent has been shown to decrease the probability of regal fritillary presence in Nebraska (although other factors may have affected this observation) (Caven et al. 2017, p. 198), although Schweitzer (1992a, p. 4) indicated switchgrass was a preferred resting habitat for regal fritillaries in some eastern locations. Schweitzer (1992a, p. 4) also noted the presence of Indiangrass (*Sorgahstrum nutans*), another warmseason bunchgrass, in eastern habitats occupied by the species.

Vegetative Litter and Grass Tussocks

Leaf litter and tussocks afforded by warm season bunchgrasses appear to be important habitat components for regal fritillaries, including for the eastern subspecies in Pennsylvania. Vegetative litter and grass tussocks provide protective areas (shelter) for larvae in winter and spring, for pupae in late spring early summer, and for adults later in the summer (Swartz et al. 2015, p. 815). These tussock structures are known to build up over years (Ferster and Vulinec 2010, p. 39). The exact time required to establish suitable tussocks for the regal fritillary has not been determined (Swartz et al. 2015, p. 814). Accumulated leaf litter has been positively associated with regal fritillary presence (Powell et al. 2007, p. 304; Davis et al. 2007, p. 1351; Vogel et al. 2007, p. 83; Helzer 2012, p. 9; Caven et al. 2017, p. 200). The positive relationship between probability of regal fritillary presence and percent litter cover can be linear when percent litter cover is 72 to 100 percent, and the probability of regal fritillary presence can be very low when percent litter is less than 72 percent (Caven et al. 2017, pp. 198, 200). Leaf litter provides shelter for eggs, larvae, and pupae. Larva may hide in bunches of grass and litter near violets (Baker c. 1932, p. 2).

Shrubs or Tall Vegetation

The regal fritillary is associated with high quality native prairie, and invasion of shrubs and other woody vegetation is typically viewed as degradation of this habitat type. Yet shrubby components within western prairie grasslands, particularly those with nectar sources, such as leadplant (*Amorpha canescens*) and western snowberry (*Symphoricarpos occidentalis*), may be important areas used by adult regal fritillaries (P. Hammond, personal communication, 2021). Shrubs or small trees (particularly those with nectar sources) have been noted as being important in eastern habitats as well (Schweitzer 1992a, p.4) including Pennsylvania (Ferster and Vulinec 2010, p. 33), Indiana, and Illinois (Illinois Department of Natural Resources 2020, p. 2) as resting areas for adult females. In addition to shrubs, or perhaps in their absence, adult regal fritillaries are also known to use tall clumps of bunchgrasses for shelter.

Violets

Violet species (*Viola spp.)* are the only host plants consumed by regal fritillary larvae. Violets must be present at an adequate level of abundance and distribution in order to ensure that at least some of the first instar larvae that survive winter diapause ultimately find violets on which to feed, allowing them to continue development through their remaining five instars, pupate, and emerge as adult butterflies that will establish the next annual population. Since the random nature of egg-laying does not guarantee proximity to host plants, larvae that survive the winter may exhaust their reserves before they can access violets (Selby 2007, p. 30). If these plants are not available to the larvae, the site cannot serve as a natal area for the species.

Different violet species may grow in different conditions and soil types along a gradient from wet to dry, thus occupying a variety of microhabitats on the landscape. Additionally, preferences by regal fritillary larvae for certain violet species have been reported in different regions, but they are known to use a variety of species and will likely use whatever local native species is present (Schweitzer 1989, p. 135; Royer and Marrone 1992, p. 21; Selby 2007, p. 29; Ferster and

Vulinec 2010, p. 32; S. Ellis, personal communication, 2021). While larvae may use any local violet they find, not all violets are equally nutritious for the larvae (E. McKinney, personal communication, 2021). Violets are typically sparsely distributed and there is a limit on the number of larvae that a finite set of violets can support. Violets do regenerate leaves if there's enough soil moisture, but without enough violets in drought years, the larvae may not acquire adequate nutrition to develop to their full potential, resulting in stunted adult butterflies. Individual larvae may consume 3 or 4 leaves per day, perhaps up to 10 leaves per day depending on both the size of the violet leaves and the larvae: equating to possibly 30 to 40 plants during the larval stage for an individual (P. Hammond, personal communication, 2021). Scaled up to population size, this would equate to 20,000 violets consumed by a population of 500 regal fritillaries (considered a relatively healthy population size [Hanski and Thomas 1994, p. 169]).

Regal fritillary populations cannot exist without violets, but while violets have been correlated with regal fritillary presence or abundance (e.g., Kelly and Debinski 1998, p. 272; Caven et al. 2017, p. 202; Henderson et al. 2018, p. 46), the presence or high density of violets does not always equate to presence or greater abundance of regal fritillary populations (Swartz et al. 2015, p. 822). Other factors, such as recent fires, isolation, local climatic variation, or need for other resources (e.g., nectar sources or bunchgrasses) may play a role (Swengel 1996, p. 80; Swengel 1997, p. 7; Debinski and Kelly, 1998, p. 19; Kelly and Debinski 1998, p. 272, Swengel 2004, p. 3; Ferster 2005 p. 9; Ferster and Vulinec 2010, p. 39; Swartz et al. 2015, p. 822, McCullough et al. 2019 p. 13).

However, if regal fritillaries are present in an area, relatively more violets and violet patches within a habitat would serve to increase the likelihood of larval survival, particularly the vulnerable first instar that must search for young violets in the spring (Wagner et al. 1997, p. 271). The density of violets necessary to establish and maintain regal fritillary populations has been suggested to be 2 to 3 plants per square meter (11 square feet) (Henderson et al. 2018, p. 46).

Diverse Floral Resources (Nectar)

As with violets, nectar sources are an important resource need as food for adults. Abundant diverse nectar resources (quality and quantity) must be available for adult regal fritillary nutrition from early summer through early fall in order to ensure survival and eventual reproduction. Absent this resource, an area cannot support regal fritillaries. Yet, as with violets, an abundance of diverse nectar sources does not guarantee presence abundance of regal fritillaries due to the potential influence of other factors but would provide for the species if present. Efforts to associate regal fritillary numbers with nectar plants have been variable – positive correlation in some cases (e.g., Vogel et al. 2010, p. 669; Huebschmann 1998, p. 25; Ries and Debinski 2001, p. 845), and no clear correlation in others (e.g., Mason 2001, p 13). Annual variation in regal fritillary numbers may occur on the same sites between years, despite little differences in nectar sources, indicating complexities in the relationship exist beyond simple quantifications of flowering plants (Huebschmann 1998, p. 27). Variation also occurs within years, as the butterflies shift in response to availability of nectar sources (Schweitzer 1992a, p. 7).

Preferences for specific nectar sources by adult regal fritillaries have been documented, particularly milkweeds (*Asclepias*), bergamots (*Monarda*), and thistles (*Cirsium*) (Huebschmann 1998, p. 29; Swartz et al. 2015, p. 822); however, the butterflies appear able to use a variety of other nectar sources, including coneflowers (*Echinacea*), blazing-stars (*Liatris*), goldenrods (*Solidago*), clovers (*Trifolium*), and ironweeds (*Vernonia*) (Selby 2007, p. 28). Many of these plants exhibit pink or purple flowers, preference for which has been documented (Schweitzer 1992a, p. 7; Swengel 1993, p. 6; Antonson 2020, p. 80; Marschalek 2020, p. 894). Abundant nectar sources that provide protein and amino acids are critical to support adults, particularly females, as nutrition significantly affects reproduction into the late summer and early fall.

Moisture

Adults need some amount of moisture to satisfy their nutritional requirements. In general, moisture requirements within habitats occupied by the regal fritillary vary somewhat; historically occupied grasslands in the eastern part of the species range have been described as mesic (moist), while regal fritillary populations in the west occupy xeric (dry) habitats (Swengel 1997, p. 12; Williams 1999, p. 5; Mason 2001, p. 13; Caven et al. 2017, p. 198), but these generalities do not always hold true. Upland sites of the Pennsylvania population are mostly dry fields where the necessary vegetative habitat components can be found, such that any potential reintroduction efforts there should focus on dry fields for habitat restoration, and wet meadows should be restored to increase nectar-plant availability for populations established in nearby dry field sites (Swartz et al. 2015, p. 824). The species has been documented in both dry and wet sites within the same geographic area (Mason 2001, p. 20). The highest regal fritillary abundances may occur in dry sites, with the second highest observed in mesic sites, and the lowest abundance at wet sites (Mason 2001, p. 13). The species may persist in relatively drier sites of the tallgrass prairies of the Midwest (Swengel 1997, p. 7), while in mixed-grass prairies of the Central Great Plains, regal fritillaries have been shown to be limited to more mesic bottomlands where violets and bluestem grow (Hammond 1995, p. 4; Ratcliffe and Hammond 2002, p. 41). The species may actually be sensitive to moisture at both extremes; flooding of wet sites may drown larvae (Mason 2001, p. 20), while dry spring weather may result in starved larvae if violets senesce early due to lack of moisture (Bliss and Schweitzer 1987, p. 5). In xeric habitats, individuals may be at risk of desiccation, particularly first instar larvae which have a high ratio of surface area to body mass (Sims and Shapiro 2014, p. 163; Nail 2016, p. 17). In captive rearing situations, creating humid conditions is important for raising eggs, larvae and pupae (e.g., Becker 2018, pp. 7, 8, 11). In the wild, microhabitat characteristics appear to be important to the species (e.g., more moisture [and resources] on north-facing slopes versus south-facing slopes or in wetland or riparian zones) (Kral-Obrien et al. 2019, p. 306). The presence of bluestem may be indicative of appropriate soil moisture in regal fritillary habitat; well-drained soils with facultative upland plants have been positively associated with adult regal fritillary presence (Caven et al. 2017, p. 199).

Ambient Temperature

Suitable ambient temperatures are needed for the growth and development of larvae and for adults to survive. Minimum air temperatures for normal flight activities of the regal fritillary have been observed to be 24 to 27°C (75 to 80°F) in Nebraska (McCorkle and Hammond 1988,

p. 192), but they have been known to fly at temperatures of 41°C (105°F) or more (McCorkle and Hammond 1988, p. 192). Other similar species of adult fritillaries require high body temperature for normal activity, so they typically fly only in full sunshine but may also fly under cloudy conditions if the air temperature is over 21°C (70°F) (McCorkle and Hammond 1988, p. 190). While the regal fritillary is apparently adapted to the temperatures within its current range, extreme temperatures, particularly of extended duration, may impact development and survival of individuals. Unusually cold periods can extend larval or pupal development timeframes and reduce survival or inhibit reproductive activity (Selby 2007, p. 36) and unusually hot periods may do the same. Early developmental stages of three species of Copper butterflies (*Lycaena tityrus*, *L. dispar*, and *L. helle*) may experience stress and mortality rates when temperatures are too high (Klockmann and Fischer 2017, p. 10872).

Recent work with the monarch (*Danaus plexippus*) has identified heat thresholds that may be relevant to the regal fritillary. The monarch is of similar size and often co-occurs with the regal fritillary. In a laboratory setting, larval monarchs exhibit high-temperature induced sublethal effects at 38°C (100.4°F), significant mortality between 40 to 42°C (104 to 107.6°F), and 100 percent mortality at 44°C (111.2°F) (Nail 2016, pp. 4, 9, 13, 15). Further, observed sublethal thermal effects to monarchs include slowed development, smaller adult mass, or greater risk of failing during pupation (Nail 2016, p. 16). It may also be possible for individuals to adapt somewhat to high temperatures; heat-shock proteins which are produced in response to exposure to high temperatures, may increase thermotolerance at temperatures previously unsuitable for development (Nail 2016, p. 11). Additionally, behavioral responses to extreme conditions (e.g., larvae seeking shaded microsites for shelter, or adult females selecting such sites for egg deposition; Kopper et al. 2000, p. 657) likely reduces the risks of high daytime temperatures posed to individual regal fritillaries. Nocturnal activity may also afford some measure of mitigation during extreme heat, particularly for larvae (Schweitzer 1992, p. 8; Kopper 2001c, p. 96; McCullough et al. 2017, p. 149). Research is currently ongoing to better inform the potential impacts of temperature on the regal fritillary and other butterfly species (L. Ries, personal communication, 2021).

Periodic Disturbance and Management Considerations

Regal fritillaries require relatively large intact native prairie habitats with adequate abundances of native violets for larval food and diverse nectar sources that can sustain adults throughout the summer and into early fall. Regardless of regional location, grassland habitats with regal fritillary populations require periodic disturbance. Eastern U.S. habitats for the species may have reached peak availability during colonial times when substantial clearing of trees opened up new areas to allow population expansion (Schweitzer 1992b, p. 15; Wagner et al. 1997, p. 271). Habitats at the single remaining eastern population of regal fritillaries in Pennsylvania are the result of military activities that maintain open areas and promote regal fritillary presence (Zercher et al. 2002, p. 13). The area is also managed by prescribed burning and removal of woody vegetation by mechanical methods such as mowing and tree cutting (Ferster and Vulinec 2010, p. 39). Occasional activities by military personnel that open up patches or disturb portions of existing patches also perpetuate suitable habitat for the species (M. Swartz, personal communication, 2021).

Native grassland habitats in the Midwest and Great Plains were particularly affected by ungulate grazing and fire. These disturbances remove excessive thatch, control invasive species, inhibit woody vegetation encroachment, promote native plant growth, and generally ensure perpetuation of open native grassland habitats. Fire and grazing would often act on the landscape in tandem; herbivores roamed the landscape freely and were drawn to recently burned areas, leaving unburned areas less grazed and more susceptible to future fires, creating heterogeneity on the landscape - a shifting mosaic of vegetation to the benefit of a diverse suite of native grassland inhabitants (Fuhlendorf and Engle 2001, p. 626; 2004, p. 2; Fuhlendorf et al. 2009, p. 2). Absent the natural regimes of fire and herbivorous grazing that maintained regal fritillary habitats historically, these habitats now require active management. Prescribed burns, livestock grazing, haying, spraying of herbicides, mechanical removal of invasive species and woody vegetation, and vegetation restoration activities are surrogate mechanisms to ensure grassland ecosystems remain viable.

However, such measures need to be applied carefully with respect to timing, frequency, and intensity, as they may have negative impacts to regal fritillaries (McCullough et al. 2019, p. 12). The regal fritillary is univoltine (one brood per year; annually completes one life cycle). Individuals spend approximately three-quarters of their lives on the ground, limited to the local site where their egg was laid and they overwintered as tiny larvae, and includes the relatively small area beyond that in which the growing larvae may need to traverse to sustain themselves on violets and eventually locate a place to pupate. Thus, management actions to sustain habitats during that time (early fall to early summer) can be detrimental not only to individuals, but to populations when the actions occur to a large percentage of the population's occupied habitat patch. Haying and grazing can favor regal fritillary abundance by leaving some litter in place while opening the canopy to improve violet growth, which results in relatively higher numbers of individuals (Royer and Marrone 1992, p. 26; Swengel 1996, p. 80, Swengel 1997, p. 12; Swengel 1998, p. 79; Powell et al. 2007, p. 304). In contrast, heavy grazing can remove and eventually homogenize vegetation as well as result in trampling of eggs, larvae, and pupae, while haying can remove nectar sources, reduce larval host plant availability, and perhaps harm the same early life stages (McCullough et al. 2019, p. 12).

Wildland fire may be a useful management tool for regal fritillary populations. Fire typically promotes all the necessary habitat components required by regal fritillaries, such as the growth of forbs, including important nectar sources and larval host plants, and the dominant warm season grasses like big bluestem (McCullough et al. 2019, p. 9). Wildland fire may improve the availability of violets by increasing flower production, growth, and seed production (Lovell et al., 1983 *in* Henderson et al. 2018, p. 42). In some cases, violets may be observed onsite after burns within 10 days (K. McCullough, personal communication, 2021).

However, the use of fire has a geographic context based on the size and connectivity of habitat patches. In areas that are small and isolated, fires that are poorly timed and too frequent, large, or severe can reduce local regal fritillary population size and may eliminate the species from burned areas (Royer and Marrone 1992 p.26; Swengel 1993, p. 7; Swengel 1996, p. 80; Kelly and Debinski 1998, p. 272; Huebschmann and Bragg 2000, p. 387, Swengel 2004, p. 3; Powell et al. 2007, p. 304). In Iowa, regal fritillaries were positively correlated with time since burn, a trend that continued after nearly 6 years (Vogel et al. 2010, p. 668). The primary mechanism may be direct mortality of larvae in the burned vegetation, but potential additional factors involved in population losses may include the removal of vegetation that occurs with fire, including the insulating leaf litter, violets used by larvae, and nectar sources used by adults, as well as their univoltine life cycle, which may increase risk of losses (Swengel 1996, p. 80; Selby 2007, p. 34). When fire is used alone and in excess on prairie remnants, prairie plant and animal diversity, particularly of insects, may decrease (Williams 1997, p. 117).

Despite the potential detrimental effects, fire has been identified as an important driver of regal fritillary densities in areas that are large or may have highly connected habitat patches. This "prairie butterfly paradox" describes species like the regal fritillary that are known to be sensitive to fire impacts, yet they occupy habitats that include disturbances such as fire to maintain them (Moranz et al. 2014, p. 32). In Kansas the highest population levels were found on sites with moderate (3 to 5-year) prescribed burning intervals (McCullough et al. 2019, p. 9). Similarly, a 3 to 4-year burn frequency in Nebraska has been noted as a realistic management strategy for regal fritillaries (Huebschman and Bragg 2000, p. 387). In Iowa at a 40.5-ha (100-ac) prairie on the Neal Smith National Wildlife Refuge, regal fritillaries have persisted even after repeated annual burns (P. Drobney, personal communication, 2020), albeit this could be due to colonization from nearby habitats. In Indiana, an approximately 2,255-ha (5,500-ac) dry sands prairie restoration area at Kankakee Sands is "aggressively managed with fire" and the species has responded positively, expanding its occupancy into adjacent Illinois oak barrens habitats that support the species (J. Shuey, personal communication, 2015). More generally, an average 3-year fire return interval in tallgrass prairie, followed by intense grazing, may help create a mosaic of patches at variable stages that resulted in "a fully functioning, resilient tallgrass prairie landscape that provides habitat for a variety of grassland obligate species that occur in the area but have very different habitat requirements" (Fuhlendorf et al. 2009, p. 594).

Conflicting information regarding the potential impacts of fire on regal fritillary populations may be due in part to patch size and isolation. The Midwest is dominated by agriculture, and remnant tallgrass prairies containing regal fritillaries are often relatively small and surrounded by cropland. Small patches themselves may not contain the topographic and vegetative mosaic needed to support regal fritillaries long-term, and management actions needed to develop such conditions is more difficult on a small scale. Isolation by barriers and significant distances, or disturbances applied at the same level as on larger tracts can be detrimental to small populations. In these instances, unless the mosaic of habitats is maintained via diversity of additional proximal suitable habitat patches that serve as sources for recolonization of disturbed sites, populations become vulnerable to permanent extirpations, particularly when burned in their entirety (McCullough et al. 2019, p. 9). Closer analysis of fire effects on regal fritillary populations in Wisconsin using compiled data over a 20-year timeframe at 7 sites in the Midwest (Wisconsin) revealed that after burning, regal fritillary populations may be suppressed at first, but then rebound quickly (80 percent recovery in first 2 years), reach a peak in 4 years, and decline again in subsequent years without fire (Henderson et al. 2018, p. 45). Importantly, all burned sites were within 622 m (2,041 ft) of unburned regal habitat that served as refugia, allowing for recolonization (Henderson et al. 2018, p. 45). Research in other areas (e.g., Missouri) has shown regal fritillary abundance returning to pre-fire levels within the first year after burning, likely due to recolonization (Moranz et al. 2014, p. 37).

In the western part of the range, relatively larger expanses of prairie exist, maintaining grassland mosaics interspersed with row crop agriculture and rural towns, as opposed to the primarily urban and agricultural mosaics of the Midwest that contain small, isolated patches of relict grasslands. In large prairie expanses, when fire occurs on a portion of the area, refugia are available within other portions of the occupied habitat or often in patches nearby. Larvae on nearby undisturbed areas surviving to adulthood can move quickly into recently burned sites, utilizing the new growth in similar numbers as in refugia during the burn year (Huebschman and Bragg 2000, p. 387). Large areas may also accommodate haying and grazing simultaneously, which can similarly be used without detriment to species' abundance on such sites (McCullough et al. 2019, p. 11). With inherently more topographically and vegetatively diverse conditions in large areas, these habitats lend themselves more readily to the shifting mosaic of conditions exploited by regal fritillary populations. The resulting local heterogeneity promotes temporal and spatial shifts in occupation by regal fritillaries over the larger patch(es), enhancing long-term population persistence.

If fire is applied to small, isolated areas, efforts to mimic natural wildfires (inclusion of unburned patches) should be made, perhaps in concert with other disturbance mechanisms (Swengel 1998, p. 84) to promote regal fritillary abundance (e.g., a scenario where 5 percent is burned, 5 percent is grazed, 5 percent is hayed, some brush cutting occurs and some in left unmanaged) (Swengel 2004, p. 4). Where at least three occupied patches exist in proximity, burning no more than one (about 30 percent) in any given year, never consecutively, and at least 3 years (preferably longer) apart may be ideal (Schweitzer 1992a, p. 29). More specific recommendations are provided in Schweitzer (1992a, pp. 29–30). On larger habitat patches in the Great Plains, larger portions of the habitat may be disturbed (P. Hammond, personal communication, 2021) without significantly impacting regal fritillary populations.

In Wisconsin, establishment of "permanent non-fire refugium" has shown beneficial effects; refuge areas are managed with alternate methods such as mowing or brushcutting, but not fire (Swengel 2004, p. 3). These refugia at the core of habitat patches enhances vital resources and regal fritillary populations, while burning refugia had the opposite effect (Swengel and Swengel 2007, pp. 274–275). As many as 6 to 8 years of rest after a burn may be required for sites to function as regal fritillary refugia (Swengel and Swengel 2007, p. 276) and regal fritillary negative responses to fire have been documented to last 3 to 5 years in this area (Swengel 1996, p. 79). Notably, that is the same interval noted above as supporting regal fritillary populations in western sites.

Importantly, regal fritillary larvae have been documented to survive fires. If fires occur when the larvae are small and at soil level, the damaging effects may pass over some individuals; later in the season, when the larvae are present higher within the vegetation, their susceptibility to the heat and flames may increase (P. Drobney, personal communication, 2020). More mature larvae may also escape harm in some instances, as documented in Kansas when a live regal fritillary larva was observed immediately after a prescribed fire, apparently escaping the flames by sheltering under a rock (K. McCullough, personal communication, 2021).

McCullough et al. (2017, p. 149) performed larval searches in the Flint Hills of Kansas and found 54 percent of their larval observations (n=12) occurred in areas that had been burned 61 days or less prior to detection, suggesting that regal fritillary larvae, having evolved over millennia in fire-dependent ecosystems, have developed adaptations for surviving fire. Moranz et al. (2014, p. 33) also observed that fire, along with associated grazing, were common disturbances in tallgrass prairie habitats in which the regal fritillary evolved, and these served as major selective forces.

The natural fire and grazing synergies that occurred historically on the prairie landscape have been replaced with land use activities that are not necessarily conducive to healthy grassland ecosystems. As a result, actions and events today can result in unintended changes to the vegetation: some native plants such as sunflowers may proliferate after a fire and shade out native violets; native grasses like big bluestem can also proliferate in the first year after a fire and suppress violet growth (it may take 2 to 3 years for post-fire growth to subside somewhat and allow native forbs to grow); areas heavily overgrazed for long periods of time may not necessarily reveal preferred results after a burn; native patches left alone for perhaps a dozen years produce bluestem thatch that may crowd out other plants (P. Hammond, personal communication, 2021). When fire is used in areas such as Wisconsin where prairie remnants are not within a surrounding matrix of prairie, commonly applied early season burns to control invasive grasses may not have the desired effect of resetting the native prairie vegetation. The fires may instead allow recruitment of weeds at the expense of native prairie floral diversity; subsequent vegetation growth can crowd out violets; and the burns may kill above-ground woody plants that sprout from roots and ultimately spread further (A. Swengel, personal communication, 2015). Thus, while periodic disturbance is required to maintain regal fritillary habitat, site-specific complexities, such as geographic location, existing matrix, and past land use must be considered in order for those disturbances to benefit the regal fritillary.

Large Blocks of Native Grasslands (Patch Size)

The regal fritillary needs large blocks of native grasslands, and they may not prefer small habitat patches (Schweitzer 1989, p. 134). Small habitat patches lack the area needed to support the required shifting resources and disturbance regimes that maintain regal fritillary habitats long-term, and occupants of small patches are also vulnerable to localized stochastic events or inbreeding depression (P. Hammond, personal communication, 2021). Generally, small colonies on prairie remnants are considered likely dispersers from other localized fragments, with most individuals remaining in their natal patch, yet some interactions may occur among patches separated by unsuitable habitat (Mason 2001, p. 5). Such areas can support regal fritillaries, but populations and metapopulations that occur in large blocks of relatively unfragmented habitat are key to supporting high numbers of regal fritillaries that can persist, even in “bad” years when local conditions reduce their numbers.

Large areas of habitat are more likely to:

- Encompass topographic variability that allows for various microhabitats that may support regal fritillaries;
- Support the shifting habitat mosaic of varying successional stages that occur with periodic disturbance on different portions of the habitat; and

- Allow individuals to move freely and quickly among these habitats without having to move across unsuitable areas.

In an agricultural landscape, large areas also provide a buffer against pesticide drift from the surrounding agricultural landscape that may directly or indirectly affect regal fritillary survival. Additionally, large areas can support high numbers of regal fritillaries, creating populations and metapopulations that are better able to withstand stochastic events that cause large fluctuations in annual numbers.

While the term "large" is used to describe suitable habitats for the regal fritillary, the exact area required to support the species long-term is difficult to quantify and the term itself is somewhat relative, viewed differently in the context of highly fragmented versus less fragmented landscapes. Regal fritillary population sizes appear to correspond with patch size (large habitats support large populations; Kelly and Debinski 1998, p. 272), but a diversity of patch sizes and ranges of sizes may be needed to support regal fritillary populations (Table 8). Notably, populations in areas considered to be relatively large patches are not immune to extirpation. For example, drought conditions in Iowa beginning in 2012 caused the extirpation of some isolated populations in that state occupying areas sized approximately 162 ha (400 ac) and recolonization had not yet been noted as of 2019 (P. Hammond, personal communication, 2021).

Table 8. Suggested sizes of regal fritillary habitat patches necessary for long-term population or metapopulation persistence and source. Abbreviations used in this table: ac = acres, ha = hectares.

PATCH SIZE	NOTES	LITERATURE SOURCE
50/65 ha (124/161 ac) or more	Minimum hectares needed for viable populations versus the size defined as "large"	NatureServe 2021, entire
101 ha (250 ac) or more	Hectares of high quality to semi-degraded prairie with topographic diversity and moisture gradient needed for viable populations	Schweitzer 1992a, p. 28
"Hundreds of acres"	Area needed for long-term population persistence (100 ac = 40 ha)	Schweitzer 1989, p. 134
405 ha (1,001 ac)	Area of continuous extensive prairie to ensure stable populations	Royer and Marrone 1992, p. 25
104 ha (257 ac)	Minimum area to sustain viable populations	Zercher 2001 *in* Shepherd and Debinski 2005, p. 245
41/19-39 ha (101/47-96 ac)	Occupied patches described as "large" versus range of sizes considered "small"	Henderson et al. 2018, p. 43
24/1,350 ha (59/3,336 ac)	Observed size of patches in a complex of eight sites composed of about 5,000 ha (12,355 ac) of public land and a large amount of surrounding private grassland that support reliably persistent populations	Swengel and Swengel 2017, p. 4
0.9-53/4.7–21.1 ha (2.2-131/11.6-52.1 ac)	Observed available patch size range versus size of occupied patches harboring the five largest populations (200+ individuals); likely part of larger metapopulation with 38% of surrounding landscape as grassland	Powell et al. 2007, p. 300
67+ ha (166+ ac)	Patch sizes harboring the largest populations	Debinski and Kelly 1998, personal observation *in* Shepherd and Debinski 2005 p. 245
50/118 and 500 ha (124/292 and 1,236 ac)	Occupied patch size with "small" populations versus patch sizes of sites used as sources for reintroductions, the latter harboring a "large and thriving" population	Shepherd and Debinski 2005, p. 245
Approximately 2,225 ha (5,498 ac)	Available habitat supporting "a robust mainland and island structure" that has expanded to satellite populations	J. Shuey, personal communication, 2015

Needed patch size is likely variable, with numerous confounding factors and local context affecting patch occupancy (Niemuth et al. 2021, pp. 4–5). In general, small, isolated patches may contain colonies, but generally are not optimal habitats. However, the FTIG eastern subspecies population in Pennsylvania is likely an exception, with habitats across 444 ha (1,097 ac) within a span of about 10 km (6.21 mi). Despite the small patch size, this population at FTIG has persisted while other populations in the east were extirpated, likely due to habitat disturbances afforded by military action and management activities.

Regardless of the lack of an exact patch size threshold, the larger the habitat patch (assuming an active disturbance regime), the better it can support abundant and resilient regal fritillary populations. Large contiguous grasslands tend to have more variable site conditions that support more diverse plant life; their greater area encompasses more habitat overall, and they are more likely to exhibit the shifting mosaics of heterogeneous habitats that favor regal fritillary presence. For the purposes of this SSA (and for evaluation of current and future conditions), 1,000 ha (2,471 ac) is considered a large patch size, adequate to support regal fritillaries long-term.

There are caveats to the "larger is better" generalization. Grassland habitats, particularly those occupied by the western subpopulation in the Great Plains portion of the regal fritillary's range, are prone to stressors such as periodic drought. Suitable habitat in some years may exist only at the microhabitat level (e.g., wetland edges or riparian zones where a higher water table creates a hydric zone that supports violets and nectar sources) that allow populations to persist, albeit in relatively low numbers (P. Hammond, personal communication, 2021; Kral-Obrien et al. 2019, p. 306). These areas are more likely to be available to the species in large, diverse, grassland areas, but populations are known to contract significantly under drought conditions and become more susceptible to localized stochastic events. Some violet species are tolerant to relatively dry conditions and potentially could support larvae; however, nectar plants can be entirely absent from dry uplands (P. Hammond, personal communication, 2021).

At the westernmost edge of the range, riparian zones and other relatively wet habitats, such as spring-fed wetland sites that support both more mesic violet species and nectar sources may be the sole means by which regal populations exist, and some occurrences at the western boundary of the species' range may not be due to extant populations at all, but rather dispersals from locations in adjacent areas (S. Ellis, personal communication, 2020). Large native grassland patches are key to long-term persistence of regal fritillaries, but such areas will not support resilient regal fritillary populations unless the species' other life history needs are also present.

Contiguous Blocks of Native Grasslands

Another key need – whether at the colony, population, or metapopulation level – is appropriate configuration of habitat patches (Niemuth et al. 2021, p. 4). The matrix dictates the ease by which dispersing regal fritillaries can access the shifting mosaic of required resources. As noted above, contiguous large habitats present the best conditions supporting regal fritillaries and abundant, proximal patches easily accessible to dispersing adults may also support populations. With connectivity comes increased genetic diversity of populations. When habitats become more isolated via habitat fragmentation, the ability of individuals to move between populations may be significantly reduced (Williams 1999, p. 4) and inbreeding or other negative effects of low genetic diversity may occur. While the species may be capable of moving 100 mi (161 km) or more, most individuals likely do not move that far. Local daily movements occur to access resources as needed. As with patch size threshold, determining the ideal level of connectivity at which colonization of isolated habitats or inter-patch dispersals are likely versus unlikely to occur is difficult and variable. The proximity required among populations to ensure movements between them has not been (and perhaps cannot be) determined precisely, but with greater distance comes reduced likelihood of that movement.

Mark-recapture studies have been conducted documenting regal fritillary movements and other studies have suggested proximity among patches that facilitate, versus inhibit, movements by regal fritillaries; Table 9 summarizes some reported values. Mark-recapture studies are often limited partly by the size of the study area. Attempts to recapture butterflies on a broad landscape scale becomes increasingly difficult as area increases, thus any individuals moving beyond the study area may never be redocumented to determine distance traveled. Additional information on dispersal and genetic exchange among sites would be helpful in determining population dynamics and persistence along with relationships between habitat quality and size (Henderson et al. 2018 p. 47).

Table 9. Suggested distances needed between occupied habitat patches to facilitate regal fritillary movements and connectivity among patches, and their respective literature sources.

DISTANCE	NOTES	SOURCE
0.8 km (0.5 mi)	Distance at which colonization of extirpated patches may become limited or take significant time, particularly if barriers such as tree lines exist	Caven et al. 2017, p. 200
Approximately 1–7.5 km (0.6–4.7 mi)	Approximate range of movements documented between demes	Ferster and Vulinec 2010, (see map, p. 32, and patch movements, p. 36)
0.15–0.4 km (0.09–0.3 mi)/ 0.25–3.5 km (0.16–2.2 mi)	Distances between patches facilitating ease of movement versus distance that may reduce movements (may include barriers such as tree lines)	Henderson et al. 2018, p. 43
2.9–3.4 km (1.8–2.1 mi)	Minimum inter-patch distance moved by two individuals (also noted that some individuals flew out of the study area, out of sight)	Marschalek 2020, p. 894
4 km (2.5 mi) / 10 km (6.2 mi)	Separation distance for unsuitable habitat versus separation distance for suitable habitat	NatureServe 2021

The distances that regal fritillaries may traverse within, among, or between colonies, populations, and metapopulations is likely highly variable and influenced by many factors. The above distances between patches suggested or documented to have been traversed by regal fritillaries are relatively short, but as noted previously, the species is characterized by strong and rapid or tireless flight (Royer and Marrone 1992, p. 4; Selby 2007, p. 26). A single metapopulation in

Indiana has recently expanded in response to habitat improvements and resulted in colonization of multiple, previously unoccupied habitats, one of which is 40 km (25 mi) away from its source (J. Shuey, personal communication, 2015). Notably, the new Indiana colony is connected to that metapopulation source by a riparian corridor, and the same river (with tributaries) extends into two adjacent Illinois counties that now have regal fritillary colonies as well.

A recent recolonization of an extirpated site in Iowa may have been repopulated by a southern Minnesota population located approximately 48 km (30 mi) to the north; the sites are linked by the Des Moines River in Iowa (P. Hammond, personal communication, 2021; J. Petersen, personal communication, 2021). Riparian areas contain intact grasslands with moisture levels that support nectar sources and violets and are not typically sprayed with herbicides. They are not the block habitats typically occupied by regal fritillaries, but in an agriculturally dominated or dry landscape, they can represent the only habitat available and likely serve as natal habitat, provide refugia, and act as natural dispersal corridors. In Wisconsin, regal fritillary records in isolated habitat patches have been observed to be close to waterways (Swengel and Swengel 2017, p. 16), thus colonization of sites that exist in the vicinity of these corridors may occur more frequently or sooner than sites far removed from these habitats. Large riparian corridors, such as the Platte River in Nebraska or the Missouri River bluffs spanning several states, are known to be relatively reliable in supporting regal fritillaries (Nagel 1992, p. 10; P. Hammond, personal communication, 2021). Such connectivity among habitats allows regal fritillaries to access the shifting mosaic of resources, and whether it be via traversable distances or the existence of riparian habitat corridors, that connection is another key need for persistence of regal fritillary populations.

AU Needs

The resiliency of an AU depends on landscape-level availability of individual resource needs, as described above, include a shifting mosaic of grassland habitats across differing successional stages that are available annually. We identified the following seven demographic factors that influence the resiliency of AUs for both the eastern and western subspecies:

- **Survival** of all life stages, particularly larvae and adult females, due to their contribution to recruitment and abundance;
- **Recruitment**;
- **Abundance**, or the number of individuals in an AU;
- **Growth trend**;
- **Fecundity**;
- **Connectivity**; and
- **Genetic diversity**;

We added these demographic needs of AU to our conceptual model for viability in Figure 20.

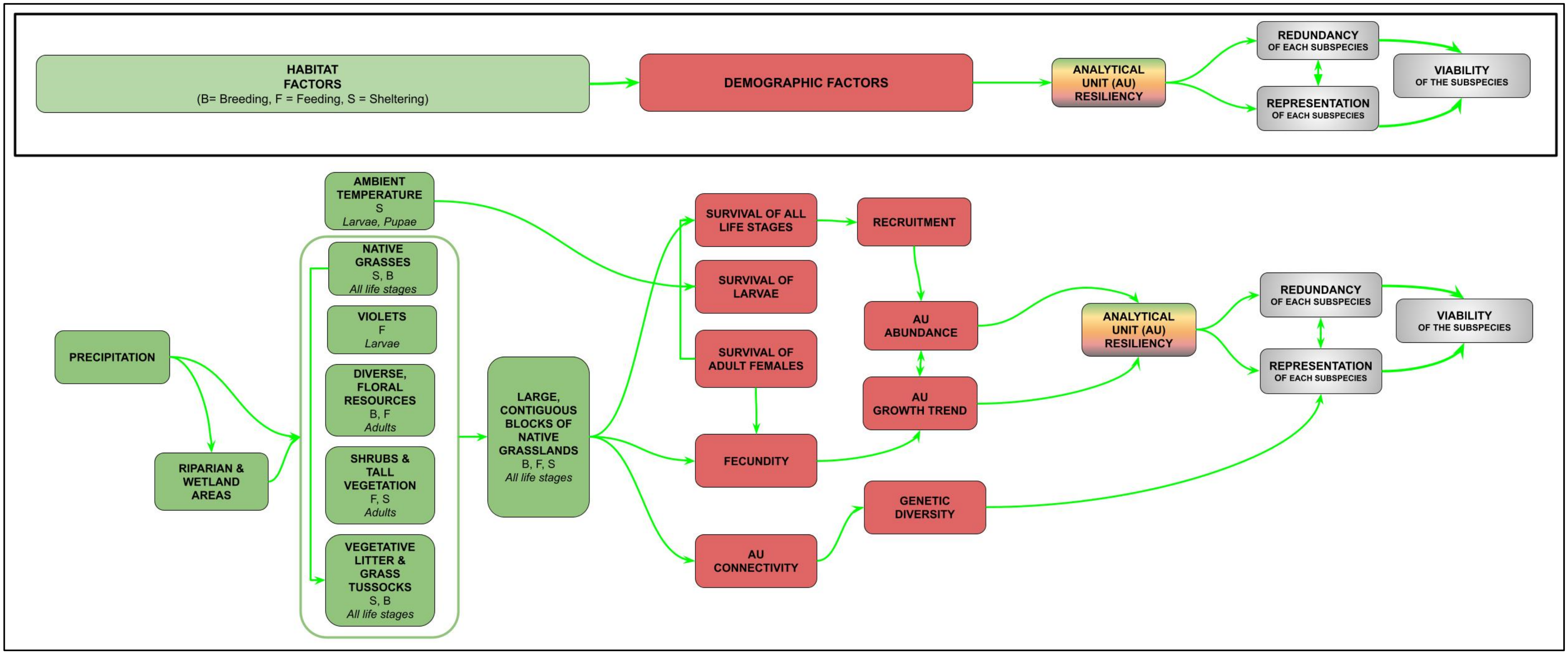

Figure 20. Conceptual model for regal fritillary AU resiliency, in terms of habitat factors (green boxes) needed by individuals to breed (B), feed (F), and shelter (S), and demographic factors (red boxes) that AUs need to be resilient. Green arrows represent positive relationships between nodes. The core conceptual model is provided at the top of the figure for reference.

Many of these demographic factors that we identified as needs for the AUs cannot be directly measured due to the small size and cryptic nature of the species' early life stages, susceptibility to unpredictable stochastic events, high mobility of adults, and overall boom-and-bust nature of the species. Although some information is available from captive rearing efforts, factors within wild populations such as fecundity rates, hatching rates, survival rates at any life stage, and recruitment rates, may be highly variable within and between years and by local area or region. Additionally, individuals may not be distributed equally across the landscape, and may be clumped within habitat patches based on the availability of microhabitat variables (Selby 2007, p. 21; Marschalek 2020, p. 895). Therefore, many of these demographic factors may be difficult to measure or approximate.

Further, as with many insect species, there may be substantial changes in numbers from one year to the next, perhaps at an order-of-magnitude level (Panzer 1988, p. 83). If diverse nectar sources are abundant and available to females before laying their eggs, the resulting high number of surviving females and high fecundity rates will establish a positive start for the next annual generation. Alternatively, if few females survive to oviposit and their nutrition is lacking, fecundity will be lower. Egg hatching success may depend on numerous local and unpredictable environmental factors (e.g., internal stores, temperature extremes, solar radiation exposure, predation), but egg fertility is not known to be limiting. A significant reduction in population numbers is thought to occur after hatching, during the first instar stage. The mortality rate of these tiny larvae is presumed to be very high, as they are faced with surviving harsh weather conditions from autumn to spring and finding a host plant (Selby 2007, p. 32); even in laboratory settings, the mortality of this life stage can be significant (over 80 percent) (Wagner 1995, p. 4). The subsequent maturing larval instars (2^{nd}–6^{th}) and pupa, being restricted to leaf litter with low or no mobility, also represent relatively more vulnerable stages than the adult phase; disease, predation, adverse weather conditions, competition for violet host plants, land use impacts (e.g., fire, pesticides) are known to impact them. The number of adult females surviving to reproduce then becomes key to establishing the baseline once again for the next generation, but again, this is highly variable and often cannot be predicted with accuracy.

In general, AUs need a sufficient number of individuals (abundance) in order to withstand environmental and demographic stochasticity. Resiliency of the AU increases with a greater number of individuals, yet the dynamics may be somewhat variable. Populations within an AU that have high abundance populations may increase exponentially in good years when conditions are most favorable, and they may still support high or at least stable numbers of individuals even in poor years. Such sites may serve as sources for recolonization of less viable satellite areas that may become extirpated in poor years.

An estimated 200 to 400 adults in a population may be needed to maintain genetic diversity; when numbers consistently fall below that level, reductions in fertility and fecundity may occur and populations may be lost (P. Hammond, personal communication, 2021). Modeling has shown that isolated butterfly populations with an equilibrium population size of less than 50 individuals are unlikely to last 100 years (typically much less), 500 individuals or more are needed to persist 100 years, and many thousands are required for populations to persist indefinitely, not considering stochastic events or changes in habitat (Hanski and Thomas 1994, p. 169). Few local populations of any butterfly species meet the highest thresholds and are subject to natural extirpation (Hanski and Thomas 1994, p. 170). Unless they are on very large sites with diverse habitat and high occupancy, isolated regal fritillary populations are at risk of local extirpation.

AU resiliency is defined by populations of adequate size, the ability to withstand order-of-magnitude annual fluctuations, and typically occurs on large contiguous native grasslands (or adequate numbers and sizes of proximal smaller patches that function collectively in a similar, albeit less ideal, manner) that exhibit a shifting mosaic of resources on a landscape scale and provide for the life-history needs of the egg, larvae, pupae, and adults over time. Such populations are better able to recover from stochastic events and withstand annual variation in the environment. The larger the populations are, and the larger and more connected their occupied suitable habitats are, the more resilient they will be over time. Connectivity among healthy populations, particularly relatively small ones, is important to ensure gene flow and recolonization of areas after population contractions or extirpations occur. Exact thresholds for these factors, at which declines, or extirpations begin to exceed expansions and recolonizations, are not known.

Subspecies Needs

The subspecies need an adequate number and distribution of AUs that are sufficiently connected with large abundance in order to withstand stochastic events (resiliency), catastrophic events that may impact a broader area (redundancy), and AUs must exhibit genetic or environmental adaptive capacity to withstand future biological and physical changes to their environments (representation). The 3Rs are discussed further below and summarized in Table 10.

Subspecies Redundancy Needs

Redundancy for the regal fritillary is characterized as having numerous resilient populations distributed across a breadth of geographic regions (i.e., representation units) to improve the ability of the subspecies to withstand catastrophic events, such as a widespread drought in the Great Plains. Depending on drought location, duration, and severity, such an event could be devastating to the species. If reservoirs of regal fritillaries persist in areas typically not impacted by drought (e.g., wetter tallgrass prairies of the Midwest, riparian areas or those with higher density of wetlands within the Great Plains (Kral-Obrien et al. 2019, p. 306)), the risk of extirpation is mitigated. Changes in climate are expected to differ in various portions of the regal fritillary’s current range, potentially altering long-term suitability of habitats; adequate number and distribution of populations is key to species persistence.

Subspecies Representation Needs

Representation is the ability of the regal fritillary to adapt to changes in its physical and biological environments. This adaptive capacity is essential for viability, and can be gauged by the breadth of genetic, phenotypic, and ecological diversity of species, as well as the ability to disperse and colonize new areas. This variation is evaluated at both a large scale (i.e., morphological, behavioral, or life history differences and environmental or ecological variation across the range), and a smaller scale (e.g., measures of interpopulation genetic diversity). It is also important to examine natural levels and patterns of gene flow, degree of ecological diversity occupied, and effective population size. The more representation or diversity (genetic, morphological, or behavioral) a species has, the more capable it is of adapting to changes (natural or human caused) in its environment. Both subspecies need sufficient ecological and genetic diversity to maintain or improve adaptive capacity.

Table 10. Summary of ecological needs of the regal fritillary at the AU (resiliency) and subspecies levels (redundancy and representation).

THE 3Rs	NEEDS FOR VIABILITY	DESCRIPTION
RESILIENCY The ability of AUs to withstand environmental stochasticity, periodic disturbances within the normal range of variation, and demographic stochasticity	High population abundance	Overall high numbers of individuals present; exponential population growth occurs when conditions are favorable; numbers may drop, but remain relatively stable at high levels when conditions are less favorable; able to withstand order-of-magnitude population fluctuations
	Large tracts of suitable habitats or complexes of multiple patches of proximal suitable habitat patches	Contiguous grasslands dominated by native species with required resources (i.e., adequate violets, nectar sources, grassland structure, and adequate environmental conditions); patch sizes that reach thousand(s) of hectares size represent highest resiliency albeit hundreds of hectares may support (less) viable populations
	Connectivity among suitable habitats	Distances among habitat patches are not prohibitive, and the matrix facilitates movements (e.g., riparian corridors, grasslands) that allow access to necessary resources on landscape scale and facilitates relatively frequent genetic exchanges and recolonizations
REDUNDANCY The ability of the subspecies to withstand catastrophic events that could lead to population collapse regardless of population health and for which adaptation is unlikely	Numerous resilient occupied AUs distributed broadly across the species' range	Adequate numbers and distribution of AUs reduce catastrophic losses occurring via regional scale events (e.g., drought), allowing for subspecies viability.
REPRESENTATION The ability of the subspecies to adapt to both near-term and long-term changes in its physical and biological environments (i.e., adaptive capacity)	Genetic diversity	High genetic diversity allows higher potential for adaptive capacity; regular genetic exchanges preclude problems such as inbreeding depression or genetic bottlenecks
	Ecological diversity	Diverse ecological settings allow for local adaptations that may buffer against stochastic or catastrophic events

Summary of Needs

Individuals of the eastern and western subspecies need sufficient quantities and qualities of native grasses, violets, diverse floral resources, shrubs and tall vegetation, vegetative litter and grass tussocks, and suitable ambient temperatures in order to breed, feed, and shelter. Native grassland patches should be large with sufficient violets, abundant nectar sources that are connected or relatively close to other large, similar patches that offer a shifting mosaic of successional stages accessible to individuals. AUs need a sufficient abundance of individuals, with survival of all life stages and sufficient recruitment, abundance, growth trend, fecundity, genetic diversity, and connectivity to be resilient. For redundancy, the subspecies need numerous resilient populations distributed across a breadth of geographic regions (i.e., representation units) to improve the ability of the subspecies to withstand catastrophic events, such as a widespread drought in the Great Plains. For representation, both subspecies need sufficient ecological and genetic diversity to maintain or improve adaptive capacity.

Local regal fritillary populations are naturally prone to local extirpations, recolonizations, population expansions, and contractions. Historically, habitat was not limiting, and entire landscapes were available to the species to access opportunistically; today, habitat loss and fragmentation have significantly altered the species' ability to access the shifting mosaic of habitats with which it has evolved, affecting the population dynamics. The species exhibits typical island biogeography characteristics, with the smallest occupied sites (colonies) being most at risk and ephemeral and the largest areas (populations and or metapopulations) being the most stable or persistent. When resilient sites are destroyed (e.g., converted to agriculture or development) or become degraded (e.g., encroachment of woody vegetation or invasive grasses; reduced in size and isolated), regal fritillary populations may not persist, and reductions to AU resiliency and the redundancy and representation of the subspecies may occur. Conversely, when the habitat needs of individuals and the demographic needs of AUs are met, the subspecies may have sufficient levels of the 3Rs to withstand stochasticity, catastrophes, and environmental change.

Chapter 4 – Cause-and-Effects: Stressors and Conservation Measures

In order to evaluate the current and future conditions for the eastern and western regal fritillary subspecies, we first explore the environmental changes, whether natural or anthropogenic, that may have occurred to result in the subspecies' current condition and that may influence condition into the future, in terms of the 3Rs (Service 2016, p. 14). In this chapter, we describe the cause-and-effect factors, or influences, that may positively or negatively influence the viability of the regal fritillary subspecies. These may directly impact the subspecies by influencing the demographic factors, or indirectly by influencing the habitat factors that we identified as subspecies needs in Chapter 3. In order to inform our evaluations of current and future conditions, we evaluated the sources, stressors, and activities that can positively (conservation actions) or negatively (stressors) affect the regal fritillary at the individual, AU, or subspecies levels, either currently or into the future. By identifying the anthropogenic and natural factors that influence the habitat and demographics of the subspecies, we can evaluate the current and future resiliency of each AU, and the cumulative effects on those AUs determine conditions related to redundancy and representation for each subspecies.

A stressor is a change in a habitat or demographic resource, such as a decrease in violets or decrease in abundance. Some stressors may directly influence the demographics of an AU through mortality of individuals resulting from actions or activities, such as agricultural conversion, while others, such as drought, may affect habitat factors that may indirectly affect individuals by influencing demographic factors. Some stressors may directly affect individuals and habitat factors at the same time, and stressors may act cumulatively. The stressors that we evaluated for the eastern and western subspecies of regal fritillary include:

- **Grassland conversion**, resulting from agricultural and urban development;
- **Pesticide** use and drift;
- **Invasive plants**, including the encroachment of woody vegetation;
- **Drought;**
- **Climate change** and **local climate events;**
- **Periodic disturbances**, such as from fire, haying, and grazing;
- **Disease;**
- **Parasitism;**
- **Competition and hybridization with sympatric fritillaries;** and
- **Collection.**

Conservation efforts that may either reduce a stressor or improve the condition of habitat or demographics for the subspecies include:

- **Reintroduction programs;**
- **Land use management plans**, such as the Integrated Natural Resource Management Plan (INRMP) at FTIG; and
- **Voluntary conservation efforts.**

Figure 21 updates our conceptual model for subspecies viability with these stressors and conservation efforts that we evaluated. Our evaluation of these sources and stressors revealed that while some may have relatively minor effects to individuals, such as specimen collecting, some factors may more strongly influence the habitat and demographic factors that we identified as needs, such that they influence the resiliency of AUs. We called stressors that may influence the current or future resiliency, and as a result, redundancy and representation of the subspecies, "drivers." For example, aerial application of herbicides across large areas that eliminate forbs from grasslands, or grassland conversion that permanently eliminates habitat, invasive plants that crowd out native species, may reduce the quality and quantity of habitats and reduce the resiliency of AUs. Some factors that we identified may have both positive and negative influences, such as periodic disturbances from fire, haying, and grazing. Climatic factors, particularly drought, may directly affect individuals by reducing the availability of suitable ambient temperatures and the availability of native grasslands and violets.

Below, we provide background on each stressor and conservation effort. Then, we summarize our analysis for each factor in terms of its potential current and future effect to individuals, AUs, and the subspecies. During this evaluation, we considered the factor's exposure, immediacy, magnitude, and geographic scope to assess the response by individuals, AUs, and then the subspecies. Appendix H provides the cause-and-effect tables that document this analysis. Due to their influence on viability, we carried forward those stressors and conservation efforts that we identified as drivers in our consideration of current and future conditions in Chapters 5 and 6, respectively.

The factors that we carried forward as drivers are climate change, drought, invasive plants, woody encroachment, and periodic disturbance for both subspecies. Additional stressors carried forward for the western subspecies are conversion and herbicide use. The eastern representative unit receives relatively more moisture than habitats of the western subspecies to the west and is less susceptible to drought. The eastern subspecies is vulnerable to woody encroachment and periodic disturbances are necessary to ensure the grasslands do not become reforested.

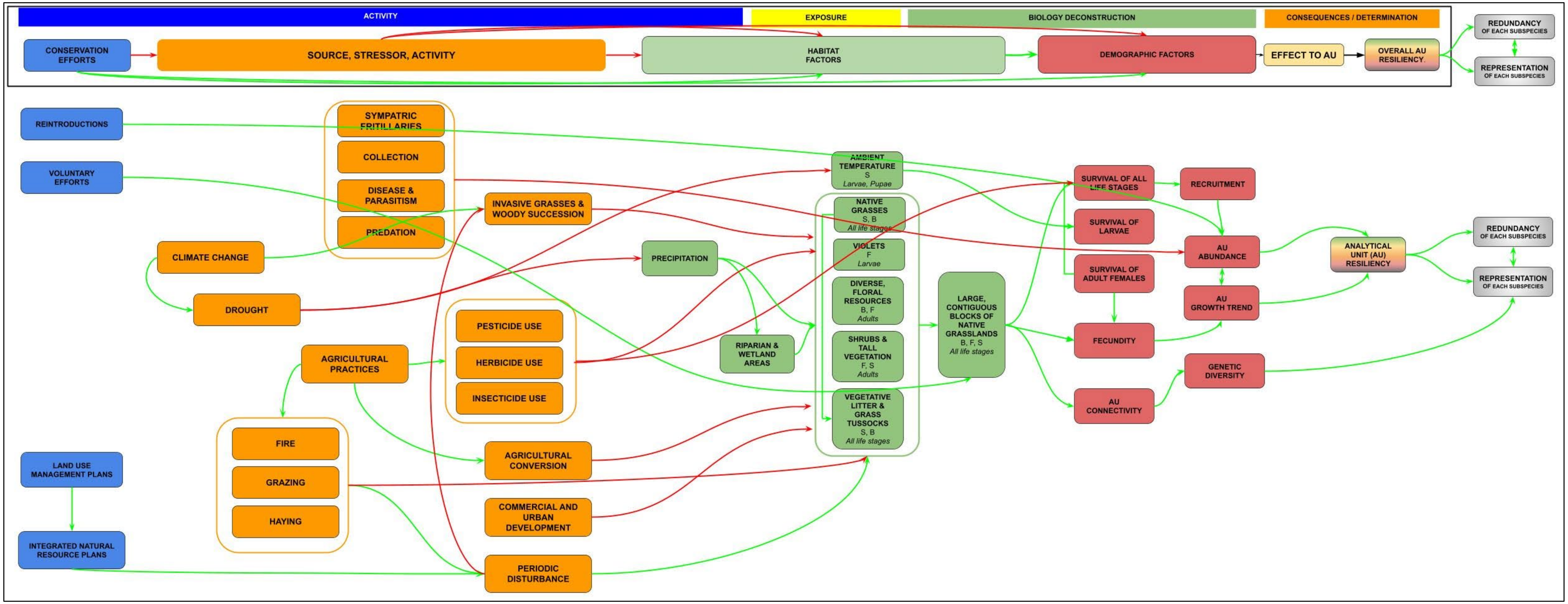

Figure 21. Conceptual model illustrating the influence of stressors and conservation measures on habitat and demographic factors contributing to regal fritillary resiliency, redundancy, and representation.

Grassland Conversion

Conversion of grasslands to other uses, such as for agriculture, reduces the amount, availability, connectedness, size, and quality of native grasslands required by the regal fritillary. Numerous authors identify habitat loss as a primary stressor for the regal fritillary (e.g., Hammond and McCorkle 1983(84), p. 218; Davis et al. 2007, p. 1342; Powell et al. 2007, p. 124; Selby 2007, p. 3;Selby 2007, p. 3; Sims 2017, p. 1; Swengel and Swengel 2017, p. 2; Marschalek 2020, p. 891; Niemuth et al. 2021, p. 2). Agriculture and development are two primary conversion activities. In the majority of AUs within the regal fritillary's overall range, the percentage of habitats converted to agricultural land use is greater than the percentage converted to human developments (Table 11). We note that the Ridge and Valley AU in Pennsylvania contains a relatively low percentage of cultivated crops compared to the percentage comprised of development; however, cover types are eclipsed by the total hectares of forested land cover. In this AU, agriculture is not a grassland conversion stressor for the eastern subspecies, but woody encroachment is a stressor, as discussed below.

Table 11. Percentage of cultivated crops versus developed land use cover in analytical units within the regal fritillary's current range. Cultivated crops include "areas used for the production of annual crops, such as corn, soybeans, vegetables, tobacco, and cotton, and also perennial woody crops such as orchards and vineyards. Crop vegetation accounts for greater than 20 percent of total vegetation. This class also includes all land being actively tilled" (Dewitz 2021, entire) while developed cover includes all four 2019 NLCD "developed" cover classes: developed open spaces and low, medium, and high intensity developed areas.

ANALYTICAL UNIT (AU)	CULTIVATED CROPS (percent)	DEVELOPED COVER (percent)
PA Ridge and Valley	8.9	21.1
Central Corn Belt Plains	73.3	13.9
Central Great Plains	50.5	4.6
Central Irregular Plains A	31.7	5.1
Central Irregular Plains B	21.2	9.5
Cross Timbers	2.1	6.4
Driftless Area	34.8	6.3
Flint Hills	15	4.2
High Plains	42.6	4.3
Interior River Valleys and Hills	40.8	9.4
Lake Agassiz Plain	76.5	4.8
Middle Rockies	1.3	1.7
Nebraska Sand Hills	4.1	0.8
North Central Hardwood Forests A	23.6	6.8
North Central Hardwood Forests B	35.3	11.5
Northern Glaciated Plains	62.5	4
Northwestern Glaciated Plains	43.4	3.3
Northwestern Great Plains	13.2	1.5
Ozark Highlands	2.9	8.1
Southeastern Wisconsin Till Plains	46.9	17.3
Southern Rockies	0.1	0.6
Western Corn Belt Plains	72.5	6.7

Currently, small remnant grassland patches represent islands of habitat for the regal fritillary that exist isolated within a matrix dominated by agriculture, particularly in the Midwest. Such sites may be occupied with relatively low numbers of regal fritillaries. The tendency to encounter edges in these relatively small areas is greater than in larger sites, perhaps resulting in higher emigration rates, draining small populations where they exist, and causing individuals that disperse into highly fragmented landscapes to have difficulty finding new, suitable patches (Ries and Debinski 2001, p. 849). Factors limiting recolonization may include isolation from other occupied sites, relatively impermeable barriers, and a surrounding landscape with few other similar patches (so as to make them unlikely to be detected by randomly dispersing regal fritillaries). Regal fritillaries can be temporarily reestablished on small, isolated prairie remnants via dispersing females that randomly find the suitable habitat, lay eggs, and establish larvae that survive to adulthood. However, unless additional dispersing adults find the site as well, inbreeding issues may again lead to local extirpation as regal fritillaries are generally known to be highly vulnerable to genetic inbreeding depression based upon laboratory experiments with brother-sister crossbreeding (P. Hammond, personal communication, 2021).

Small grassland tracts containing regal fritillary colonies may be more vulnerable to extirpation than larger blocks of native grasslands, but multiple colonies occurring as part of a collectively larger group may function together as a population. When adults in colonies can move across the matrix to reach other suitable habitat patches, the collective occupied habitats may exhibit diverse conditions that can better support the subspecies' life-history needs. Similarly, large contiguous habitat patches that accommodate the shifting mosaic of habitats at varying successional stages within their boundaries that allow regal fritillaries to access necessary resources may also support populations.

Suitable regal fritillary habitat is a shifting resource, and the subspecies are well adapted to use it on a landscape scale. Habitat loss and fragmentation of grasslands, and lack of natural historical processes to maintain the habitats, may reduce the ability for individuals to access needed habitat resources. Grassland conversion may result in small habitat patches that exist in a matrix dominated by agriculture and development. If source populations do not exist nearby, extirpated sites may or may not be recolonized by individuals.

The larger the patch size, the more opportunity for these local patches to exist and support resilient populations over time. Formerly continuous grasslands in the central U.S. have been reduced in quantity, size, quality, and/or proximity via human activities that have altered the ability of the regal fritillary to use those resource requirements and sustain healthy viable populations. We discuss grassland conversion from agriculture and development activities below.

Agriculture

An estimated 162 million ha (400 million ac.) of native prairie historically existed in North America prior to European settlement in the 1800s; these biomes have since been converted primarily to agriculture, resulting in as much as 99.9 percent reduction in native prairie ecosystems, with the most severe declines among former tallgrass habitats (Samson and Knopf 1994, p. 418). Those tallgrass prairies of the eastern Great Plains and Midwest represented the core of the regal fritillary's historical range. The majority of tallgrass prairie today, particularly in the Midwest, is limited to small, isolated remnant tracts that are fractions of their former size and extent. Further west, mixed-grass prairie has also been impacted by conversion and other uses; mixed-grass has been reduced to 30 percent of historical amounts with about 7.8 million ha (19.3 million ac.) remaining (World Rangeland Learning Experience 2021, entire). Much of the mixed-grass prairie is also fragmented and isolated. Shortgrass prairies at the western edge of the western subspecies' range are the most intact among the three prairie types, but it is not apparent that regal fritillary populations occur there (Selby 2007, p. 24). Small, ephemeral colonies may be found in scattered moist habitats within these relatively dry grasslands (e.g., riparian zones, wetland edges, seeps or springs) (S. Ellis, personal communication, 2020).

The direct loss of grassland habitats continue today as agricultural and economic opportunities may continue to convert grasslands, even when agricultural yields are relatively low (Hoekstra et al. 2005, p. 25; Lark et al. 2020, p. 1). Grasslands (native and planted) were the source for 77 percent of all new croplands (primarily corn and soybeans) from 2008–2012 (Lark et al. 2015, p. 5). There was substantial geographic variation in the distribution of ongoing cropland conversions, identifying "hotspots" of change; the greatest amount of new cultivation occurred in the Prairie Pothole Region of eastern South Dakota and North Dakota, as well as the lesser-cultivated areas of southern Iowa and northern Missouri (Lark et al. 2015, p. 3). Approximately 647,500 ha (1.6 million ac) of long-term (more than 20 years) unimproved grasslands (longstanding prairie and range-like locations) were converted to cropland during a 4-year study period, much of it in the plains between North Dakota and Texas (Lark et al. 2015, p. 5). Revisiting conversion rates again and adding another 4 years (2013–2016) to the previous 2008–2012 analysis, Lark et al. (2020, p. 3) identified the impacts to grasslands, excluding planted areas, to examine potential impacts to wildlife and found the same hotspots identified above as well as additional areas, including along the Canadian border of the Northern Great Plains (Figure 22). Across the U.S., grasslands, including those used for pasture and hay, constituted 88 percent of the land converted to crop production; the highest rates of natural landcover relative to its remaining area occurred in the western Corn Belt and western Plains (Lark et al. 2020, p. 3). Notably, these areas are within the range of the regal fritillary. That study also specifically estimated loss of milkweed plants – a significant nectar source for regal fritillaries – and determined a loss of 8.5 percent of milkweed stems due to conversion since 2008 (Lark et al. 2020, p. 4).

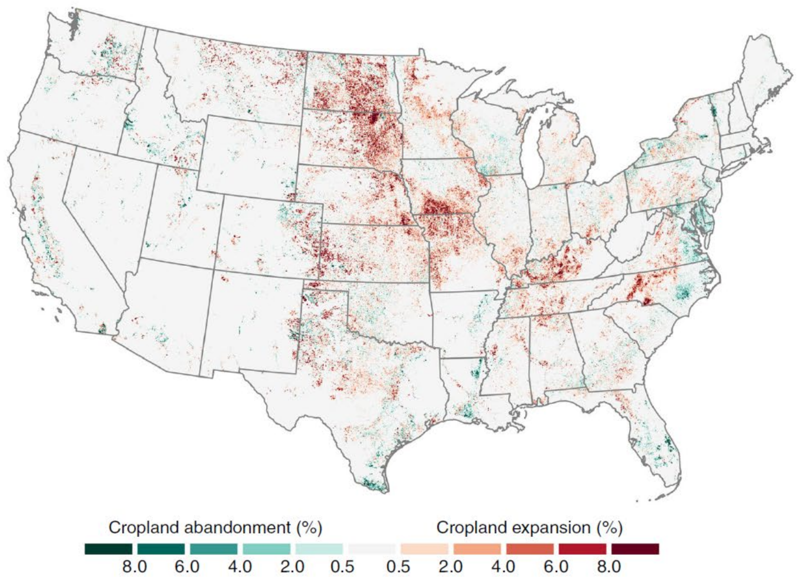

Fig. 1 Net cropland conversion 2008–16. Rates of net conversion calculated as gross cropland expansion minus gross cropland abandonment and displayed as a percentage of total land area within non-overlapping 3 km × 3 km blocks. Net conversion was most concentrated in the eastern halves of North and South Dakota, southern Iowa, and western portions of Kansas, Kentucky, and North Carolina.

Figure 22. Excerpt from Lark et al. (2020, p. 2) identifying hotspots of crop expansion across the United States which encompasses the range of the regal fritillary, 2008–2016. Abbreviations used in this figure: km = kilometers

In addition to the obvious reduction in the amount of habitat available for the regal fritillary, grassland conversion may fragment and isolate regal fritillary populations, which could increase risk of local extirpations. Regal fritillaries thrive in large native grassland habitats. Small habitats lack required diversity, and periodic disturbances that would otherwise not be a threat to regal fritillary populations can result in permanent extirpations. When habitat patches become small, fragmented, and isolated, the species' access to these resources, at the scale they need to flourish, deteriorates; dispersals are less successful, recolonizations become less likely, genetic diversity of populations is reduced, inbreeding may suppress expansion of populations, the capacity of populations to adapt to their changing environment is reduced, and local extirpations may begin to outpace recolonizations, which could reduce the viability of the subspecies.

Development

While agriculture is the dominant activity that has impacted North American grasslands, any other activity that removes native prairie sod may be a contributing factor to the loss and fragmentation of regal fritillary habitat. Besides agriculture, grassland conversion activities include road construction, road maintenance, gravel mining, housing and commercial developments, and energy projects (Selby 2007, p. 3). Historically, these activities cumulatively have had a significant impact on the prairie landscape, although not at the magnitude comparable to agriculture. Like agriculture, development is an ongoing source of loss and fragmentation of grasslands.

Some anthropogenic activities occur throughout the range of the regal fritillary, but only impact populations at the local scale. Road construction may occur nearly anywhere. While the footprint of roads may be cumulatively large, the level of impact to regal fritillary populations due to this activity is likely to be at a local scale. Previously intact large prairie tracts may be divided into smaller patches by roads, and risks to individuals may be imposed by traffic on those roads, but regal fritillaries would not be precluded from occupying remaining adjacent suitable habitats. Gravel mining may occur nearly anywhere as well (at least where gravel resources are available), and while gravel mines may be large and involve complete removal of grassland habitat, the impacts are typically finite and at a local scale. Similarly, a majority (but not all) of new housing and commercial developments are likely to incur finite impacts at a local scale, and these actions are most likely to occur near larger cities on the outskirts of expanding urban areas, not typically in large intact grassland habitats, which tend to exist in more rural areas.

In contrast, energy projects such as oil and gas drilling, wind energy, or solar projects, may have a disproportionate impact to regal fritillary habitats, as undeveloped areas may be impacted – even targeted – by these activities. Economic and political policies often drive these actions, sometimes resulting in frenetic periods of development. The impacts of energy projects to insects, however, is not well studied. For some energy development projects, if the footprint is small and native grasslands remain intact with necessary resources in adjacent areas, the action may incur impacts similar to highways – loss of some habitat and addition of new mortality risks, but unlikely to completely displace populations or preclude regal fritillary occupation of nearby suitable habitats. This may be the case for projects like oil/gas drilling or wind energy facilities and their associated infrastructure. Solar may present a different issue, particularly with ground-based photovoltaic cell projects that involve placement of numerous, closely spaced panels on the landscape as these projects may involve large-scale removal or degradation of grasslands that occurs during installation of solar panels (Chiabrando et al. 2009, p. 2445). Potential exists for significant impacts, depending on project size, potentially rending large habitat patches unsuitable for regal fritillaries, but recent research on this issue is lacking.

Cumulatively, grassland conversion due to non-agricultural development has in the past, is currently, and is expected to continue to reduce the amount of habitat available to regal fritillaries and may directly impact individuals. Specific, local projects may not be as impactful to the species as widespread agriculture has been, but the effects to individuals in the immediate footprint and adjacent areas can be detrimental. Exact effects may vary by individual site circumstances and the surrounding land-use matrix. Additionally, other stressors may act in tandem with energy projects to impact regal fritillaries, including the introduction of invasive plants, alteration of disturbance regime, or pesticide use, which are discussed below.

To summarize, grassland conversion, whether due to agriculture or development, historically contributed to the loss and fragmentation of the habitat needs of both subspecies, and is likely to continue to reduce the availability of the subspecies' habitat needs in the future. Agriculture historically had the most severe impact to regal fritillaries, while numerous other forms of conversion have contributed to the lost and fragmentation of needed habitats. This stressor may result in the permanent loss of available habitats, increased fragmentation, and isolation that may reduce numbers of local populations and the overall abundance of AUs, resulting in potential declines in AU resiliency and overall decreases in the viability of the subspecies. As a result, we considered grassland conversion as a current and future driver of resiliency for AUs and potentially the viability of both subspecies.

Pesticide Use and Drift

Individuals of either regal fritillary subspecies may be exposed to pesticides. Pesticides, which include herbicides, insecticides, and fungicides are commonly used on crop fields throughout the regal fritillary's range. They are chemicals used to control plant and animal pests that would otherwise impact crop yields, and have been used intensely throughout the Midwest. In the western part of the range of the western subspecies, herbicides may also used be used to control weeds in grasslands and native prairies of the mixed-grass and shortgrass prairies. Pesticides come in many forms: liquid, granular, dust, and as seed coatings, and are applied by multiple vehicles such as airplanes and helicopters, farm equipment, all-terrain vehicles (ATVs), and on foot. Timing and frequency of pesticide use varies by type and purpose; however, pesticides are generally used throughout the primary growing season (spring through fall) which coincides with the egg, larvae, pupae, and adult stages of the regal fritillary life cycle. Regal fritillaries may be impacted directly by pesticides by foliar application, and indirectly by exposure to contaminated seed, plant tissue, and soil, as well as consuming contaminated plant tissue. More individuals may be exposed to pesticides in already fragmented or isolated habitats. Additionally, if applicators are not attentive to wind conditions, end-row spacing, or droplet size, for example, pesticide drift onto adjacent lands can occur. Among the pesticide types, herbicides and insecticides may kill individuals, reduce habitat quality and quantity, may reduce the abundance of local populations, and may reduce the resiliency of AUs. We discuss herbicides and insecticides below.

Herbicides

Herbicides are chemicals that may be used at least once in a growing season to control broadleaf weeds or grasses in crop fields. Herbicides are also commonly used to control woody vegetation and weeds in both public and private grasslands, including native prairie. If not used carefully, herbicides can indirectly impact regal fritillary populations by eliminating or reducing nectar and foodplants, especially if applied during critical periods of the lifecycle. Adverse effects can occur when herbicides are applied within regal fritillary habitat or nearby via drift (Dana 1997, p. 3; Stark et al. 2012, pp. 25, 27; Cordova et al. 2020, p. 5). The effects of herbicide use may be especially problematic in areas where nectar food sources are already limited. Herbicide drift from adjacent croplands into regal fritillary habitats may have limited and temporary effects to individuals and habitats by temporarily reducing the availability of the habitat needs.

Generally, herbicides are considered "safe" because active ingredients target plants and not insects. Direct effects of herbicide active ingredients on regal fritillary are not well known or understood, but in general, any population declines from herbicide use may be due to changes in the plant community or from inert ingredients in the herbicide, which are not tested for toxicity (Fox 1964, p. 407; Stark et al. 2012, p. 26). Herbicides are often mixed with a surfactant (surface active agent that reduces the surface tension of water) and solvents (collectively referred to as adjuvants), so individuals may not only be exposed to the active ingredient, but also the adjuvants, which are often not included in the risk assessments required for pesticide registration (Mullin et al. 2015, p. 2). For example, 4 out of 11 commercially available spray adjuvants were toxic to honeybees (Goodwind and McBrydie 2000, p. 232). Active ingredients and inert ingredients may interact synergistically, which may result impacts that would not occur by exposure to the active ingredients alone (Mullin et al. 2015, p. 3). It is possible that similar direct or cumulative impacts from active and inactive ingredients may affect individual regal fritillaries. However, there is no information regarding the extent of exposure or the magnitude of effect from herbicide use specifically for regal fritillaries.

Herbicides may also be aerially sprayed across large areas of native grasslands to reduce forbs so that more grasses are available to graze livestock. This practice is ongoing, particularly on private lands in eastern South Dakota, the Flint Hills of Kansas, and Oklahoma, and may dramatically reduce the quantity of violets and nectar sources available for individual regal fritillaries. Unlike the potentially limited or temporary effects to habitats and individuals from herbicide drift, this practice directly exposes native grasslands to herbicides, so could dramatically reduce numbers of violets and nectar sources. The reduction and removal of violets and nectar source in native grasslands may extirpate local colonies (Selby 2007, p. 36), and if more widespread, could also decrease population abundance and the resiliency of AUs. Although the extent of this practice is unknown, it has been observed and reported, and may be increasing.

Herbicide use may decrease the availability of the subspecies' resource needs, especially if used directly and broadly to decrease forbs in native grasslands. Specifically, herbicides may reduce the availability of violets and nectar sources, but the reduction may be temporary or localized if herbicides drift into native grasslands from adjacent croplands. The effects may be more permanent and widespread if herbicides are applied aerially and broadly in native grasslands. Active and inert ingredients in herbicides may also interact and could be toxic to individuals. Herbicide use is ongoing and will continue into the future. If widespread, such that numbers of violets and nectar sources decline dramatically, herbicide use could decrease the resiliency of AUs. The best available information indicates that herbicide use may affect individuals, colonies, and local populations, and currently or in the future may reduce the resiliency of AUs and the viability of the western subspecies.

Insecticides

Insecticides are chemicals developed and applied to crops to kill insects that may reduce crop yields. Most insecticides that may kill or retard the growth of individual regal fritillaries are "broad-spectrum," so are designed to kill a wide range of insect species. The larvae of Lepidopterans are considered "pests" in agriculture, so many insecticides are tested specifically on Lepidopteran species to ensure their effectiveness. Regal fritillaries exposed to insecticides may be killed or affected in other ways. For example, the Gettysburg National Military Park population of regal fritillaries disappeared after spraying local wooded areas with *Bacillus thuringiensis* (BT), a bacterial insecticide**,** for spongy moths (Schweitzer 1993, p. 9). How severely the regal fritillary individual is impacted by insecticide use likely depends on the concentration of the insecticide to which it was exposed and/or whether the insecticide became incorporated into plant tissues (e.g., leaves, pollen, nectar) used by the regal fritillary larvae or adults.

Both the timing and method of insecticide application may influence how individual compounds affect target and non-species. Soybeans are a major crop planted throughout the historical overall range of the western subspecies, and insecticide application on soybean crops may coincide with the emergence of regal fritillary larval and egg-laying periods. When applied aerially, insecticides may drift as far as 60 m (197 ft), the longest distance measured, and may result in high mortality rates for larvae. Regal fritillary eggs are most often deposited near the ground on the underside of senesced vegetation, and therefore, may be less susceptible to pesticide residue (Krishnan et al. 2021a, p. 1772). High boom applications reduced the effects of pesticide drift for larva and eggs while still maintaining efficacy on crops. Finally, late instar larvae exposed via cuticle or dietary pathways may not die in that stage, but individuals display arrested pupal ecdysis (i.e., they fail to properly metamorphosize and die as pupae) (Krishnan et al. 2021b, p. 1).

Insecticides registered for use within the overall ranges of both subspecies are represented by three major classes: organophosphates, pyrethroids, and neonicotinoids. Lepidopteran toxicity data are not available specifically for the regal fritillary, but recent work with monarch butterflies detail effects from both acute and chronic exposure to compounds from all three classes (Krishnan et al. 2020, p. 932; Krishnan et al. 2021a, p. 1763). Additionally, all three classes have been detected in native prairie habitats in North and South Dakota and in western Minnesota that are used by the regal fritillary and the federally threatened Dakota skipper (*Hesperia dacotae*) (Runquist 2017, pp. 1–2, 3–7;; Skadsen 2010, 2011, 2012, 2013, 2016, 2017, 2018, 2019, 2020, 2021; Skadsen and Backlund 2014, 2015; Goebel et al. 2022, p. 7). Insecticide use in these areas may have resulted in local extirpations, followed by recolonizations, of regal fritillary populations, although there is no information to indicate that insecticide use affects more than individuals. Below, we summarize the three classes of insecticides.

Organophosphates and Pyrethroids

Several laboratory studies have examined the toxicity of select organophosphates and pyrethroids to nontarget lepidopteran species within the families Nymphalidae, Lycaenidae, Papilionidae, Hesperiidae, and Pieridae (Eliazar and Emmel 1991 p.19; Salvato 2001 p.13; Hoang et al. 2011 p.1001; Bargar 2012 p. 2126). In general, while toxicity was exhibited across all species and chemicals, no consistent patterns emerged either within or across studies that demonstrated sensitivity was related to species (or species group), lifestage, or size of adults, though inconsistency in testing regimes may limit the ability to detect patterns that exist. However, working with both larvae and adult monarch butterflies, Krishnan et al. (2020, p. 927) showed that beta-cyfluthrin (pyrethroid) and chlorantraniliprole (anthranlic diamide) displayed higher toxicities based on LD50s for both cuticle and dietary exposure compared to chlorpyrifos (organophosphate) and imadaclorphid and theomethoxam (both neonicotinoids). Chlorpyrifos is leaving the market (EPA press release, 18 August 2021: https://www.epa.gov/newsreleases/epa-takes-action-address-risk-chlorpyrifos-and-protect-childrens-health), and the pyrethroids are being phased out due to the soybean aphid (*Aphis glycines*) resistance to these compounds (Hanson et al. 2017, p. 2242).

Neonicotinoids

Neonicotinoid insecticides are neurotoxins designed to kill invertebrates by overstimulation. Use of neonicotinoids, especially as crop seed treatments, has grown exponentially both worldwide and in the U.S. in the last decade (Stone 2013, entire; Hladik et al. 2014 p.191). Because of their widespread use and persistence, neonicotinoids are frequently found in the soil, surface and ground water, and air samples (Mineau and Palmer 2013, pp. 37–42; Van der Sluijs et al. 2014, pp. 151–153; Simon-Delso et al. 2014, p. 26). Use of many neonicotinoids peaked starting in 2004, but their indiscriminate use started to decline sharply in 2015 (Pesticide national synthesis project 2021, entire).

Neonicotinoids persist and accumulate in soils, so may be found in nectar and pollen of treated crops and landscapes (Goulson 2013, pp. 979–981) and in guttation droplets (drops of xylem sap on the tip or edges of leaves) (Girolami et al. 2009, pp. 1811–1814). Reported levels of neonicotinoids in soils, waterways, field margins, and floral resources overlap substantially with concentrations that are sufficient to control pests in crops, and commonly exceed the LC50 (the concentration that kills 50 percent of individuals) for non-target insects (Goulson 2013, p. 985). While neonicotinoids may kill insects, there is little evidence of regal fritillary mortality from neonicotinoids. Drift of neonicotinoids from adjacent crop applications into regal fritillary habitats are likely restricted to immediate field borders. However, unlike population collapses observed in Dakota skippers that co-occur with regal fritillaries, regal fritillary populations may be less susceptible to population and AU-level impacts from neonicotinoids due to relatively higher numbers of regal fritillaries on the landscape and their comparatively high dispersal ability, which allows recolonization of areas that have been impacted by insecticide use but still harbor nectar sources. Therefore, insecticide use may affect individuals, but there is no information to indicate that it currently or in the future may reduce the resiliency of AUs or the viability of either subspecies.

Invasive Plants and Woody Encroachment

Invasive, exotic (non-native) plants and woody vegetation may degrade the quality and quantity of native grasslands needed by the regal fritillary. These may be plants that spread into native habitats from purposefully planted areas to form self-perpetuating populations (Fulbright et al. 2013, p. 505). The regal fritillary is a native grassland specialist, and needs specific vegetation, such as grasses and violets to breed, feed, and shelter, which makes it vulnerable to changes in vegetation. The invading plant species of concern and the magnitude, scope, and exposure to the regal fritillary and its habitat varies by location. Conservation efforts that target invasive plants, generally some type of disturbance regime such as fire, grazing, or mechanical/chemical controls, may reduce the stressor. However, invasive grasses and woody plant encroachment are known to degrade native grassland quality and quantity and may become more widespread, and potentially problematic, in the future. Invasive grasses and woody encroachment are discussed further below.

Invasive Grasses

Invasive grass species include Kentucky bluegrass (*Poa patrensis*) and smooth brome (*Bromus inermus*), which are the two primary species invading the Midwestern and Northern Great Plains prairies (Royer and Marrone 1992, p. 28;). These invasive grasses may make the conservation, rehabilitation, and restoration of native grasslands challenging, as smooth brome and Kentucky bluegrass are aggressive, highly competitive species that form dense monocultures that may exclude all other vegetation. These grasses may easily invade native prairies, have proliferated on lands that were idled or overgrazed, and may have been bolstered by longer growing seasons and increased precipitation that has occurred due to climate change (Bahm et al. 2011, p. 195; Murphy and Grant 2005, p. 353; Kral-Obrien et al. 2019, p. 302; DeKeyser et al. 2015, p. 258–259). Encroachment of these species in native prairies is pervasive and has continued over the last several decades, with a 35 percent increase of Kentucky bluegrass in North Dakota over 23 years, occupying approximately 82 percent of private rangelands in the Prairie Pothole Region of North Dakota and 61 percent in South Dakota (DeKeyser et al. 2015, pp. 256–257). In South Dakota, native grass cover may average 39 percent and cover by native forbs may average 11 percent but cover by smooth brome and Kentucky bluegrass may average 41 percent and 24 percent, respectively (Bahm et al. 2011, p. 191). Both species of invasive grass may dominate the vegetative cover and seedbank where established (Cully et al 2003, p. 994; Setter and Lym 2013, p. 158). Kentucky bluegrass may affect nitrogen cycling, pollinator diversity, and hydrology (Toledo et al. 2014, entire). Kentucky bluegrass is now a major component of the Northern Great Plains and may decrease species richness and diversity (DeKeyser et al. 2015, p. 259). Kentucky bluegrass and smooth brome may have initiated potential population-level impacts to regal fritillaries in the 1990s (Royer and Marrone 1992, p. 28).

A recent study in North Dakota, South Dakota, and Minnesota specifically examined the amount of invasive Kentucky bluegrass to the abundances of several butterfly species, including the regal fritillary (Kral-Obrien et al. 2019, entire). Sites with higher bluegrass cover were found to have lower plant species diversity and flowering forb richness (Kral-Obrien et al. 2019, p. 305). As with other native grassland obligate species, regal fritillary abundance was associated with greater plant species richness and less bluegrass cover; Kentucky bluegrass was characterized as having a strong negative influence on the regal fritillary (Kral-Obrien et al. 2019, pp. 304–305).

Similar issues with other invasive species are occurring in southern parts of the subspecies' range. Exotic warmseason species include Old World bluestem species, namely yellow bluestem (*Bothriochloa ischaemum*) and Caucasian bluestem (*Bothriochloa bladhii*), are becoming more problematic in areas of the Central Great Plains (Condos 2021, entire). Currently these species are more prevalent in the Southern Great Plains but are being planted as livestock forage in Kansas, and once established, are highly difficult to eradicate (Condos 2021, entire). These are bunchgrasses that outcompete native grass species (perhaps partially by changing microbial resources in the soil among other mechanisms; Wilson et al. 2012, p. 335), converting diverse native grasslands into monocultures that lack forbs and support fewer insects, particularly native pollinators (USFS 2018, p. 2). Over time, the continued degradation due to invasive grasses is likely to increasingly fragment and isolate habitats, posing additional risks to regal fritillary populations. Therefore, invasive grasses may have reduced the resiliency of AUs currently and in the past and may continue to do so in the future. As a result, we considered invasive grasses a current and future driver of resiliency for AUs and potentially the viability of both subspecies.

Woody Plant Encroachment (Succession)

In general, woody encroachment describes an advancement of grassland succession into forests. Woody plant encroachment and woody forbs can reduce the quality and quantity of available grasslands for regal fritillaries (K. McCullough, personal communication, 2021). Like invasive grasses, woody encroachment leads to altered plant communities and loss of grassland and savannah ecosystems, and efforts to control it often have limited success (Ratajczak et al. 2012, p. 702; Miller et al. 2017, p. 2298). Woody plants and forbs may encroach and establish, resulting in the declines of the regal fritillary's needed bunchgrasses, violets, and nectar sources. The shrubs, trees and other woody plants tend to grow taller than the grass, shade out the grassland understory, and if left unchecked, may eventually replace the prairie vegetation that the subspecies' need. Although regal fritillaries need shrubby, tall vegetation for shelter and as nectar sources, too much woody vegetation may decrease the density of regal fritillary colonies and local populations by reducing the availability of other needs (McCullough et al. 2019, p. 8). Succession (reforestation of previously cleared open pastures) of forests that replaced grasslands may have historically contributed to the widescale reduction of the eastern subspecies' overall range (Wagner et al. 1997, p. 271). The exact threshold at which woody vegetation becomes a detriment to the habitat versus a beneficial feature is unknown, and the threshold may vary depending on a variety of landscape features.

Woody plant encroachment may be more severe in certain areas, but it is not necessarily ubiquitous. It is unclear why woody plants proliferate in some areas and not others (Barger et al. 2011, p. 3), but encroachment may be influenced by livestock grazing, climate, topography, soils, and increased carbon dioxide in the atmosphere (Archer et al. 2017, p. 31). Grassland and savanna communities have experienced an average 45 percent decline in species richness (Ratajczak et al. 2012, pp. 699–700), and woody encroachment may reduce species abundance (Archer et al. 2017, p. 59). This appears to be problematic particularly in the Central Great Plains ecoregion, where woody encroachment rates were five to seven times higher across the Central Great Plains relative to ecoregions outside of the Great Plains (Barger et al. 2011, p. 3). Eastern red cedar (*Juniperous virginiana*) exhibits some of the highest encroachment rates in the regal fritillary's overall range (Barger et al. 2011, p. 3). In 30 years, woody vegetation invaded 34 percent of the Flint Hills of Geary County, Kansas, on unmanaged areas (Bragg and Hulbert 1976, p. 22).

Woody encroachment may be particularly problematic for the eastern subspecies, where forested ecosystems are more prevalent. At FTIG, the surrounding landscape is primarily forested. At FTIG, prescribed fire, mowing and targeted brush cutting are used frequently to suppress shrub and tree sprouts, and without this important vegetation management, habitat for the eastern subspecies would be rapidly reforested (M. Swartz, personal communication, 2020, 2021). The coverage of the Deciduous Forest land cover type in the Ridge and Valley analytical unit is 69,890 ha (172,701 ac), and Evergreen Forest cover type area is an additional 1,430 ha (3,535 ac) (Dewitz 2019, entire). These two cover types represent 45 percent of the overall land cover in the Ridge and Valley AU, so woody encroachment is a prevalent issue at FTIG and for the eastern subspecies. As with invasive grasses, over time, the continued degradation due to woody encroachment is likely to increasingly fragment and isolate habitats, posing additional risks to regal fritillary populations. Therefore, woody encroachment may have reduced the resiliency of AUs currently and in the past, and may continue to do so in the future, particularly for the eastern subspecies. As a result, we considered woody encroachment a current and future driver of resiliency for AUs and potentially the viability of both subspecies.

Drought

Drought is a naturally occurring event across North America, but it is considered one of the defining characteristics of grasslands (Tucker et al. 2011 p. 1; Wishart 2011, entire). The percentage of area in drought can be highly variable annually, but in some areas, such as the Great Plains, it is considered rare for severe drought not to occur somewhere in the region every year (Wishart 2011, entire). Due to climate change, droughts have been occurring more frequently, and across greater spatial scales, but few studies examine extreme drought effects on insects (Forister et al. 2018, p. 1), and there is little specific research on the response of regal fritillary to drought, although negative effects have been observed (Selby 2007, p. 10). In general, drought may have positive effects on insects, such as more rapid development and less exposure in vulnerable stages, and negative effects, such as reduced nectar availability and early senescence of host plants (Forister et al. 2018, p. 1). While butterflies are typically considered warm-loving species, extreme hot or dry periods can drastically reduce population sizes directly through heat stress to larvae or by reducing the plant quality and quantity of host and nectar plants (Oliver et al. 2015, p. 941; Forister et al. 2021, p. 1044).

The regal fritillary appears to be drought-sensitive, as observations may be reduced during prolonged dry periods and in some cases, extirpations may occur, particularly in small, isolated habitats that lack heterogeneity (P. Hammond, personal communication, 2021). With their long flight period and relatively long-life span (several months), adult regal fritillaries require a nearly continuous supply of nectar summer through fall in order to survive and reproduce (Wagner et al. 1997, p. 266). Drought may decrease the availability of these needed flowering nectar plants (Royer and Marrone 1992, p. 25), so drought may increase an adult's risk of starvation, reduce breeding success, and increase risks associated with forced emigration in search of food. Spring droughts may reduce the availability of violets, so larvae may starve or their growth may be stunted (P. Hammond, personal communication, 2021).

Regal fritillary larvae are also vulnerable to drying that may accompany droughts. In laboratory settings, humidity is kept high, as much as 96 percent, to improve the overwintering survival of first instars (Becker 2018, p. 8). Regal fritillary pupae may also be vulnerable to desiccation (Becker 2018, p. 11), and eggs require some amount moisture (Klockmann and Fischer 2017, p. 10875; Becker 2018, p. 7). Thus, prolonged and extended dryness associated with drought during any season could negatively impact regal fritillary individuals of all life stages.

The subspecies' adaptation to drought lies primarily in its high mobility and fecundity that allows it to access many areas to lay hundreds to thousands of eggs. Large, connected, heterogenous tracts of grassland with low-lying areas such as riparian zones and wetland edges, may also reduce the negative effects of drought. When conditions become unfavorably dry, larvae, pupae, and eggs in upland areas may be the most vulnerable to desiccation, while those existing in low-lying areas, spring-fed sites, or riparian areas with adequate moisture may be less vulnerable. Adults are drawn to such areas because they often support nectar sources that are absent in uplands during drought. It is in these moisture refugia that populations may survive in drought-impacted areas, although potentially at reduced levels, until more favorable conditions return. These individuals may then become a source for population expansions into adjacent areas, and repopulation of extirpated sites may occur (P. Hammond, personal communication, 2021). Semi-natural, relatively unfragmented landscapes may help reduce the effects of drought and promote recolonization (Oliver et al. 2015, pp. 941, 943, 944)

In combination with habitat loss and fragmentation from grassland conversion and expected increases in the frequency and severity of drought across the overall range in the future, drought has and will continue to be an important driver of regal fritillary populations. As a result, drought may reduce the current and future resiliency of AUs and the viability of both subspecies.

Local Climatic Events and Climate Change

Regal fritillary individuals may experience a wide range of climatic and environmental factors, such as severe storms, heat waves, unusually cool or wet periods, and frosts. These events may occur at a local or larger scale, and they may result in temporary or longer impacts to individuals and local populations. Additionally, global climate change may exacerbate the frequency and magnitude of these stochastic events. We discuss local climatic events and climate change below.

Local Climatic Events

Local climatic events within the current range of normal variability, may occasionally occur that negatively affect numbers of regal fritillaries. Severe or unusual storms can cause mortality; a population in the east was thought to have been impacted by salt spray from hurricanes (Schweitzer 1993, p. 9; Selby 2007, p. 32). Hail has the potential to cause mortality via physical impact or freezing temperatures and can destroy local floral resources. Heavy rain or flooding may impact habitat as well or result in drowning of vulnerable early life stages (Mason 2001, p. 17). Unusually cold spring temperatures after larvae become active could also cause mortality. Similarly, excessive heat (see Temperature section) can be fatal for larvae in particular, as well as other life stages (Nail 2016, pp. 4, 9, 13, 15). These types of events may cause reduction in individual butterflies or temporary extirpation at a given site if the event is large or the population is small. Generally, these are not of the extent or magnitude affecting significant numbers of individuals or populations of regal fritillaries, but potentially could affect relatively large areas, i.e., cold damp springs, harsh winters, early or late hard frosts (Schweitzer 1993, p. 9; Selby 2007, p. 32).

Climate Change

Global climate change is broadly accepted as one of the most significant risks to biodiversity world-wide (Staudinger et al. 2013, p. 465). Specific impacts of climate change on pollinators are not well understood; however, expected changes forecasted for terrestrial species and communities include increased ambient temperature, changes to annual and seasonal precipitation patterns, increased frequency of extreme events, and changes to hydrologic regimes

(Staudinger et al. 2013, p. 466). These climate changes may lead to decreased resource availability (due to mismatches in temporal and spatial co-occurrences), decreased availability and suitability of larval habitat (due to increased flooding or storms), and increased stress from over-heating (due to higher temperatures) (Cohen et al. 2018, p. 226; Zografou et al. 2021, p. 3283). Based on the known biology and life history of the species, increasingly warmer temperatures may have effects such as interruption of winter diapause resulting in energy expenditure and potentially reduced first instar survival; alteration of violet and/or nectar plant phenology, availability, or abundance impacting food resources at larval and adult stages; unusual post-winter diapause cold periods impacting larval survival; and direct mortality of regal fritillaries at all life stages due to excessive heat, drought, or severe storms. Despite having a wide climatic tolerance based on its range, the regal fritillary experiences very large fluctuations in annual numbers – even in populations with stable to increasing trends – suggesting that extreme weather can negatively impact regal fritillary abundance (Swengel and Swengel 2017, p. 19). Several populations in western Iowa, for example, were extirpated during extreme drought in the mid-2010s, with no perceived recovery as of the summer 2021 (P. Hammond, personal communication, 2021).

Climate variability may lead to shifts in geographic range, reported for regal populations in Wisconsin and North Dakota (Swengel and Swengel 2017, p. 19; P. Hammond, personal communication, 2021), as well as decoupling pollinators from matching both host plant and nectar plant phenologies (Memmott et al. 2007, p. 712), as demonstrated in other butterfly species (Forister et al. 2010, pp. 2088–2089; Hickling et al. 2006, p. 452). Spring larval emergence may rely on heating degree days, photo period or a combination of both, leading to larvae emerging when violets are older and less palatable. Drier summers could force regal fritillaries to leave otherwise suitable habitat in search of nectar sources. Other potential effects from climate change include increased flooding and storm events, which may directly reduce available larval (violet) habitat (Goulson et al. 2015, p. 4) by inundating those areas reliant on wetland associated violets. Finally, effects from climate change may add increased stress in the future, further compounding pressures from other factors, including pathogens, non-native species, and habitat loss (Goulson et al. 2015, pp. 4–5; Kerr et al. 2015, pp. 178–179; Williams and Osborne 2009, p. 371).

For the AUs of the western regal fritillary, under RCP 4.5, all models display a seasonal increase in spring precipitation, with the greatest uncertainty occurring in the summer months during adult emergence through pre-egg laying period, depending on the global circulation model (GCM) used. This stable-to-decreasing trend from some GCMs could be beneficial when considering invasion of coolseason exotic grasses such as Kentucky bluegrass and smooth brome, which are associated with abundant annual precipitation (Stubbendieck et al. 1985, pp. 38–42). The decreasing trend may not be significant enough to shift the competitive edge back to native prairie plants without human intervention, such as through prescribed burning and grazing. However, prescribed fire and prescribed grazing behave differently on sites dominated by coolseason exotic grasses compared to native plant dominated sites. There are numerous data gaps associated with reducing Kentucky bluegrass and smooth brome and understanding how management treatments such as fire and grazing behave under invasion scenarios. Likely, the timing of treatments, along with short-term and long-term precipitation trends are factors that will affect a land manager's ability to increase native plant representation. Based on recent data analysis, different treatment (i.e., burning, grazing, and rest) outcomes are affected by geographic differences in temperature and precipitation (Grant et al. 2020, p. 16). In the mixed-grass prairie, it appears that burning and grazing treatments are more effective at increasing native plants compared to the traditional season long grazing practices (Dornbusch et al. 2020, p. 90). Long-term data trends show warmer and wetter winters and springs across the Great Plains, which may exacerbate the invasion of coolseason exotic grasses. Comparatively, cooler and drier areas composed of shortgrass and mixed-grass prairies of the Western Great Plains remain resistant to coolseason grass intrusion due to greater percentage of native plant cover, even with the proposed increases to winter and spring precipitation (Grant et al. 2020, p. 19).

Some studies suggest a possible east to west shift in the forest-prairie transition zone due to increasing suitability for woody species to inhabit what is currently grassland and shrubland (Bachelet et al. 2003 p. 8). The primary drivers in this "tension" between prairies and forest are climate, soils and topography (Andersen 2005, p. 131). There is evidence that woodlands existed along riparian areas at wetland edges, on escarpments and on sand hills prior to Euro-American settlement (Severson and Sieg 2006; Grant and Murphy 2005, p. 361). These trees and shrubs existed because of wetter soils and protection from the wind; however, spread of woody plants was limited primarily by drought, flood, fire, and herbivory (Severson and Sieg 2006). The loss of bison, fire suppression, tree plantings (Grant and Murphy 2005 p. 365), and wet cycles in recent years have allowed for the expansion of woody vegetation. As an example, at J. Clark Salyer National Wildlife Refuge (North Dakota), the cover of aspen woodland has doubled since European settlement. With this continued encroachment, it is possible that remaining grasslands could be converted within 75 to 130 years, eliminating habitat for grassland obligate species, such as the regal fritillary (Grant and Murphy 2005, p. 366). It is possible that a decreasing summer precipitation trend in most of the Great Plains could indirectly benefit prairie habitats by creating lengthier windows for management of woody vegetation; however, it is highly likely that land managers would not be able to keep up with removing woody invasion with the possibility of addressing invasive coolseason grasses (Gannon et al. 2013, p. 6).

Periodic Disturbances: Fire, Haying, Grazing

Fire, haying, grazing, and other activities (e.g., manual or chemical removal of weeds or woody vegetation) are common disturbances in grasslands that have the potential to negatively impact regal fritillaries yet are necessary to conserve the habitat on which the species depends (Selby 2007, p. 3). Grasslands left unmanaged will become overgrown, invaded by woody vegetation or exotic species, and/or covered in thatch that inhibits floral diversity and suppresses violets and nectar sources. Periodic disturbances ensure native grasslands remain suitable for regal fritillaries by removing excessive thatch, controlling invasive species, and stimulating native plant growth, but can result in mortality of regal fritillaries via trampling, crushing, burning, poisoning, and removal of shelter and food resources. When these actions occur in patches in a landscape dominated by large native grassland tracts that are well-connected, mortality typically does not rise to the level of permanent population loss. Temporary impacts to occupied habitats are incurred, and some occupants of the habitat may be lost, but disturbances including fire, grazing, and haying, can ultimately serve to maintain habitat suitability and disturbed areas are quickly recolonized by regal fritillaries from adjacent areas. Larvae may also survive these disturbances (McCullough et al. 2017, entire). When these actions occur on smaller, more isolated patches, however, local extirpations may occur as the actions can impact the entire occupied area, effectively eliminate all of the occupants, and the lack of nearby refugia can potentially preclude recolonization or cause population impacts lasting several years (Swengel 1996, p. 73). Timing and intensity can also determine the level of impact: moderate to light grazing is generally considered beneficial to regal fritillaries, while heavy grazing is not (Royer and Marrone 1992, p. 28). Fires perhaps on a 3- to 5-year rotation (Henderson et al. 2018, p. 41; McCullough et al. 2019, p. 9) may be beneficial, while shorter or longer intervals between burns are more detrimental (McCullough et al. 2019, p. 9) (albeit annual burning can still provide some benefits to habitat compared to no burning [Henderson et al. 2018, p. 41]). When applied on a landscape scale appropriately (proper timing, extent, intensity, frequency), these disturbances can minimize regal fritillary mortality while creating a shifting mosaic of habitats in various successional stages that provide a net benefit to the population growth rate. When applied inappropriately, they may affect individuals and populations, particularly those already at risk due to other factors.

Currently the Midwest populations are vulnerable to negative impacts of improperly applied periodic disturbances due to their small, isolated patches. Many populations in the Great Plains are also small, but the landscape is less fragmented; thus, disturbed sites are more easily recolonized when favorable vegetative conditions return. At FTIG, following the INRMP, the eastern subspecies is intentionally managed to enhance populations at the known occupied SCAs. Should these actions cease, resiliency of the eastern subspecies could decline significantly.

Disease

Pathogens are ubiquitous in insect populations, but few studies examine their impacts on populations (Myers and Cory 2015, p. 231). There are no known diseases specific to the regal fritillary, but the species is susceptible to many of the same diseases as other Lepidoptera (Royer and Marrone 1992, p. 28). Regal fritillary larvae, particularly the first instars, are extremely susceptible to disease, parasitoids, viral or fungal pathogens, and predation (Selby 2007, p. 30).

Nuclear polyhedrosis viruses (NPV) may infect regal fritillaries and can be transmitted via females to offspring during egg-laying or if larval frass (excrement) is ingested by others (Wagner 1995, p. 4). NPV has been problematic in captive rearing efforts, in one case causing the loss of 80 percent of captive larvae (Wagner 1995, p. 4), although high larval density in captive settings are not representative of field conditions. Widespread infection by NPV (or a similarly devastating virus or disease) is a concern for the eastern subspecies (M. Swartz, personal communication, 2021) due to the endemic nature of this population.

An intracellular bacteria, genus *Wolbachia*, is common in insects, particularly butterflies. About 25 to 33 percent of individuals may be infected with *Wolbachia*, and around 80 percent of Lepidoptera species are infected at a non-negligible frequency (Ahmed et al. 2015, p. 1). A recent study at FTIG detected *Wolbachia* in Aphrodite fritillaries (*A. Aphrodite*) and great spangled fritillaries (*A. cybele*) but did not find *Wolbachia* bacteria in 200 FTIG field specimens of regal fritillary (Rutins et al. 2022, p. 3). *Wolbachia* lives in the cells of insects and is usually (not always) transmitted to the next generation when females lay their eggs, thus can parasitically manipulate its host's reproductive system (Ahmed et al. 2015, pp. 1–2). This has been known to be problematic in captive rearing situations for other fritillary species (e.g., the Oregon silverspot [*Argynnis zerene hippolyta*]) as the bacteria is known to reduce viability, fertility, and fecundity in captive reared family lines, but little is known about its affects to regal fritillaries, particularly in the wild (P. Hammond, personal communication, 2021).

Thus, as with other Lepidoptera, regal fritillary individuals may be susceptible to disease. However, there is no information to indicate that disease currently or in the future may reduce the resiliency of AUs or the viability of either subspecies.

Parasitism

Regal fritillary larvae are known to be parasitized by Hymenoptera (a group including ants, bees, sawflies and wasps, among others). In Kansas, Kopper (2001c, p. 96) reported the collection of 12 individual regal fritillary larvae, three (25 percent) of which were parasitized by Hymenoptera. The tachinid fly (*Compsilura concinnata*) is a parasitic fly introduced to North America from Europe to control the spongy moth, but is known to parasitize other butterflies and moths, including Nymphalidae species (Arnaud 1978, p. 46). This forest fly may have used regal fritillaries in the east as hosts historically, although evidence of this impacting regal fritillary populations is lacking (NatureServe 2021).

The potential exists for pathogen outbreaks to affect regal fritillary populations, and this has been speculated to have played a role in the collapse of populations in the east, but there is no information to support that claim (Wagner et al. 1997, p. 271; Selby 2007, p. 32). Given the much larger distribution of the western subspecies, disease or parasitic outbreaks would likely occur at a local scale and not affect the entire western subspecies. This is not necessarily the case for the eastern subspecies, given its small size and isolation, where a severe outbreak of disease or parasitism at FTIG has the potential to eliminate or significantly reduce the size of the sole remaining population. In 2002 and 2003, hundreds of regal fritillary larvae were observed to have climbed to the tops of shrubs or other tall vegetation at FTIG and died there, but researchers were unable to determine the cause of the mortality (Ferster and Vulinec 2010, p. 37; M. Swartz, personal communication, 2021). Such an event has not since been observed and could not be definitely attributed to a parasite. Therefore, parasites may affect individuals, but there is no information to indicate that parasitism currently or in the future may reduce the resiliency of AUs or the viability of either subspecies.

Predation

Although no predator specifically targets regal fritillary (Royer and Marrone 1992, p. 28), a variety of birds, small reptiles, amphibians, mammals, and other invertebrates may prey upon individual regal fritillaries of all life stages. Eggs, larvae and pupae are relatively immobile and confined to the ground, so may be at greater risk of predation than adult regal fritillaries. In the winter, predation risk to first instar larvae may be relatively low as many predators may also be in overwintering diapause. Spiders, such as crab spiders, are known predators, likely for all regal fritillary life stages (Selby 2007, pp. 32–24; Becker 2018, p. 10; P. Hammond, personal communication, 2021). Deer and other herbivores may inadvertently feed on regal fritillary larvae while browsing on grasses and forbs (Jim Bissel, Cleveland Museum of Natural History, personal communication, 2020). There is no information to indicate that either subspecies is more susceptible or more resistant to predation than other lepidopterans. Therefore, although predators may prey on individuals, there is no information to indicate that predation currently or in the future may reduce the resiliency of AUs or the viability of either subspecies.

Competition and Hybridization with Sympatric Fritillaries

Both subspecies of regal fritillary may compete with other *Argynnis* species (Barton 1995, p. 12; P. Hammond, personal communication, 2021). All regal fritillary larvae require violets as host food plants, and when multiple *Argynnis* species are present in a patch, competition for limited violet and nectar resources may occur. The Aphrodite fritillary in particular has been observed to dominate the regal fritillary in some instances, perhaps partly because it develops faster than the regal fritillary (Barton 1995, p. 12; P. Hammond, personal communication, 2021). However, the level of competition, the species potentially competing for resources, and the violet plant species themselves may vary regionally. On the wet tallgrass prairie in the Midwest and eastern edge of the Great Plains, regal fritillaries share common blue violet (*V. papilionacea*) as a larval foodplant with the Aphrodite fritillary (*Argynnis aphrodite*). At the western edge (dry shortgrass) of the Great Plains, regal fritillaries may compete with Edwards' fritillary (*A. edwardsii*) for Nuttall's violet (aka yellow prairie violet), a larval foodplant.

On the dry tallgrass and mixed-grass prairies between these extremes, regal fritillaries may be the only species of *Argynnis* present, with the prairie violet the primary larval foodplant (P. Hammond, personal communication, 2021). In the northern Great Plains region, the Aphrodite fritillary, the meadow fritillary (*Boloria bellona*), and the silver-bordered fritillary (*B. selene*) may be competitors (Royer and Marrone 1992, p. 24). The great spangled fritillary overlaps with regal fritillaries but may not compete for the same violet species in Illinois (A. Moorehouse, personal communication, 2021).

At FTIG in Pennsylvania, the eastern regal fritillary may compete with both the Aphrodite fritillary and the great spangled fritillary for the same violet and nectar sources. At least one population estimate of all three species revealed regal fritillaries as the minority and Aphrodite by far the dominant species, with great spangled fritillaries second in abundance (Barton 1995, p. 12). At FTIG, these species may attempt to mate with regal fritillaries and may harass them at nectar sources (M. Swartz, personal communication, 2021).

Various species of fritillary could produce hybrids with regal fritillaries, with laboratory crosses including the great spangled fritillary, nokomis fritillary (*A. nokomis*), Diana fritillary (*A. diana*), zerene fritillary (*A. zerene*), and Edwards' fritillary (*A. edwardsii*) (Hammond et al. 2013, pp. 267, 269, 271). However, non-viable hybrid females were severely stunted, usually died in the pupal stage or were unable to expand their wings upon eclosion (hatching) and were rarely able to develop into a deformed adult; male hybrids were sterile, thus hybrid back-crosses with any other species were not possible (Hammond et al. 2013, p. 271). In some cases, larvae showed defects such as altered feeding behavior, chewing randomly on leaf surfaces instead of feeding efficiently along leaf margins, resulting in slow growth and stunting of larvae with most dying as pupae or being unable to expand their wings as adults (Hammond et al. 2013, p. 272). These laboratory studies indicate that pheromone mating system is likely 99 percent effective in precluding hybridization in the wild, it does occur occasionally, though hybrid crosses with regal fritillaries are an evolutionary dead end (Hammond et al. 2013, p. 272).

Despite local observations of interspecific competition and the known dependence by all *Argynnis* larvae on violets and adults on nectar sources, there is no information to indicate that these are a significant or widespread factor limiting regal fritillary population growth in both subspecies (Selby 2007, p. 33). Therefore, although competition and hybridization with sympatric butterflies may affect individuals, there is no information to indicate that these stressors currently or in the future may reduce the resiliency of AUs or the viability of either subspecies.

Collection

Collection of regal fritillaries is not a widespread problem for the species or either subspecies, but overcollection could affect small colonies (Selby 2007, p. 36). For example, a historical population in West Virginia may have been either greatly depleted or eradicated by an "unscrupulous collector" (Bliss and Schweitzer 1987, p. 5). Additionally, regal fritillaries may be more attractive to collectors and have more potential commercial value that other prairie-specialist butterflies (Selby 2007, p. 36). Limited collection for scientific purposes is unlikely to affect the subspecies at a population level unless it is already significantly depressed. Additionally, scientific collection permits are required in states with legal protections for the regal fritillary, and protected areas usually require permission to access (Selby 2007, p. 36). The population at FTIG exists wholly on military property that is not accessible to the public, so collection, other than for scientific purposes by trained biologists, does not occur there. Wildlife biologists collect around 8 to 12 adult females every year at FTIG for their captive rearing program, and population data are considered carefully before collecting (Pollard 1977, entire; E. McKinney, personal communication, 2022). Therefore, although individual regal fritillaries of either subspecies may be collected, and overcollection may have historically reduced a population of the eastern subspecies, there is no information to indicate that collection currently or in the future may reduce the resiliency of AUs or the viability of either subspecies.

Conservation Actions

Conservation Actions for the Eastern Subspecies

Reintroduction Efforts

Since 2011, FTIG Wildlife staff, in coordination with ZooAmerica North American Wildlife Park in Hershey, Pennsylvania, developed a regal fritillary captive rearing program and have reintroduced regal fritillaries into off-site suitable habitats annually with no data indicating success of a viable population at these locations to date. Reintroduction sites need to meet certain criteria before reintroduction is considered including size of grasslands, availability of caterpillar and adult nectar plants, site management goals that are compatible with regal fritillary vegetation requirements, and partner commitments to manage and monitor the site for multiple years.

Gravid females collected from FTIG in mid-August are brought to the ZooAmerica laboratory where eggs are laid and hatched, and the larvae collected (Becker 2018, pp. 3–9). From 2011–2013, all larvae were kept overwinter and reared to adults in the lab. Adult regal fritillaries were released to unoccupied potential regal habitat at FTIG in 2012 and 2013, until a suitable off-post reintroduction site on Pennsylvania Game Commission (PGC) state game lands was identified in 2014. While there is hope of expansion to additional locations in the future, PGC state game lands have been home to the only reintroduction sites from 2014 to the present (E. McKinney, personal communication, 2021).

In the fall of 2014, after several years of poor overwinter survivorship in the lab, staff began releasing a portion of the first instar larvae on or near violets at reintroduction sites, while simultaneously continuing to modify the lab overwintering protocols. From 2014–2019, all adult regal fritillary reared in the lab were released to PGC state game land reintroduction sites. In 2020, overwinter lab survival of larvae significantly increased after adapting a draft protocol of a butterfly rearing lab in the Midwest. This increase in survival prompted a switch from fall larval releases to spring caterpillar releases with the intent of mitigating overwintering larval mortality in the field. Staff continued to keep a small portion of caterpillars in the lab to rear to adulthood for research purposes during this time. Though it is likely that adult output would be maximized by rearing all surviving caterpillars to adults in the lab, that task has not been feasible due to limitations in staffing, time, and lab space needed for current protocols employed. There may be better site fidelity with release of larva; adults appear to be more likely to stay in the area where they developed as caterpillars (Becker 2016, p. 6). Larval versus adult introduction is monitored at FTIG and the reintroduction sites, but due to the large size of the reintroduction sites, the tendency for adults to disperse, and the inability to distinguish unmarked adults (released as caterpillars at FTIG), it has been difficult to evaluate the findings and draw conclusions (V. Tilden, personal communication, 2021).

Reintroduction efforts of the eastern subspecies have been attempted at 8 sites within Pennsylvania since 2014, though to date no viable populations have been established. With additional habitat assessment, it was determined most of these reintroduction sites have been too small and over-run with undesirable vegetation. Restoration continues at several potential sites, and in 2021, releases and reintroduction efforts focused on one PGC state game lands site (E. McKinney, personal communication, 2021). The efforts at this PGC site are ongoing. In addition to the hope of creating additional populations, the rearing program helps support and augment the population at FTIG; reared caterpillars (fall 2018, 2019; spring 2020, 2021) and adult regal fritillaries (summer 2021) have been used to supplement populations within FTIG SCAs when numbers have dropped, possibly improving the resiliency of the FTIG populations (M. Swartz, personal communication, 2021).

Although no viable populations of the eastern subspecies are currently known to exist outside of FTIG, the program is ongoing and has the potential to result in expansion of the range of the eastern subspecies in the future. Currently, however, the reintroduction efforts offsite of FTIG do not appear to influence the current or future 3Rs for the eastern subspecies. The western subspecies has also been reintroduced in restored prairie habitats at the Neal Smith National Wildlife Refuge in Iowa (Shepherd and Debinski 2005, entire).

FTIG National Guard Training Center Lebanon and Dauphin Counties, Pennsylvania, Integrated Natural Resource Management Plan (INRMP)

Under the Sikes Act (16 U.S.C. 670a-670f, as amended), staff at FTIG are responsible for carrying out programs and implementing management strategies to conserve and protect biological resources on their lands. In coordination with the Service and other partners, they developed an INRMP that serves as a guidance document to achieve conservation goals (Pennsylvania Department of Military and Veterans Affairs [PADMVA] 2021, entire). The INRMP is updated every 5 years. The current version (2022–2027) outlines several objectives for the conservation of the regal fritillary eastern subspecies. Primary objectives include increasing or maintaining population levels and nectar sources/larval host plants. Actions include extensive seasonal monitoring and reintroduction efforts, and as outlined above, FTIG staff have been working with partners to achieve their objectives.

Land management activities are necessary to ensure open areas are sustained for the military operations at the training center and also provide essential benefits to the regal fritillary. Disturbance is provided by fires, either set by prescribed fire techniques or initiated by training activities. Fire serves to remove duff and to initiate germination of and provide space for host violet and nectar-plant seeds. Fire also invigorates and favors clump-forming grasses over mat-forming cool season grasses. Grasslands are also maintained with mowing and spot treatments of herbicide (PADMVA 2021, entire). Without management activities at FTIG, woody vegetative succession would overtake the nectar sources that are crucial for this subspecies, and the population would decline or vanish. Regal fritillary conservation efforts have been ongoing since the early 2000s and, because of the long-standing monitoring and management, FTIG has been able to successfully maintain the last population of the regal fritillary eastern subspecies in the world (PADMVA 2021, entire).

The PA National Guard anticipates continued use of FTIG in perpetuity and is committed to implementing ecosystem management with the intention of demonstrating the interrelationships between military mission and natural resource management (PADMVA 2021, entire). Commitment by FTIG to continue management of the SCAs in the long-term will help to ensure the conservation of this species. Although the INRMP provides conservation objectives, the regal fritillary currently has no legal protections within the State or on FTIG. SCAs are still an active part of FTIG military operations, thus not all actions occurring within them are to the benefit of regal fritillaries. Even though FTIG has an approved INRMP, it serves as a guidance and best management practices document. At times, instances have occurred where tenants or outside organizations do not adhere to or follow what is outlined in the INRMP. Regal fritillary mortality as a result of routine military actions on FTIG does occur; incidences of fire are known to impact SCAs as a result of military actions on adjacent lands, and chemical or mechanical clearing on SCAs is also completed for military purposes. Incentive to conserve the eastern population of regal fritillaries exists, but there is no mandate, and military needs at FTIG take precedent. The INRMP discusses management actions from the perspective of military needs: "The management plan does not interfere with training and has been fully integrated into our program for over a decade." (PADMVA, 2021, p. F-16). Further, the INRMP states:

> "Areas identified as being part of the 219 acres of Regal [Fritillary] Research Area (RRA) [now called "SCA" as of 2023] and delineated as such in the field are not available for heavy or wheeled maneuver, bivouacking, helicopter landing, or artillery firing positions. These areas are not removed entirely from training, as they can be used for a variety of purposes including, but not limited to, being downrange as part of the impact area for that range, light maneuver (on foot), drop zones, and simulation use as minefield or other operational impediment. Restrictions have not impacted military training because work arounds have ensured military training is not compromised. In addition, eastern regal fritillary occupied acreage outside the 219 acres has maintained persistent populations whose range has expanded over the last 20+ years of monitoring." (PADMVA 2021, p. G-1).

Due to military operations, SCAs may be burned too frequently, which can negatively impact the species needs, such as violet sources, and can also affect different life stages of the species. To date, the two have coexisted and the regal fritillary population has persisted. See Appendix F of INRMP at: https://www.dmva.pa.gov/dmvaoffices/Environmental-Resources/Pages/Environmental-Documents.aspx for more information about ongoing monitoring, management, rearing, and habitat management efforts at FTIG (PADMVA 2021). However, the management activities at FTIG have maintained habitats and the eastern subspecies, and if they continue as currently implemented, are likely to maintain habitats and the subspecies into the future. We discuss our current and future conditions analysis for the eastern subspecies in Chapters 5 and 6.

Additionally, FTIG completed a Candidate Conservation Agreement (CCA) in partnership with the Service (FTIG and Service 2024, entire). The CCA helps codify regal fritillary butterfly conservation intentions at the military installation. The agreement will be appended to the INRMP.

Conservation Actions for the Western Subspecies

The western subspecies currently ranges from Indiana to Colorado and from Oklahoma to North Dakota. Periodic disturbances of native grasslands in this range are ongoing on lands owned by the Federal government, states, non-governmental organizations, corporations, and private entities. However, these actions are typically economically driven, not necessarily conducted with the needs and life history of the regal fritillary in mind and may or may not be beneficial to the species. Appropriate haying, grazing, and burning are generally known to be beneficial to regal fritillaries by promoting native grassland habitats, and these actions do occur under all types of land ownership. However, as previously discussed herein, land use activities conducted without knowledge or consideration for the species' life history can be detrimental to individuals and populations, particularly on small, isolated habitat patches. Additionally, activities are not typically conducted in a coordinated manner among landowners or on a scale large enough to improve the resiliency, redundancy, or representation of the western subspecies.

The regal fritillary is a focus species in some areas, but typical sites are local and small in scale. Entities such as The Nature Conservancy, for example, have focused successful restoration and management activities on prairie butterflies (and the regal fritillary, specifically) on preserves such as Platte River Prairies in Nebraska. As a Species of Greatest Conservation Need per State Wildlife Action Plans (see Appendix A, *State Designations*), the species is monitored by many state wildlife agencies. This monitoring, however, does necessarily lead to focused management actions; many state-owned lands are managed for game production purposes that may be incompatible with regal fritillary needs. Additionally, state-owned lands represent a very small fraction of landownership in the western subspecies' range. Habitat preservation programs are in place in some areas. Iowa, for example, has a system of local prairie preserves designed to protect remaining tallgrass native prairie patches; albeit these preserves are typically small, isolated, and not necessarily conducive to long-term regal fritillary conservation. State endangered or threatened status has afforded some special management and protection measures for the subspecies, such as providing refugia in Wisconsin prairies (Swengel and Swengel 2007, p. 264), but similarly, they are often isolated sites on a small, local scale.

Large-scale Federal programs such as the Service's grassland easement program (Service 2020, entire) have prevented conversion of habitats to agriculture or other developments, and the Natural Resource Conservation Service also has an easement program (*https://www.nrcs.usda.gov/wps/portal/nrcs/main/national/programs/easements*) to protect grasslands. However, management is left to private landowners. The U.S. Forest Service, which currently recognizes the regal fritillary as a Sensitive Species, administers large tracts of land within the species' range where beneficial actions such as grazing are ongoing, but not necessarily with the species' needs in mind. Some habitat restoration and reintroduction efforts (e.g., Neal Smith National Wildlife Refuge in Iowa) have occurred that have been successful and have even resulted in population expansions into adjacent areas (e.g., The Nature Conservancy's Kankakee Sands in Indiana); such projects are evidence that population declines or losses can be reversed or restored.

Of overarching consideration, however, is that none of these local successes are known to outpace concurrent loss of habitat via mechanisms identified above in this chapter, such as conversion to agriculture and developments, invasive vegetation, and herbicide applications. Ongoing conservation actions are incorporated in our analysis of current conditions. We are currently not aware of any large scale, coordinated, legally binding, long-term conservation actions that have the potential to improve any of the 3Rs for the western subspecies of regal fritillary.

Cumulative Effects

Many of the stressors faced by the eastern and western subspecies of regal fritillary are interrelated and could be synergistic, or act cumulatively. When stressors act synergistically, or in concert with one another, the potential combined effects on the species are called cumulative effects. Principal stressors discussed above include drought, herbicide use over large areas, invasive plants, woody encroachment, and conversion, and the conservation mechanisms, such as an INRMP that may direct habitat and population management. The principal stressors assessed in previous sections may cumulatively impact individuals and AUs beyond the scope of each individual stressor.

We note that, by using the SSA framework, we have not only analyzed individual effects of stressors on individuals, AUs, and the subspecies, but we have also analyzed their potential cumulative effects. Because the SSA uses metrics for demographics (resiliency), distribution (redundancy), and diversity (representation), the effect of multiple stressors is inherent in the assessment and helps to assess how populations and, ultimately the species, responds cumulatively to the interactive effects of stressors and conservation efforts included in the future scenarios (Smith et al. 2018, p. 6). We incorporate the cumulative effects into our analysis when we characterize the current and future condition of the subspecies across the AUs. Our assessment of the current and future conditions encompasses and incorporates the stressors individually and cumulatively. Our current and future condition assessment is iterative because it accumulates and evaluates the effects of all the factors that may be influencing the subspecies, including negative influences from stressors and positive influences from conservation efforts. We evaluate potential effects from these influences consistently across the same subset of habitat and demographic needs for the subspecies, both currently and into the future. Because the SSA framework considers not just the presence of the factors, but also to what degree they collectively influence risk to the entire subspecies, our assessment integrates the cumulative effects of the factors and replaces a standalone cumulative effects analysis.

Summary of Cause and Effects

Appendix H provides our detailed analysis of cause-and-effects for the eastern and western subspecies of regal fritillary. While the list of stressors affecting the regal fritillary includes many factors known to negatively affect the butterfly, for several of these, we lack specific evidence, information, and/or estimates of risk to determine whether they are significant drivers of AU resiliency. The factors that we identified that may influence the viability of both subspecies are climate change, drought, invasive plants, woody encroachment, and periodic disturbance. Additional stressors carried forward for the western subspecies are conversion and herbicide use. The eastern representative unit receives relatively more moisture than habitats of the western subspecies to the west and is less susceptible to drought. The eastern subspecies is vulnerable to woody encroachment and periodic disturbances are necessary to ensure the grasslands do not become reforested.

We concluded that disease, predation, collection, insecticide drift are not significant risks to either the eastern or western subspecies. While collections or insecticides could arguably rise to the level at which they could impact populations, it is not clear they have or will, and mitigating factors (e.g., the mobility of the species may mask the impacts of insecticide drift, whereas other prairie pollinators with limited dispersal capabilities have displayed local population crashes or extirpations perhaps in concert with the use of neonicotinoids [Runquist and Heimpel 2017, p. 6-7]). Given the prevalence of insecticide applications in agricultural practice, this factor in particular may represent a relatively bigger risk regal fritillary populations, yet we lack documentation in that regard. An additional stressor – competition with sympatric fritillaries – is not a significant stressor to either subspecies.

In contrast, other factors present a clearer risk to regal fritillary populations, specifically those that have in the past, and are expected to continue, to degrade or eliminate habitat or resources within habitat. The loss of native grasslands, particularly due to agricultural concersion, have led to associated synergistic effects that occur with resulting fragmentation and isolation and this has clearly been detrimental to regal fritillary populations. This factor has been implicated in subspecies-level impacts as habitat loss in the east likely resulted in breakdown of metapopulation dynamics and the historical collapse of populations there, resulting in the near-elimination of the eastern subspecies. Ongoing efforts to eliminate forbs by aerial spraying herbicides in native grasslands for the perceived benefit to livestock has the potential to permanently eliminate the western regal fritillary from large areas of otherwise suitable habitats, and this practice appears to be expanding. Invasive vegetation, whether it be monoculture grass species or woody species encroachment, is a widespread problem that continues to reduce the availability of feeding, breeding, and sheltering resources required by both subspecies, throughout their respective ranges. Periodic disturbances, such as fire, grazing, and mowing, are necessary to set back the progression of these invasives, but if conducted improperly (e.g., too frequently, poorly timed, or of too high intensity), may instead have detrimental effects, both on the regal fritillary as well as its habitat, and in some cases, can exacerbate invasive vegetation problems. Climate change, specifically warming temperatures and increasing frequency and duration of drought, is also expected to further reduce the amount of suitable habitat available to this species via fewer violet and nectar sources and may incur direct impacts via intolerable heat.

Conservation measures are occurring to reduce the effects of some of the stressors impacting regal fritillary habitat. In some areas, such as Indiana, without concerted efforts specifically directed at restoring historical habitats for the regal fritillary and pollinators in general, the species might otherwise have been extirpated from the State. Ongoing periodic disturbances – haying, grazing, and burning – across the range on private and public lands may be compatible with regal fritillary conservation, although they can also be detrimental when inappropriately applied. However, most of these actions are for purposes other than conservation of the regal fritillary, are not conducted with the life-history needs of the species in mind (e.g., lack of management plans for the regal fritillary), are not legally binding, not necessarily funded, or are not ensured in perpetuity. For example, the INRMP at FTIG recommends how to protect, manage, and conserve the eastern subspecies, but the document is also somewhat limited in its protections, and military priorities at FTIG may take precedent over impacts to regal fritillaries. FTIG completed a CCA to conservation intentions for the eastern regal fritillary at the military installation, which will be appended to the FTIG's INRMP.

Chapter 5 – Current Conditions

In this chapter, we describe the current condition of the eastern and western subspecies of the regal fritillary in terms of their current resiliency, redundancy, and representation. We do this by evaluating the current conditions of a subset of the habitat and demographic factors that we identified as needs in Chapter 3. Additionally, in Chapter 4, we summarized our evaluation of causes-and-effects, or the sources and conservation efforts that may influence the resiliency of each AU. We begin our evaluation of current condition with a description of the methodology that we used to evaluate resiliency consistently across the 22 AUs, with additional detail provided in Appendix I. In short, we developed a categorical model, called a condition category table, to calibrate resiliency in terms of stochastic risk to AUs based on the condition of five habitat factors and two demographic factors that we identified as needs in Chapter 3. We then used this condition category table to evaluate resiliency for each AU and summarized our evaluation of current condition for the eastern and western subspecies in terms of the 3Rs.

Methodology to Evaluate Current and Future Conditions

As summarized in Chapter 3, we identified a variety of habitat and demographic needs for the regal fritillary. For our analysis of current and future conditions, we selected a subset of these needs, five habitat factors and two demographic factors that are most influential to AU resiliency and that we could measure consistently across all 22 AUs. The five habitat factors and two demographic factors that we used to evaluate resiliency were:

Habitat factors:

- **Native grasslands**;
- **Riparian and wetland areas**;
- **Ambient temperature**;
- **Moisture**, or **precipitation**; and
- **Large, contiguous blocks of native grasslands**.

Demographic factors:

- **Abundance**; and
- **Growth trend.**

We described each of these habitat factors needed by individuals and demographic factors needed by AUs in more detail above in Chapter 3. Using our conceptual model for resiliency as a guide, we then developed a categorical model, called a condition category table, for these five habitat factors and two demographic factors, to calibrate our evaluation of resiliency in terms of a plausible range of stochastic risk, from highest to lowest risk, for each factor (Table 12). The categories we used to describe resiliency are very high, high, medium, low, very low, and extirpated, which represent relative levels of stochastic risk for each factor, with very high being the best condition, or contributing the most to resiliency, and extirpated being the worst condition, or not contributing to resiliency, based on the condition of the factor described in the table's rows. We used the condition category table to calibrate our understanding of resiliency and to evaluate the condition of each habitat and demographic factor for each AU. As we considered the condition, we used metrics that were available consistently for all AUs, including compiled information from peer-reviewed literature, surveys and reports, geospatial information, and input from scientific experts. When available, we used scientific reports (published white and gray literature) and analyses to quantify and describe these conditions for the AUs. In the absence of published data, we relied on scientific experts from a variety of backgrounds to help assess the condition of the factors. Three of nine current condition factors (native grasslands quality, abundance, and growth trend) were evaluated qualitatively, while the remaining six factors (native grasslands quantity, riparian/wetland area, ambient temperature, precipitation, and patch size and connectivity of large, connected blocks of native grasslands) were quantitatively evaluated. Appendix I presents additional detail regarding the condition category table and the metrics that we used to evaluate the condition of each of the nine factors.

Table 12 Condition category table (categorical model) used to evaluate the resiliency of AUs based on the current and future projected conditions of five demographic factors and two habitat factors. If any demographic factor is in extirpated condition, the AU has no resiliency, regardless of the condition of the habitat factors.

FACTORS	**HABITAT FACTORS**							**DEMOGRAPHIC FACTORS**	
	NATIVE GRASSLANDS (Quantity and Quality)		**RIPARIAN & WETLAND AREAS**	**AMBIENT TEMPERATURE**	**PRECIPITATION (Available Moisture)**	**LARGE, CONTIGUOUS BLOCKS OF NATIVE GRASSLANDS**		**ABUNDANCE**	**GROWTH TREND**
Summary of Evaluation Method (see Appendix I)	***Quantity:*** *Quantitative evaluation of the percent of AU that is native grasslands using geospatial data*	***Quality:*** *Qualitative evaluation of existing native grass types, violets, diverse floral resources, shrubby/tall vegetation, vegetative litter, and grass tussocks*	*Quantitative evaluation of the percent of AU that is potentially suitable habitat using geospatial data.*	*Quantitative evaluation of ambient temperatures*	*Quantitative evaluation of relative moisture supporting floral resources, individual health using climate data.*	*Quantitative evaluation of **patch size**, or percent of AU composed of patches sized 1,000+ ha (2,471 ac) using geospatial data.*	*Quantitative evaluation of **connectivity**: percent of AU comprised of grass patches using geospatial layers.*	*Qualitative evaluation of abundance using expert input.*	*Qualitative evaluation of abundance using expert input.*
VERY HIGH (5 points)	≥51% of AU is composed of native grasslands	75-100% of AU grasslands are high quality, native tallgrass dominant (75% or more), and diverse with a heterogenous mosaic of successional stages. Vegetative litter/tussocks always available within the majority of patches at ideal level (several years buildup). Violets are highly abundant (e.g., 5 or more plants per 1 m^2 (11 ft^2 [50,000+ per 1 ha or 2.5 ac]) throughout AU. Diverse floral resources are always abundant and available. Shrubs/tall vegetation is available (without woody encroachment concerns) in nearly all patches.	Potentially suitable habitat within riparian and wetland 100-m (328-ft) buffer represents 16.1% or more of AU	<1% of area of AU exceeds 41 °C (105 °F) for 2+ days during spring and summer	Spring precipitation ≥254 mm (10 in); summer precipitation ≥254 mm (10 in); <0.5 droughts/decade	≥81% of AU is composed of habitat patches sized1000+ ha (2471+ ac)	≥81% of AU is composed of connected habitat patches (within 3-5 km [1.9-3.1 mi])	Adults are abundant in nearly all populations throughout the AU in most years; approximately 25 per 1 ha (2.5 ac) or more and nearly ubiquitous throughout habitat; the AU is a consistent source for satellite areas within the AU or adjacent AUs	All populations in AU consistently exhibit exponential growth in good years and stable trend during poor years.
HIGH (4 points)	26-50%	50-74% of AU grasslands are high quality (limited degradation), native, tallgrass dominant (~50-74%), and heterogenous with a mosaic of successional stages. Vegetative litter/tussocks are available in most habitats at ideal levels (several years buildup). Violets are generally plentiful (2-4.9 plants/m^2) in most areas. Diverse floral resources are abundant and available annually. Shrubs/tall vegetation is available (without woody encroachment concerns) in most patches.	8.1-16%	1-20%	Spring precipitation 216-254 mm (8.50-9.99 in); summer precipitation 216-254 mm (8.50-9.99 in); 0.6-0.9 droughts per decade	61- 80%	61- 80%	Adults are abundant in most populations throughout the AU in most years; ubiquitous with ~10-24 individuals per 2.5 ac (1 ha) in good years; more patchily distributed and less common (5-10 individuals/ha) in poor years; the AU is a consistent source for satellite areas	Most populations in AU exhibit exponential growth in good years, and stable trend in poor years. Some smaller areas may be extirpated in poor years, but repopulation and growth happen quickly.
MEDIUM (3 points)	11-25%	25-49% of grasslands in the AU are native, diverse, and high-quality mixed grass (25-49% tallgrass composition). On average grasslands are of moderate quality, generally a mix of heterogenous native grasslands and homogenous nonnative grasslands or with woody encroachment. Vegetative litter/tussocks may or may not be available in most patches - about as likely to be present as not (buildup may be limited in many habitats with less than 2 years buildup or excessive with a decade or more of no disturbance). Violets are available, but at relatively lower densities (1-1.9 plants/m^2) in most areas. Diverse floral resources may be widely available some years but limited in others. Shrubs/tall vegetation may/may not be available or woody encroachment (succession) may be occurring in a few areas to the detriment of native grasses and floral resources.	4.1-8%	21-40%	Spring precipitation 152-216 mm (6.0-8.49 in); summer precipitation 152-216 mm (6.0-8.49 in); 1.0-1.9 droughts per decade	41-60%	41-60%	Adults are common to locally abundant in populations across some areas of the AU but absent in other areas most years; ~5-10 individuals per 1 ha (2.5 ac) in good years, 1-5 individuals per 1 ha (2.5 ac) or less in poor years - typically not a source for satellite areas	Populations in AU exhibit exponential growth infrequently. May provide some refugia and act as a source, but repopulation and growth of satellite areas is relatively slow.
LOW (2 points)	6-10%	5-24% of grasslands in the AU are diverse, native, and high quality. Overall, grasslands are low quality, often homogenous, non-native, shortgrass dominant (5-24% tallgrass). Vegetative litter/tussocks are usually not adequate or available in most habitats (e.g., denuded or overgrown and rarely ideal condition). Violet density is low (0.5-0.9 plants/m^2) in most areas. Floral resources are not diverse or abundant and are a limiting factor most years. Shrubs/tall vegetation may either not be available, or woody encroachment (succession) may become dominant over grasslands in some areas.	2.1-4%	41-60%	Spring precipitation 114-152 mm (4.50-5.99 in); summer precipitation 114-152 mm (4.50-5.99 in); 2.0-2.9 droughts per decade	21-40%	21-40%	Adults occur in low numbers within populations throughout the AU in most years; very few locally common/abundant populations exist; 1-4 individuals per 1 ha (2.5 ac) in good years, less than 1 individual 1 ha (2.5 ac) or absent in poor years; not a source for adjacent AUs, many areas may be sinks	Populations in AU typically do not exhibit exponential growth, even in good years. The AU does not act as refugia or source – repopulations are reliant on dispersers from adjacent AUs
VERY LOW (1 point)	1-5%	< 4% of grasslands in the AU are native, diverse, and high quality; the AU is dominated by homogenous nonnative or low-quality habitats; <4% tallgrass composition or shortgrass dominant. Vegetative litter/tussocks are extremely limiting; almost never available at appropriate level (denuded or overgrown). Violet densities are very limiting; 0.9 plants/m^2 or less) in most areas. Floral resources are a limiting factor nearly every year. Shrubs/tall vegetation are either not available or woody encroachment (succession) dominates in many/most areas.	1.1-2%	61-80%	Spring precipitation 76-114 mm (3.0-4.49 in); summer precipitation 76-114 (3.0-4.49 in); 3.0-3.9 droughts per decade	1-20%	1-20%	Adults typically occur in very low numbers within populations; uncommon to rare throughout the AU in most years; no locally abundant populations; less than 1 individual per 1 ha (2.5 ac) in good year, no individuals detected in poor years; most areas act as sinks	Populations in AU consistently exhibit little to no growth in most years, many are extirpated in poor years. Repopulation may take years, if it occurs at all. AU may be a population sink or may only harbor dispersing adults occasionally with few to no populations most years.
EXTIRPATED (0 points)	< 1%	No good quality grasslands present	≤1.0%	81-100%	Spring precipitation <76 mm (3 in); summer precipitation <76 mm (3 in); ≥4.0 droughts per decade	<1%	<1%	Absent	Absent

Current Conditions

To evaluate resiliency, we used the condition category table like a key and assigned conditions for each of the habitat and demographic factors for all 22 AUs, from 5 points for very high condition to 0 points for the lowest, extirpated condition (Table 13). For all factors, a higher score reflects a more favorable condition, and a lower score reflects a less favorable condition. We scored each AU consistently for each factor, and then calculated an unweighted average, with resiliency assigned across an evenly distributed range of values among the six condition categories (Table 14). Any AU with the lowest condition, extirpated, as a condition for either the demographic factors, Abundance or Growth Trend, automatically received a resiliency of extirpated regardless of the condition of any of the other factors. Once condition categories were assigned to each habitat and demographic factor in every AU, we calculated their average point values and compared the results to the ranges of values in Table 13 to assign resiliency to each AU.

Table 13. Condition categories and associated point values and range of values for each category.

CONDITION CATEGORIES	POINT VALUE	RESILIENCY *Unweighted average of points across nine factors* (RANGE OF VALUES)
VERY HIGH	5	VERY HIGH 4.170 - 5.00
HIGH	4	HIGH 3.336 - 4.169
MEDIUM	3	MEDIUM 2.502 - 3.335
LOW	2	LOW 1.668 - 2.501
VERY LOW	1	VERY LOW 0.834 - 1.667
EXTIRPATED (X)	0	EXTIRPATED (X) 0.00 - 0.833

The results of applying the condition category table to the current resiliency of the AUs are provided in Table 14 and displayed on a map in Figure 23.

Table 14. Current conditions of the eastern and western subspecies of regal fritillary per quantitative and qualitative evaluations of nine habitat and demographic factors outlined in the condition category table.

CURRENT HABITAT AND DEMOGRAPHIC CONDITIONS AND CURRENT RESILIENCY OF 22 AUs												
SUBSPECIES	REPRESENTATION UNIT NAME	ANALYTICAL UNIT NAME	HABITAT FACTORS							DEMOGRAPHIC FACTORS		RESILIENCY
			NATIVE GRASSLANDS (Quantity and Quality)		RIPARIAN/ WETLAND AREAS (Refugia)	AMBIENT TEMPERATURE	PRECIPITATION (Available Moisture)	LARGE, CONTIGUOUS BLOCKS OF NATIVE GRASSLANDS		ABUNDANCE	GROWTH TREND	
			Quantity: *Percent of AU that is native grasslands (surrogate: NLCD 2019 Herbaceous)*	***Quality:*** *Evaluation of existing native grass types, violets, diverse floral resources, shrubby/tall vegetation, vegetative litter, and grass tussocks*	*Percent of AU that is potentially suitable habitat (surrogates: NLCD 2019 Herbaceous, Pasture/Hay, Scrub/Shrub, Emergent Herbaceous Wetland) within 100 m (328 ft) buffer of streams and wetlands*	*Temperatures relative to key thermal tolerance of larval and pupal stages: percent of AU with Temperatures over 41 °C (105 °F) for 2+ days*	*Relative moisture supporting floral resources, individual health: spring and summer precipitation in mm (in) and number of droughts per decade*	*Patch size: percent of AU composed of patches sized 1,000+ ha (2,471 ac)*	*Connectivity: percent of AU comprised of grass patches within 5 km (3 mi) of 500+-ha (1,236+-ac) patches or within 3 km (1.9 mi) of 101-to 500-ha (247- to 1,236-ac) patches*			
EASTERN	**EAST**	**Ridge and Valley**	X	Low	Low	Very High	High	X	Very Low	Very Low	Very Low	**LOW**
WESTERN	**MIDWEST**	**Central Corn Belt Plains**	X	Medium	Very Low	High	Very High	X	Very Low	Low	Medium	**LOW**
WESTERN	**MIDWEST**	**Central Irregular Plains - A**	X	Medium	Medium	High	High	X	Low	Medium	Medium	**LOW**
WESTERN	**MIDWEST**	**Driftless Area**	Very Low	Low	Medium	High	High	Very Low	Low	Very Low	Very Low	**LOW**
WESTERN	**MIDWEST**	**Interior River Valleys and Hills**	X	Very Low	Medium	High	High	X	Very Low	Low	Medium	**LOW**
WESTERN	**MIDWEST**	**North Central Hardwood Forests – A**	Very Low	Low	High	High	High	Very Low	Very Low	Low	Low	**LOW**
WESTERN	**MIDWEST**	**North Central Hardwood Forests – B**	X	Low	High	Very High	High	X	Very Low	Low	Low	**LOW**
WESTERN	**MIDWEST**	**Southeastern Wisconsin Till Plains**	X	Very Low	High	High	High	Very Low	Low	Very Low	Very Low	**LOW**
WESTERN	**MIDWEST**	**Western Corn Belt Plains**	Very Low	Low	Medium	High	High	X	Very Low	Low	Low	**LOW**
WESTERN	**NORTHERN GREAT PLAINS**	**Lake Agassiz Plain**	Very Low	Low	Medium	High	Medium	Very Low	Very Low	Low	Low	**LOW**
WESTERN	**NORTHERN GREAT PLAINS**	**Middle Rockies**	Medium	Low	High	Very High	Medium	Low	Low	Very Low	Very Low	**MEDIUM**
WESTERN	**NORTHERN GREAT PLAINS**	**Northern Glaciated Plains**	Low	Medium	High	High	Medium	Very Low	Low	Medium	Medium	**MEDIUM**
WESTERN	**NORTHERN GREAT PLAINS**	**Northwestern Glaciated Plains**	High	Medium	High	High	Low	Low	Medium	Medium	Medium	**MEDIUM**
WESTERN	**NORTHERN GREAT PLAINS**	**Northwestern Great Plains**	High	Low	High	High	Medium	High	High	High	Medium	**HIGH**
WESTERN	**CENTRAL GREAT PLAINS**	**Central Great Plains**	High	Low	High	Medium	High	Low	Low	Low	Very Low	**MEDIUM**
WESTERN	**CENTRAL GREAT PLAINS**	**Central Irregular Plains - B**	Very Low	Medium	High	Medium	High	Very Low	Medium	Medium	High	**MEDIUM**
WESTERN	**CENTRAL GREAT PLAINS**	**Cross Timbers***	High	Low	Medium	Medium	Very High	Low	Medium	Extirpated	Extirpated	**X***
WESTERN	**CENTRAL GREAT PLAINS**	**Flint Hills**	Very High	Medium	Very High	Medium	Very High	Medium	High	Medium	Medium	**HIGH**
WESTERN	**CENTRAL GREAT PLAINS**	**High Plains**	High	Low	Medium	High	Low	Low	Medium	Very Low	Low	**MEDIUM**
WESTERN	**CENTRAL GREAT PLAINS**	**Nebraska Sand Hills**	Very High	High	High	High	Medium	Very High	Very High	Low	High	**HIGH**
WESTERN	**CENTRAL GREAT PLAINS**	**Ozark Highlands**	X	Low	Medium	High	Very High	Very Low	Medium	Low	Low	**LOW**
WESTERN	**CENTRAL GREAT PLAINS**	**Southern Rockies**	Medium	Very Low	High	Very High	Low	High	High	Very Low	Very Low	**MEDIUM**

*Cross Timbers average is within Low point range but in Extirpated condition for both Abundance and Growth Rate, thus analytical unit resiliency is Extirpated.

Figure 23 is a map of the current conditions for the eastern and western subspecies in terms of resiliency, redundancy, and representation. Currently, AU resiliency ranges from high to extirpated, and there are no AUs current with very high resiliency.

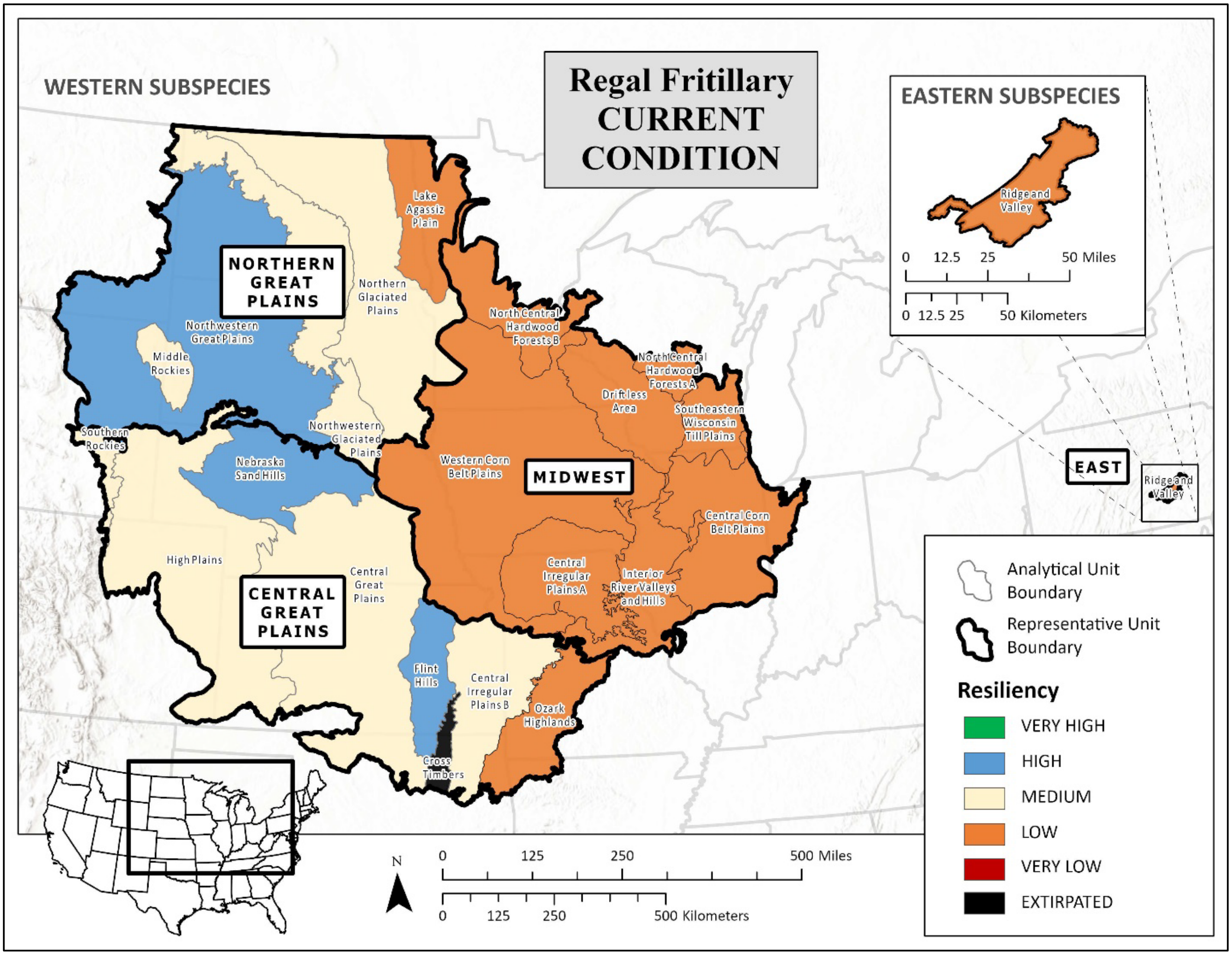

Figure 23. Current conditions for the eastern and western subspecies of regal fritillary in terms of the 3Rs.

Redundancy describes the subspecies' ability to withstand catastrophic events, from which adaptation is unlikely, such as a large wildfire or large, extended drought. Redundancy can be described as the number and distribution of AUs for each subspecies, and visually illustrated in Figure 23, above. Redundancy across both subspecies, with associated resiliency for the AUs is also summarized in Table 15.

Table 15. Current redundancy of the eastern and western subspecies of regal fritillary expressed as the number of analytical units (AUs) within each resiliency category, for each subspecies.

CURRENT REDUNDANCY BY SUBSPECIES (SEE MAP IN FIGURE 22 FOR DISTRIBUTIONAL SPREAD)		
RESILIENCY	EASTERN SUBSPECIES AUs	WESTERN SUBSPECIES AUs
Very High		
High		3
Medium		7
Low	1	10
Very Low		
Extirpated (X)		1
TOTAL AUs	1	21

Representation describes the full suite of ecological, genetics, and life history diversity of the eastern subspecies across one AU and one representative unit, and of the western subspecies across 21 AUs and 3 representative units. Representation is illustrated in the map in Figure 23, above, with the representative unit polygons and summarized below in Table 16 with the numbers of AUs in each resiliency category.

Table 16. Current representation of the eastern and western subspecies of regal fritillary, expressed as the number of analytical units (AUs) within each condition category by the single representation unit of the eastern subspecies, and among the three representation units of the western subspecies.

CURRENT REPRESENTATION - EASTERN AND WESTERN SUBSPECIES				
RESILIENCY	EASTERN SUBSPECIES	WESTERN SUBSPECIES		
	EAST	MIDWEST	NORTHERN GREAT PLAINS	CENTRAL GREAT PLAINS
Very High				
High			1	2
Medium			3	4
Low	1	8	1	1
Very Low				
Extirpated (X)				1

Summary of Current Conditions for the Eastern Subspecies

Currently, the eastern subspecies has one AU, the Ridge and Valley AU in Pennsylvania, with low resiliency. Although this AU currently has very high and high conditions for the habitat factors of temperature and precipitation, respectively, the lower conditions of the other habitat factors, and the very low condition of both demographic factors, results in its low resiliency.

Specifically, the percent native grasslands and patch size for the AU are currently in extirpated condition, and the quality and connectivity of habitats are in low and very low conditions, respectively. The quantity of habitats available in the AU is limited by the primary land cover of deciduous forest, at nearly 40 percent coverage (Dewitz 2021, entire).

Currently, habitats for the eastern subspecies are maintained by suitable disturbances, often from military exercises in some areas, and beneficial management actions in others (PADMVA 2021, entire), which has helped maintain resiliency in this AU. Actions by FTIG, including those outlined in the INRMP, have helped maintain suitable habitats for the eastern subspecies. Without these actions and activities, habitat occupied by the eastern subspecies could be quickly overtaken by woody plants, which would reduce the resiliency of the AU. The AU is more mesic and least likely among all regal fritillary AUs to be prone to drought and conversion. With low resiliency, this AU is more at risk to stochastic events, such as bad years or harsh winters, than AUs with more resiliency, but resiliency is not currently very low or extirpated. The AU has maintained resiliency, although at low levels, and demonstrated the capacity to withstand stochastic events in the past.

Redundancy for the eastern subspecies is described as the one, Ridge and Valley AU, with low resiliency, found as distributed on FTIG. With only one, narrowly distributed AU with low resiliency, the eastern subspecies is inherently more at risk to catastrophic events, such as uncontrolled and widespread wildfire, than if there were additional and more widely distributed AUs.

Current representation for the eastern subspecies is similarly limited to the single, isolated population at FTIG. The AU is small and isolated, so genetic diversity for the eastern subspecies is limited. The nearest population of the western subspecies is about 869 km (540 mi) west, and natural genetic exchange between the subspecies is unlikely. Genetically, regal fritillaries of the East representation unit exhibit distinct haplotypes that are not present in any other known extant regal fritillary population (Williams et al. 2001b, p. 146). Allelic diversity level and expected levels of heterozygosity in the East unit are lower than those in either the Great Plains or Midwest (Williams et al. 2003, p. 16). The eastern subspecies has distinct genetic structure at a small spatial scale (i.e., among the SCAs on FTIG), reflecting high rates of genetic drift among its small colonies (Keyghobadi et al. 2006, p. 3). A reduction in dispersal tendency at FTIG may be in response to habitat fragmentation, via either behavioral response or selection against unsuccessful migrants, but further analysis is needed (Keyghobadi et al. 2006, pp. 3–4). As a result, the current adaptive capacity for the eastern subspecies is challenging to describe.

To summarize, given its current resiliency, redundancy, and representation, the eastern subspecies is inherently more at risk to stochastic events, catastrophic events, and environmental change than the western subspecies. The eastern subspecies is small, isolated, has unique habitat and genetic characteristics and stressors, and is the only known remnant of a subspecies that was historically more broadly distributed. However, resiliency is currently low, and the subspecies has demonstrated the capacity to withstand stochastic and catastrophic events, although ecological and genetic diversity are low. Ongoing activities and conservation actions for the eastern subspecies continue to reduce habitat loss and fragmentation associated with woody encroachment, which has helped maintain low resiliency in its single AU. As a result, the current viability for the eastern subspecies depends largely on these beneficial actions and activities to control woody encroachment and improve habitats.

Summary of Current Conditions for the Western Subspecies

Current resiliency of the 21 AUs for the western subspecies ranges from high to extirpated. Three AUs currently have high resiliency, 7 have medium resiliency, 10 have low resiliency, and one AU is currently extirpated. There are no AUs currently with very high or very low resiliency.

The Northwestern Great Plains AU in western North and South Dakota, eastern Montana and Wyoming, and the Nebraska Sandhills AU in west-central Nebraska, and the Flint Hills AU in eastern Kansas, currently have high resiliency. The high resiliency in these AUs reflects the generally high condition of their habitat and demographic factors. We note that the Northwestern Great Plains AU is quite large and extends westward into the shortgrass biome. While some conditions (native grasslands, forbs) may be intact in these westernmost regions, we acknowledge that such areas may become too dry during droughts to consistently support regal fritillaries; yet the subspecies is able to take advantage of the resources during wetter years. The same caveat of drier conditions may be applied to the Middle Rockies, Southern Rockies, and High Plains AUs.

The Cross Timbers AU in northwestern Oklahoma and southeastern Kansas is the only AU of the western subspecies that is currently extirpated, with no resiliency. Although the Cross Timbers AU has low conditions for the habitat factors, its abundance and trend are currently in extirpated condition, so the AU currently has no resiliency. This AU has documented observations since 2010, so was included in our analysis, but currently the demographic factors are in extirpated condition.

Western subspecies AUs currently with medium resiliency include the Middle Rockies (Black Hills region of South Dakota), Northern Glaciated Plains (primarily eastern North and South Dakota) and Northwestern Glaciated Plains (primarily central North and South Dakota). Further south, the following AUs also have medium resiliency: Central Great Plains (southern Nebraska, central Kansas, northern Oklahoma), Central Irregular Plains-B (eastern Kansas, western Missouri and northeastern Oklahoma), High Plains (western Kansas and Nebraska, east-northeast Colorado and southeast Wyoming) and Southern Rockies (primarily Wyoming). Notably, all of these units are west of the historical tallgrass prairie core of the species' range. This means these areas are relatively drier with progressively less tallgrass vegetative composition in a westly direction, becoming more mixed-grass or short-grass dominant.

The rest of the AUs for the western subspecies were determined to have low resiliency, including all of those in the Midwest (the tallgrass prairie region). One commonality among these AUs, with exception one AU in the Central Great Plains (Ozark Highlands, which scored medium for Connectivity), was that they all had condition category ranks at or beneath the low level for the habitat factors of Native Grass Quantity, Patch Size and Connectivity.

In localized areas in some AUs (not the entire AUs themselves), there are existing populations observed that may appear to have greater resiliency than that reflected by the conditions or resiliency of their respective AUs. Such populations may be in parts of the AUs where lands offer relatively good habitat conditions, and patches may be large or in close proximity, promoting good connectivity. Due to habitat restoration actions in Indiana, for example, a former at-risk population has grown and become a source of recolonization for adjacent areas, yet suitable habitat across the rest of the larger AU is very limited. In western Missouri, seemingly resilient populations exist on local habitat patches that are relatively small and within an agricultural landscape, but many are also proximal, which promotes successful adult dispersal. Ongoing periodic disturbances (primarily grazing) sustain those habitats. In some areas of Wisconsin, although habitats are typically small and fragmented, significantly higher abundance values than those used for our condition category criteria for that demographic need have been reported (A. Swengel, personal communication, 2022). In contrast, in the Flint Hills of eastern Kansas, surveys for the butterfly typically result in abundance values on the low end of our condition category scale, despite the considerable amount of large, connected habitats (albeit this may be a sampling method issue). However, resiliency is evaluated at the AU scale.

Three representation units with several AUs within each compose the range of the western subspecies: (1) Midwest (eight AUs), (2) Northern Great Plains (five analytical units), and (3) Central Great Plains (eight AUs). All eight Midwest AUs are currently in Low condition. Those of the Northern Great Plains range from High to Low, while those of the Central Great Plains range from High to Extirpated; both representation units exhibit an overall average of Medium. We discuss current representation for each of the representative units below.

Midwest Representation Unit

Like the other representation units, the habitats occupied by the western regal fritillary in the Midwest provide the same basic resources required by regal fritillaries as elsewhere in the range (bunchgrasses, violets, nectar, etc.), but the Midwest's historically vast tallgrass prairie ecosystem was the heart of the regal fritillary's former range. Tallgrass prairies generally receive less precipitation than eastern forested areas but relatively more than mixed and short-grass prairies farther west. Woody encroachment can be problematic here (albeit to a lesser degree than the East), as are invasive grass encroachment that reduces the diverse native prairie vegetation on which regal fritillaries rely. Kelly and Debinski (1998, p. 273) noted significantly smaller regal fritillaries in Iowa compared to South Dakota, North Dakota, and Kansas specimens, and suggested this could be the result of genetic drift influencing small populations. Although the regal fritillary is described as a strong flier, within highly fragmented habitats, there could be strong selection pressure against individuals that disperse and are unsuccessful, while individuals that do not disperse may be predisposed to be more stationary, such as smaller individuals, potentially leading to rapid genetic evolution (Kelly and Debinski 1998, p. 273). In the Midwest, the matrix of agriculture currently presents a barrier to dispersal and gene flow among populations, either preventing individuals from attempting dispersal and/or reducing the likelihood that attempted dispersals will result in successful colonization elsewhere. Williams et al. (2003, p. 13) evaluated the genetic effects of fragmentation that has occurred since the 1860s among regal fritillary populations, comparing populations in the East (historically isolated), Midwest (fragmented), and the Great Plains (unfragmented) using four microsatellite loci (identified in Williams et al. 2002, entire). Previous work (Williams et al. 2001b, entire) used mitochondrial DNA analysis to differentiate the subspecies; these microsatellites allowed for comparisons within these groups. Allelic differentiation was detected between these groups, but at each microsatellite locus, significant differentiation was least common in the Great Plains samples, relatively more common in the Midwest, and most in the East (FTIG) (Williams et al. 2003, p. 14). When comparisons were made within these groups, pairwise comparisons within Midwest samples showed higher differentiation than those in Great Plains samples (and differentiation was highest in comparisons within the East population at FTIG) (Williams et al. 2003, pp. 14–15). Measures of genetic diversity revealed a similar pattern with allelic diversity level and expected levels of heterozygosity highest in the Great Plains, relatively less in the Midwest, and least in the East. This general pattern is consistent with predicted genetic effects of habitat fragmentation, whereby populations may experience restricted gene flow and genetic bottlenecks (Williams et al. 2003, p. 16). In short, habitat fragmentation has clearly disrupted the level of gene flow observed among Midwestern and the Eastern populations (Williams et al. 2003, p. 16).

Northern Great Plains Representation Unit

Current genetic information indicates that regal fritillaries across the entire Great Plains (Northern and Central) exhibit less differentiation than those in the Midwest or Pennsylvania and are considered as one (homogenous) genetic population with high gene flow over hundreds of kilometers (km) (Williams et al. 2003, pp. 13, 14). A key factor in this is the post-breeding dispersal capabilities of females (i.e., potentially more than 100 mi [161 km]) (P. Hammond, personal communication, 2021), as well as the open habitats of the Great Plains with their relatively larger tracts of grasslands that are typically better connected than habitats of either the Midwest or East. The species can be locally common in the Northern Great Plains, but numbers vary considerably on an annual basis, and plummet when the climate becomes hot and dry, particularly over multiple years.

In terms of habitat, the Northern Great Plains and Central Great Plains representation units currently support relatively more intact and better-connected grasslands used for livestock grazing or haying than the Midwest unit, but the plains units are drier, more prone to drought, and with less tallgrass species comprising the grasslands. The Northern Great Plains representation unit experiences shorter growing seasons and colder weather patterns than those in the Central Great Plains. Habitats in the Midwest representation unit are primarily small, isolated patches in an agriculturally dominated landscape, and many sites exist as conservation preserves – small remnants of the once-vast tallgrass prairie.

Current genetic information indicates that all western subspecies units have 22 unique haplotypes among populations sampled across the Midwest and Great Plains, with no apparent geographical associations detected among them (Williams 2001b, p. 146). However, regal fritillaries across the entire Great Plains (Northern and Central) exhibit less differentiation than those in the Midwest and are considered as one (homogenous) genetic population with high gene flow over hundreds of kilometers (Williams et al. 2003, pp. 13, 14). Further, allelic diversity and expected levels of heterozygosity are relatively lower in regal fritillaries from the Midwest compared to the Great Plains, consistent with predicted genetic effects of habitat fragmentation (Williams et al. 2003, p. 16).

Summary of Current Conditions

Historically, populations of the regal fritillary functioned on a vast scale and were abundant and broadly (albeit patchily) distributed, particularly in the Midwest and Great Plains; likely millions of individuals occupied the North American prairies prior to establishment of European agriculture (Hammond and McCorkle 1983(84), p. 219). Occupancy was dictated by local habitat suitability – not availability – as natural processes including climate, grazing, and fire, maintained the open grassland habitats with native violet and nectar components required by the regal fritillaries. This vast range may have facilitated eastward expansion, perhaps via coastal grasslands, where the butterflies opportunistically moved into inland habitats created and maintained by human activities (P. Hammond, personal communication, 2021).

Today, patches of adequate size and diversity are significantly reduced, both in number and proximity, interrupting the landscape-level scales at which the regal fritillary historically functioned. Accessibility to suitable habitats has become increasingly restrictive. The eastern subspecies is extirpated from nearly every formerly known occupied eastern location and a small fraction of the historically vast tallgrass prairies of the Midwest are left, mostly existing as remnants that are severely fragmented and isolated. Less severe conditions exist westward in much of the mixed-grass prairie range, but much of these grasslands have been converted to agriculture and other human uses as well. Dispersals from occupied habitats today have the potential to be dead ends as individuals move into a matrix that may be composed of unsuitable agricultural fields where they are unable to find the resources they need to survive and establish the next annual generation. Risk of genetic collapses increases without regular successful dispersal events. Natural periodic disturbances that historically maintained the shifting mosaic of habitats on the landscape scale have been replaced with land use and management regimes that, when applied inappropriately, can reduce or eliminate regal fritillary populations. Stochastic events and synergistic processes related to habitat loss have significantly greater potential to cause population extirpations that may outpace recolonization rates.

Chapter 6 – Future Conditions

In this chapter, we consider how the viability of the eastern and western subspecies could change from their current conditions, described in Chapter 5, into the future. To assess future conditions, we conducted a future scenario analysis. In that analysis we developed three scenarios to represent different plausible futures in terms of climate conditions. Each scenario represents a plausible, yet simplified representation of the climatic stressors likely to influence subspecies viability either directly or indirectly. The future scenarios help capture the full range of uncertainty associated with the future, bounded by the least and most risk to the subspecies. The future outcome in the future could include a combination of factors from any of these scenarios, but our analysis has captured a full portfolio of risk to the subspecies.

We projected future conditions 50 years into the future, to year 2075. We selected this timeframe because climate models reveal potentially significant change during that time period, and it also incorporates over 50 annual generations of the regal fritillary. Additionally, the timeframe is biologically meaningful because it also represents the approximate timeframe during which the historical range of the eastern subspecies contracted to its current distribution in one AU.

Future Scenarios

We developed three future scenarios based on projected climate conditions and plausible states of the primary drivers (stressors) of viability for the eastern and western subspecies. During our cause-and-effects analyses (Chapter 4 and Appendix H), we identified several significant drivers for the viability of the eastern and western subspecies. These vary somewhat by subspecies. Drivers for the western subspecies include grassland conversion, herbicide application, climate change factors (particularly drought), invasive grasses/woody encroachment (succession), and periodic disturbances (fire/haying/grazing). For the eastern subspecies, grassland conversion and herbicide application are not considered risk factors on FTIG; future status is more reliant on management activities that ensure grassland habitat persists. Both subspecies are vulnerable to fragmentation and isolation that occurs with loss or degradation of habitat. Thus, the overarching trends of future scenarios for both subspecies are generally similar, but the individual scenario descriptions include subspecies-specific factors.

In developing the future scenarios, we considered the likelihood that conditions would improve for the subspecies versus the likelihood that they would continue to decline. A future condition in which the demographics of one or both subspecies could improve would require habitat restorations and enhancements that would result in increases in quantity, quality, size, connectivity of native grasslands, and/or more favorable climatic conditions. Further, habitat restorations or enhancements would have to occur at a higher rate than habitat loss and degradation, and future climate conditions would have to remain favorable rather than increase in volatility with rises in temperature and reductions in precipitation as predicted.

The future scenarios help describe the range of uncertainty in the future based on the range of plausible impacts of potential future stressors and conservation actions. Different probabilities may be associated with our future scenarios, but all three are considered equally plausible for the purposes of our SSA analysis.

The general concepts of each scenario are as follows:

- **Scenario 1: Continuation of Existing Conditions/Trends.** This scenario assumes that current stressors affecting the species do change somewhat over time but continue generally per current trends.
- **Scenario 2: Moderately Worsening Conditions/Trends.** Under this scenario, current stressors become more problematic over time, showing an increase of the stressors and trends currently observed.
- **Scenario 3: Significantly Worsening Conditions/Trends.** This is the most severe scenario, in which new stressors arise and/or current stressors are exacerbated significantly above current levels and trends.

These scenarios are described in further detail for each subspecies below, beginning with the eastern subspecies. As noted above, some overlap in factors exists, but the subspecies' different environmental and situational circumstances require a separate evaluation of future conditions.

Methods Used to Evaluate Future Conditions of Habitats

In Chapter 4, we identified the factors affecting the regal fritillary that are likely to be carried forward into the future and described how they may change for each subspecies, and under each scenario. We then used the same steps applied to develop the current conditions table, repeating the process three times for each subspecies, resulting in six future conditions tables. We used the condition category table (Table 12, above) again to evaluate the future condition of the nine habitat and demographic factors for each AU, with a straight average of the scores calculated to determine future resiliency of each AU under each future scenario. We largely used expert judgement to inform the evaluations of future conditions. Although patch size and connectivity were evaluated quantitatively for current conditions, we did not apply that analysis method to future conditions due to computational processing limitations. This also avoided the compounding of additional assumptions that would be required to accurately quantify patch size and connectivity into the future. Future patch size and connectivity were evaluated qualitatively instead. A predictive model for future grassland conversion was used to estimate various levels of change (i.e., increasing conversion risk) to native grasslands and riparian/wetland areas. Finally, additional climate models that aligned with our future condition scenarios were applied to evaluate precipitation and ambient temperatures. Specific methods to determine future conditions are described below.

Future Native Grassland Quality, Contiguous Blocks of Native Grasslands – Patch Size and Connectivity, Abundance, Growth Trend Methods

For the future conditions tables, the same factors that had been ranked qualitatively for current conditions were again evaluated for each future scenario, applying the criteria from the same condition category table. Additionally, patch size and connectivity were ranked qualitatively, as we were unable to quantify changes to these factors in the future. The methods used to calculate connectivity in current conditions were not used to project connectivity into the future in combination with the agricultural conversion risk model, as the compounding of assumptions and uncertainties would render the outcome misleading. Instead, connectivity scores stayed the same into future scenarios 1, 2, and 3 unless qualitative assessment suggested otherwise.

Future Native Grasslands (Quantity) Method

We identified grassland conversion as primary driver of current and future resiliency of AUs, so we used projections of this stressor to help estimate the future availability of grassland habitats. To determine total area of each analytical unit that was at risk of future conversion to agriculture, the NLCD 2019 data was used in combination with a probabilistic crop suitability model that estimated future cropland expansion (Smith et al. 2016, entire). The Smith et al. (2016) model results in each pixel being assigned a value between zero and one, with zero being the areas with the lowest probability of conversion and one being the highest probability of conversion. Three different thresholds, aligning with the increasing severity of conditions outlined in our future scenarios, were used to assess risk of conversion to agriculture: low (>0.98, i.e., very little future conversion risk), medium (>0.7, i.e., moderate risk of future conversion), and high (>0.3, i.e., high level of future conversion risk). The scenario approach was used, as the Smith et al. (2016) model does not incorporate market/economic conditions (i.e., commodity prices, etc.) The NLCD 2019 "cultivated crops" class was used to mask (eliminate) pixels within the Smith et al. (2016) model that were currently known to be cultivated for agriculture. This was necessary to ensure that areas which are currently cultivated were not included in the calculations for future conversion risk. The resulting layers (with currently cultivated areas eliminated) were then combined with all classes from NLCD 2019 in ArcPro to determine area for each analytical unit by NLCD class that was at risk of conversion. The summary spatial statistics for each analytical unit were obtained by using the "summarize within" function within ArcPro. Values for the Grassland/Herbaceous land cover were selected and converted to a percentage within each analytical unit to obtain the future conditions under each scenario.

Future Riparian/Wetland Areas (Refugia) Method

The methods used to quantitatively determine the amount of riparian/wetland areas within each analytical unit for future conditions were identical to those used to determine the values for future conditions of Native Grasslands (Quantity) (section 2.10.3). The riparian/wetland values calculated for current conditions (section 2.9.3 above) served as baseline data. To that baseline, we applied the probabilistic crop suitability model that estimated future cropland expansion (Smith et al. 2016).

This was done in each analytical unit to determine how much the NLCD 2019 land covers (Dewitz 2021) existing today might change under the three different thresholds that aligned with the increasing severity of conditions outlined in our future scenarios: low (>0.98, i.e., very little future conversion risk), medium (>0.7, i.e., moderate risk of future conversion), and high (>0.3, i.e., high level of future conversion risk). New percentages of the combined NLCD layers Grassland/Herbaceous, Scrub/Shrub, Pasture/Hay, and Emergent Herbaceous Wetland within the 328-ft (100-m) buffer to streams and wetlands (U.S. Environmental Protection Agency 2021) were then calculated for each analytical unit.

Future Ambient Temperature Method

The process used to calculate future ambient temperature was the same as that for current conditions but with the additional models and a future timeframe. Climate projections from the MACA Climatology Lab (Abatzoglou and Brown 2012) were again used to obtain the area within each AU over which two or more days are projected to exceed 40˚C (104˚F) between April 1 and July 15. Averaged projections under RCP 4.5 were obtained by the four different models at 30m resolution to account for variation in the models: INMCM4.0, MRICGCM3, and HadGEM2 for future scenarios 1, 2, and 3, respectively, for the eastern subspecies; INMCM4.0, HadGEM2, and MIROC5 for scenarios 1, 2, and 3, respectively for the western subspecies (Collins et al. 2008; Volodin et al. 2010; AORI 2016; Yukimoto et al. 2012). We found that, within the timeframe of our future scenarios, difference in projected ambient temperature and precipitation under RCP 4.5 and RCP 8.5 were minimal, so needed only to evaluate future scenarios under RCP 4.5. We repeated the process for each year that made up the future time frame (2066–2075) and averaged the proportions for each AU over the future timeframes to compare how the extent of potentially unsuitable temperatures in each analytical unit may change from now into the future.

Future Precipitation (Available Moisture) Method

The methods used to evaluate future scenario conditions for precipitation were similar to those for current condition with the exception of the use of additional models, and spring and summer precipitation were averaged over the projected time 2040 – 2069, while the number of times the summertime levels of the projected SPEI was below -1.5 (severe drought) was by decade. These models, all under RCP 4.5, are INMCM4.0, MRICGCM3, and HadGEM2 for future scenarios 1, 2, and 3, respectively, for the eastern subspecies; and INMCM4.0, HadGEM2, and MIROC5 for scenarios 1, 2, and 3, respectively for the western subspecies (Collins et al. 2008; Volodin et al. 2010; AORI 2016; Yukimoto et al. 2012). These models adhered to the theme of increasingly detrimental conditions under each scenario for spring precipitation, summer precipitation, and droughts per decade, at the same locality in each analytical unit identified for current conditions. The same scales used to rank these three factors in current conditions were again applied to the new values obtained from the additional climate models, and the resulting values were summed, averaged, and applied to our future conditions tables.

Eastern Subspecies Future Conditions

In this section we provide the descriptions of the three future scenarios for the eastern subspecies followed by the results of our analysis of changing future conditions under each of those scenarios, in terms of the 3Rs.

Eastern Subspecies Future Scenarios

The future scenario table below (Table 17) presents quantitative and qualitative information. Climate modeling data aligning with our future scenario themes (continuation of current trends [upper plausible-limit scenario], moderately adverse changes, and significantly worse changes [lower plausible-limit scenario]) afforded quantitative information in the form of predicted temperatures, precipitation, and frequency of drought during key life stages (larval and pupal stages). Changes in climate and drought also described qualitatively in this table, as are other population stressors affecting the eastern subspecies of regal fritillary, including encroachment of woody vegetation and periodic disturbances.

Table 17. Future scenario descriptions and the factors analyzed under each scenario to characterize the future condition of the eastern subspecies of regal fritillary.

EASTERN SUBSPECIES FUTURE SCENARIO DESCRIPTIONS				
FACTORS ANALYZED		SCENARIO 1[a] CONTINUATION Upper Plausible-Limit	SCENARIO 2[b] MODERATELY WORSE	SCENARIO 3[c] SIGNIFICANTLY WORSE Lower Plausible-Limit
CLIMATE	Percentage of Analytical Unit (AU) with 2 or more days over 41 °C (105 °F)	0%	0%	23.65%
	Spring precipitation/percent change relative to historical	302 mm (11.9 in)/2.6%	300 mm (11.8 in)/1.7%	325 mm (12.8 in)/10.3%
	Summer precipitation/ percent change relative to historical	310 mm (12.2 in)/7.0%	287 mm (11.3 in)/-0.9%	274 mm (10.8 in)/-5.3%
	Standardized Precipitation Evapotranspiration Index[d], June July August Mean, expressed as droughts per decade/change from historical	0.7/-0.1	2.0/1.9	2.3/2.2
	DROUGHT	Drought occurs infrequently, but currently increasing in frequency, duration, intensity	Drought rate more than doubles from current rate, conditions may last longer, reduced recovery periods between incidents.	Drought more than triples current rate; lack of violet/nectar resources may have population-level impact and lack of reprieve time may affect ability of population to recover.
	CLIMATE CHANGE	Heat and drought intensity/duration/ frequency is increasing rangewide. Phenology of violets/nectar sources is changing to earlier dates. Winter conditions becoming milder in some areas. Storms becoming increasingly severe/more frequent.	Heat and drought intensity/duration/ frequency intensifies moderately Phenology of violets/nectar sources become days earlier. Winter conditions become milder, may involve increase in overwintering larvae energy expenditures. Storms increasingly severe/more frequent have greater potential to extirpate small population.	Heat and drought intensity/duration/ frequency increases. Phenology of violets/nectar sources may shift permanently a week or more earlier. Late season nectar sources become unreliable. Winter conditions become milder, causing increased mortality of overwintering larvae. Storms become increasingly severe/more frequent have greater potential to extirpate small population.
INVASIVES/WOODY ENCROACHMENT (SUCCESSION)		Same rate and magnitude of succession, conservation activities continue as now, and help to maintain current rates of invasives and encroachment.	Woody encroachment increases by approximately 25% increase from current rate. Eastern SSP FTIG decreases the use of fire and herbicides to control woody succession	Woody encroachment increases by 50% from current rate. Loss of targeted management for the research zones on FTIG (88 ha total [217 ac]; account for 10% of regal habitat on FTIG) (impact zone = 810 ha [2000 ac]). Some may still be open areas, but specific management for regal fritillaries is significantly reduced
PERIODIC DISTURBANCE (Land Management Activities to benefit pollinators)		Disturbances conducted specifically to benefit regal fritillary occur on SCAs; military exercises randomly impact SCAs and impact zone.	Disturbances conducted to benefit regal fritillaries are not prioritized; random military activities become more common, impact more areas.	Disturbances specifically for regal fritillary cease; random military actions become sole source of disturbance without consideration for presence of the species; woody encroachment reduces size of habitat patches.

[a]Scenario 1 climate model: inmcm4
[b]Scenario 2 climate model: MRICGM3
[c]Scenario 3 climate model: HadGEM2
[d]SPEI Historical (1979-2020), droughts are severe and/or extreme (SPEI <-1.5)

The future scenario table, above (Table 17, above) was particularly useful to evaluate habitat and demographic needs of the eastern subspecies for which quantitative data were not available. To determine appropriate condition categories for those needs, we considered the current condition of the eastern subspecies, the changes to the needs that were assessed quantitatively, and the descriptions for each scenario above to envision how the habitat and demographic factors may change in the future.

Eastern Subspecies Future Resiliency

As with current conditions, we used quantitative methods to obtain percent native grasslands, riparian/wetland areas (refugia), ambient temperature, and precipitation values for future conditions, but used models to project the values into the future under the three scenarios, including a predicted habitat conversion model (Smith et al. 2016, entire) and several climate models (Collins et al. 2008, entire; Volodin et al. 2010, entire; Yukimoto et al. 2012, entire ; AORI 2016, entire). We were unable to obtain future quantitative information for patch sizes and connectivity under the Large, Contiguous Blocks of Native Grasslands habitat need; thus, that factor, in addition to habitat quality and the two demographic factors of abundance and growth trend, were evaluated qualitatively for the future scenarios.

Since the eastern subspecies exists in only one representation unit composed of a single AU, the three future scenarios are combined into a single future conditions table (Table 18). Where quantitative information is available, it is provided in the table below. Qualitatively assessed factors in the table include only the assigned condition category with no associated data.

Table 18. Future condition and overall resiliency of the eastern subspecies of regal fritillary anticipated under three future scenarios using the habitat and demographic factors from the condition category table.

EASTERN SUBSPECIES FUTURE CONDITIONS											
		HABITAT FACTORS							DEMOGRAPHIC FACTORS		
		NATIVE GRASSLANDS (Quantity and Quality)		RIPARIAN/ WETLAND AREAS (Refugia)	AMBIENT TEMPERATURE	PRECIPITATION (Available Moisture)	LARGE, CONTIGUOUS BLOCKS OF NATIVE GRASSLANDS				
REPRESENTATION UNIT NAME	ANALYTICAL UNIT NAME	***Quantity:*** *Percent of AU that is native grasslands (surrogate: NLCD 2019 Herbaceous)*	***Quality:*** *Evaluation of existing native grass types, violets, diverse floral resources, shrubby/tall vegetation, vegetative litter, and grass tussocks*	*Percent of AU that is potentially suitable habitat (surrogates: NLCD 2019 Herbaceous, Pasture/Hay, Scrub/Shrub, Emergent Herbaceous Wetland) within 100 m (328 ft) buffer of streams and wetlands*	*Temperatures relative to key thermal tolerance of larval and pupal stages: percent of AU with Temperatures over 41 °C (105 °F) for 2+ days*	*Relative moisture supporting floral resources, individual health: spring and summer precipitation in mm (in) and number of droughts per decade*	***Patch size:*** *percent of AU composed of patches sized 1,000+ ha (2,471 ac)*	***Connectivity:*** *percent of AU comprised of grass patches within 5 km (3 mi) of 500+-ha (1,236+-ac) patches or within 3 km (1.9 mi) of 101- to 500-ha (247- to 1,236-ac) patches*	ABUNDANCE	GROWTH TREND	OVERALL RESILIENCY
EAST	Ridge and Valley	**FUTURE SCENARIO 1**									
		0.75% - X	Low	2.56% - Low	0% - Very High	Harrisburg, PA: spring 302 (11.9); summer 310 (12.2); 0.7 droughts - High	X	Very Low	Very Low	Very Low	**LOW (1.778)**
		FUTURE SCENARIO 2									
		0.71% - X	Very Low	2.06 - Very Low	0% - Very High	Harrisburg, PA: spring 300 (11.8); summer 287 (11.3); 2 droughts - High	X	Very Low	Very Low	Very Low	**VERY LOW (1.556)**
		FUTURE SCENARIO 3									
		0.63% - X	X	0.87% - X	23.65% - Medium	Harrisburg, PA: spring 328 (12.8); summer 274 (10.8); 2.3 droughts - High	X	X	X	X	**X (0.778)**

Future Scenario 1, the continuation scenario, represents the upper plausible-limit scenario for the eastern subspecies. Habitat and demographic conditions are expected to continue at current levels under this scenario, as are conservation actions carried out by FTIG personnel on SCAs. Climate is anticipated to change, generally at the rate currently observed. Under this scenario, resiliency into the future remains the same as it is currently, low.

The resiliency outlook under **Future Scenario 2** is between the upper and lower plausible-limit scenarios. Temperatures and spring/summer precipitation may not change significantly, but the potential for drought more than doubles over current conditions under this scenario, and milder winters or late frosts could negatively affect overwintering first instar larvae. Additionally, with less prioritization on regal fritillary conservation under this scenario, woody encroachment reduces the quality of the habitat. With these changes, the resiliency is reduced to very low under this scenario.

Under **Future Scenario 3,** the lower plausible-limit scenario, resiliency falls to extirpated, and the AU is no longer resilient under this scenario. Approximately 24 percent of the unit may experience 2+ days over the 41 °C (105 °F) temperature threshold that may negatively impact larval or pupal survival, and the frequency of severe drought more than triples over the current conditions rate. Despite a predicted rise in spring precipitation, summer precipitation is expected to drop. Climate change can also affect other life stages of the butterfly. Further, as management for regal fritillaries on research units cease, woody encroachment in occupied habitats increases as much as 50 percent. While periodic disturbances would continue, they would be according to military training needs, not designed to benefit the regal fritillary. The potential exists for the eastern subspecies to be lost under this scenario.

Eastern Subspecies Future Redundancy and Representation

The summary of both redundancy and representation are combined in the table below (Table 19) for the single representation unit (East) and sole AU (Ridge and Valley AU) of the eastern subspecies.

Table 19. Future resiliency, redundancy, and representation of the eastern subspecies of regal fritillary East representation unit and Ridge and Valley analytical unit (AU) under three future scenarios.

FUTURE REDUNDANCY AND REPRESENTATION - EASTERN SUBSPECIES			
RESILIENCY	EAST (Ridge and Valley)		
	Future Scenario 1	Future Scenario 2	Future Scenario 3
Very High			
High			
Medium			
Low	1		
Very Low		1	
Extirpated (X)			1

As is the case currently, future redundancy and representation for the eastern subspecies is limited by the distribution of the single AU and its capacity to withstand catastrophes. Under the first two future scenarios, the Ridge and Valley AU that comprises the single representation unit (East) has low and very low resiliency. Under Future Scenario 3, the AU becomes extirpated, and the subspecies has no redundancy or representation. While some habitat could remain in the AU under Future Scenario 3, our evaluation rubric requires that if the abundance and growth factors are in extirpated condition, the AU is not resilient. As a result, under Future Scenario 3, the eastern subspecies has no resiliency, redundancy, or representation.

Western Subspecies Future Conditions

In this section we provide the descriptions of the three future scenarios for the western subspecies followed by the results of our analysis of changing future conditions under each of those scenarios, in terms of the 3Rs.

Western Subspecies Future Scenarios

The western subspecies future scenario descriptions table (Table 20) below provides similar information to that of the eastern subspecies beginning with climate modeling data that align with the general trend in our three future scenarios. As detailed earlier in this chapter, one climate model was used for current conditions and for Future Scenario 1 of both subspecies, however, for the remaining future scenarios, the climate models differed between the eastern and western subspecies, based on their alignment with the theme of each scenario. Overall climate and drought descriptions are provided as well, in addition to descriptions of other stressors affecting the western subspecies of regal fritillary, including conversion of grasslands, invasive vegetation, periodic disturbances, and herbicides.

Table 20. Future scenario descriptions and the factors analyzed under each scenario to characterize the future condition for the western subspecies of regal fritillary.

WESTERN SUBSPECIES FUTURE SCENARIO DESCRIPTIONS				
FACTORS ANALYZED		**SCENARIO 1[a]** **CONTINUATION** Upper Plausible-Limit	**SCENARIO 2[b]** **MODERATELY WORSE**	**SCENARIO 3[c]** **SIGNIFICANTLY WORSE** Lower Plausible-limit
CLIMATE	Range of Percentage of Analytical Units with Two or More Days Over 41 °C (105 °F)	6.5-52.2%	0.1-73.5%	0.7-88.4%
	Range of Spring Precipitation/Range of Percent Change Relative to Historical	99-411 mm (3.9-6.2 in)/2-37%	107-422 mm (4.2-16.6 in)/2-44%	94-386 mm (3.7-15.2 in)/-17.7-14.9%
	Range of Summer Precipitation/Range of Percent Change Relative to Historical	97-340 mm (3.8-13.4 in)/-20.3-10.4%	89-307 mm (3.5-12.1 in)/-19.5--6.9%	112-300 mm (4.4-11.8 in)/-17.46-4.9%
	Range of Standardized Precipitation Evapotranspiration Index/Summer Mean in June, July, and August (Droughts Per Decade/Range of Change from Historical)	0.3-2.3/-0.7-1.7	0.3-3/-0.3-1.3¯	1-5.7/0.7-5.3
	Drought	Drought occurs commonly and is typically present in some locations within the species' range at some point every year; currently increasing in frequency, duration, intensity; timing/locations are altered from historical	Drought continues to occur every year in some portions of range at any given time, but areas subjected to drought become more expansive, may affect one or more AUs particularly in southern portions of Great Plains. Conditions last longer and experience reduced recovery periods between incidents.	Large portions of the range are subjected to drought for extended periods without adequate reprieve to allow regal populations to recover and repopulate. Extirpation becomes permanent in AUs, particularly in the Central Great Plains, but other units also vulnerable
	Climate Change	Heat and drought intensity/duration/ frequency is increasing range- wide. Phenology of violets/nectar sources is changing to earlier dates. Winter conditions becoming milder in some areas. Storms becoming increasingly severe/more frequent.	Heat and drought intensity/duration/ frequency intensifies moderately range-wide. Some northern shift in range possible but may be limited by lack of habitat in northern areas. Phenology of violets/nectar sources change to earlier dates. Winter conditions become milder, may involve increase in overwintering larvae energy expenditures. Storms increasingly severe/more frequent have greater potential to extirpate small populations.	Heat and drought intensity/duration/ frequency increases significantly range-wide, precluding recovery/ recolonizations. Phenology of violets/nectar sources permanently shift to earlier dates. Late season nectar sources become unreliable. Northern shift in climate envelope results in significant impacts to Central Great Plains AUs. Winter conditions become milder in many areas, causing increased mortality of overwintering larvae. Storms become increasingly severe/more frequent have greater potential to extirpate small populations.
Conversion		Little to no change in current grassland conversion rates due to many areas in the Midwest occurring on preserves (TNC or USFWS). In the central and Northern GP commodity prices drive conversion (TNC conversion data) - current rates have slowed from historical times, but still ongoing and subject to economic drivers that increase rate periodically; 2% of at-risk lands per TNC model are actually converted, 98% remain intact	Conversion continues unabated, advances in agriculture (i.e., drought tolerant crops) increases conversion in Great Plains. Moderate conversion rates occur: 30% of at-risk lands per TNC model are actually converted; 70% remain intact	Conversion continues unabated, advances in agriculture (i.e., drought tolerant crops) increases conversion in Great Plains. Moderate conversion rates occur: 70% or more of at-risk lands per TNC model are actually converted; 30% remain intact
INVASIVES/ WOODY ENCROACHMENT (SUCCESSION)		West - Eastern Red Cedar is concerning in some local areas within Great Plains; Smooth Brome and Kentucky Blue grass are a increasing significantly in native tracts of NGPs; woody succession is increasing rangewide	Woody encroachment and invasive grass species increase by approximately 25% increase from current rate. Attempts to control eastern red cedar, smooth brome, Kentucky bluegrass or other invasive vegetation are less effective.	Woody encroachment and invasive grass species increase by 50% from current rate. Attempts to control eastern red cedar, smooth brome, Kentucky bluegrass or other invasive vegetation are reduced.
PERIODIC DISTURBANCES (Land Management Activities to benefit pollinators)		Disturbances occur in many areas; some areas are left undisturbed & become unsuitable. Disturbances detrimental to regal fritillaries (i.e., season-long grazing; complete, ill-timed burns on small areas) are common. Disturbances conducted specifically to benefit regal fritillary do occur, but relatively rarely and typically in preserved sites (e.g., Kankakee in IN). Awareness regarding disturbances to benefit pollinators is increasing, but application of appropriate activities is lacking; limited mostly to preserves.	As habitat conversion continues and remnant habitat patches are reduced in size, small, isolated patches and disturbance regimes detrimental to the species become more common/wide-spread (particularly in Great Plains). Local extirpations increase. Disturbances conducted to benefit pollinators stagnate; awareness does not reach private landowners with majority of grassland holdings; adjustments in disturbance regime as large areas convert to small-patch size do not occur.	Small, isolated patches with inappropriate disturbance regimes dominate the species' range; awareness of the need to adjust measures to benefit regal fritillary is does not reach (or is not applied by) private landowners still with majority of holdings. Extirpations become increasingly widespread and permanent.
HERBICIDE USE/DRIFT		Herbicide drift reduces the effective habitat size of native grasslands existing in agricultural matrix. Aerial herbicide application in native grasslands conducted on private lands eliminates forbs with goal of increasing grass for perceived benefit to livestock and is becoming increasingly common in at least one AU.	Herbicide drift continues unabated and becomes increasingly common with ongoing agricultural conversion; effective habitat size of small/isolated patches is reduced further; practice of aerial herbicide application on privately owned native grassland pastures expands within AU and into adjacent AUs	Herbicide drift continues unabated and becomes increasingly common with ongoing agricultural conversion; new, more effective herbicides are developed and applied broadly; application to eliminate forbs within native grasslands becomes widespread in the range, including regal fritillary strongholds (i.e., Flint Hills)

[a]Scenario 1 climate model: inmcm4
[b]Scenario 2 climate model: HadGEM2
[c]Scenario 3 climate model: MIROC5
[d]SPEI Historical (1979-2020), droughts are severe and/or extreme (SPEI<-1.5)

Similar to the methods conducted for the eastern subspecies, the future scenario descriptions for the western subspecies (Table 20, above) were applied to the baseline (current conditions) of AUs for the western regal fritillary. We kept the quantitative habitat changes within those AUs in mind to qualitatively determine appropriate condition categories for remaining habitat and demographic needs. The evaluations were again completed by our Regal Fritillary SSA Core Team members and coordinated with the species experts to ensure the results were reasonable.

Western Subspecies Future Resiliency

Due to the presence of 21 AUs in the current range of the western subspecies, individual future condition tables for resiliency are provided separately below for each future scenario. The same methodology described above for the eastern subspecies was applied to the western subspecies, with the exception of the use of different climate models, as described above.

Western Subspecies Future Scenario 1 Resiliency

The condition categories of the habitat and demographic needs of the western subspecies AUs, and their overall resiliency as anticipated under Future Scenario 1, are provided below in Table 21.

Table 21. Future conditions and resiliency of each analytical unit for the western subspecies of regal fritillary anticipated under Future Scenario 1 using the habitat and demographic factors outlined in the condition category table.

WESTERN SUBSPECIES – FUTURE CONDITIONS UNDER FUTURE SCENARIO 1											
REPRESENTATION UNIT NAME	ANALYTICAL UNIT NAME	HABITAT FACTORS							DEMOGRAPHIC FACTORS		RESILIENCY
		NATIVE GRASSLANDS (Quantity and Quality)		RIPARIAN/ WETLAND AREAS (Refugia)	AMBIENT TEMPERATURE	PRECIPITATION (Available Moisture)	LARGE, CONTIGUOUS BLOCKS OF NATIVE GRASSLANDS		ABUNDANCE	GROWTH TREND	
		Percent of AU that is native grasslands	Evaluation of existing native grass types, violets, diverse floral resources, shrubby/tall vegetation, vegetative litter, and grass tussocks	Percent of AU that is potentially suitable habitat	Temperatures relative to key thermal tolerance of larval and pupal stages	Relative moisture supporting floral resources, individual health:	Patch size	Connectivity			
MIDWEST	Central Corn Belt Plains	0.54 - X	Medium	1.39 - Very Low	17.70 - High	Bloomington, IL: spring 300 (11.8); summer 248 (11.2); 0.33 droughts - Very High	X	Very Low	Low	Medium	LOW (2.111)
	Central Irregular Plains - A	0.55 - X	Medium	10.65 - High	36.35 - Medium	Bethany, MO: spring 315 (12.4); summer 340 (13.4); 0.66 droughts - High	X	Low	Medium	Medium	LOW (2.444)
	Driftless Area	1.45 - Very Low	Low	6.20 - Medium	18.14 - High	La Crosse, WI: spring 325 (12.8); summer 269 (10.6); 1.67 droughts - High	Very Low	Low	Very Low	Very Low	LOW (2.111)
	Interior River Valleys and Hills	0.53 - X	Very Low	4.74 - Medium	31.76 - Medium	Carbondale, IL: spring 404 (15.9); summer 323 (12.7); 0.33 droughts - Very High	X	Very Low	Low	Medium	LOW (2.000)
	North Central Hardwood Forests - A	2.39 - Very Low	Low	9.39 - High	18.49 - High	Steven's Point, WI: spring 249 (9.8); summer 282 (11.1); 1 drought - High	Very Low	Very Low	Low	Low	LOW (2.333)
	North Central Hardwood Forests - B	0.91 - X	Low	14.36 - High	15.37 - High	St. Cloud, MN: spring 224 (8.8); summer 282 (11.1); 1.33 droughts - High	X	Very Low	Very Low	Very Low	LOW (1.889)
	Southeastern Wisconsin Till Plains	0.45 - X	Very Low	8.76 - High	19.10 - High	Union Grove, WI: spring 257 (10.1); summer 302 (11.9); 1 drought - High	Very Low	Very Low	Very Low	Very Low	LOW (1.889)
	Western Corn Belt Plains	3.94 - Very Low	Low	4.54 - Medium	27.64 - Medium	Spencer, IA: spring 249 (9.8); summer 315 (12.4); 2.33 droughts - Medium	X	Very Low	Low	Low	LOW (1.889)
NORTHERN GREAT PLAINS	Lake Agassiz Plain	1.28 - Very Low	Low	6.99 - Medium	6.48 - High	Grand Forks, ND: spring 135 (5.3); summer 226 (8.9); 1.333 - Medium	Very Low	Very Low	Low	Low	LOW (2.111)
	Middle Rockies	18.15 - Medium	Low	11.16 - High	24.06 - Medium	Custer, SD: spring 178 (7.0); summer 188 (7.4); 1.33 droughts - Medium	Low	Low	Very Low	Very Low	LOW (2.333)
	Northern Glaciated Plains	7.89 - Low	Medium	13.5 - High	16.80 - High	Lake City, SD: spring 178 (7.0)); summer 236 (9.3); 2.33 droughts - Medium	Very Low	Low	Low	Low	MEDIUM (2.556)
	Northwestern Glaciated Plains	32.30 - High	Medium	14.21 - High	27.35 - Medium	Mobridge, SD: spring 130 (5.1); summer 165 (6.5); 2 droughts - Low	Low	Medium	Medium	Medium	MEDIUM (3.000)
	Northwestern Great Plains	48.18 - High	Low	19.85 - Very High	38.71 - Medium	Buffalo, SD: spring 140 (5.5); summer 142 (5.6); 1.67 droughts - Low	High	High	Medium	Low	MEDIUM (3.222)
CENTRAL GREAT PLAINS	Central Great Plains	37.64 - High	Low	12.94 - High	50.65 - Low	Lebanon, KS: spring 246 (9.7); summer 257 (10.1); 0.67 drought - High	Low	Low	Low	Very low	MEDIUM (2.556)
	Central Irregular Plains - B	5.29 - Very Low	Medium	10.65 - High	45.80 - Low	Pittsburg, KS: spring 411 (16.2); summer 330 (13.0); 0.67 drought - High	Very Low	Medium	Medium	High	MEDIUM (2.778)
	Cross Timbers	27.19 - High	Low	7.01 - Medium	52.16 - Low	Ponca City, OK: spring 338 (13.3); summer 277 (10.9); 0.67 drought - High	Low	Medium	X	X	X (2.222)*
	Flint Hills	59.79 - Very High	Medium	16.58 - Very High	51.21 - Low	Manhattan, KS: spring 295 (11.6); summer 302 (11.9); 1 drought - High	Medium	High	Medium	Medium	HIGH (3.556)
	High Plains	40.13 - High	Low	7.98 - Medium	32.53 - Medium	Sterling, CO: spring 137 (5.4); summer 165 (6.5); 0.67 drought - Medium	Low	Low	Very Low	Low	LOW (2.444)
	Nebraska Sand Hills	61.37 - Very High	High	12.30 - High	38.21 - Medium	Hyannis, NE: spring 170 (6.7); summer 193 (7.6)); 1 drought - Medium	Very High	Very High	Low	High	HIGH (3.889)
	Ozark Highlands	0.85 - X	Low	8.01 - Medium	35.64 - Medium	Springfield, MO: spring 371 (14.6); summer 310 (12.2); 0.67 drought - High	Very Low	Medium	Low	Low	LOW (2.222)
	Southern Rockies	17.25 - Medium	Very Low	15.03 - High	6.39 - High	Laramie, WY: spring 99 (3.9); summer 97 (3.8); 1.33 droughts - Low	High	High	Very low	Very low	MEDIUM (2.667)

* Cross Timbers point average is in Low condition category, but Extirpated in Abundance and Growth Trend, thus entire unit is Extirpated; the climate model for Ambient Temperature and Precipitation, Scenario 2, Western Subspecies is inmcm4 (same as Scenario 1 for Eastern Subspecies)

As with the eastern subspecies, Future Scenario 1 is the upper plausible-limit scenario for the western subspecies as stressors and trends continue as they are currently. Two AUs, Flint Hills and Nebraska Sandhills, remain in the High condition category, and the Cross Timbers AU remains the sole extirpated AU for the western subspecies under this scenario. The Cross Timbers unit was included in current conditions among the number of AUs occupied due to 2010 or later records in the unit. However, under each of the future scenarios, this AU is considered to have no resiliency and to be no longer occupied.

Three other AUs dropped to the next lowest resiliency level, including the Middle Rockies and High Plains (which both dropped from medium to low resiliency) and the Northwestern Great Plains (dropped from high to medium resiliency). Generally, habitat and demographic conditions may decline in most AUs under Future Scenario 1, but not all of them are to a degree that would cause a change to their resiliency. The climatic conditions continue to warm under this scenario with more frequent and severe storms and droughts, and while these conditions may not significantly impact much of the western subspecies' range, localized effects may be observed. Habitat loss and degradation via ongoing conversion, invasive herbaceous and woody vegetation contribute to lowering resiliency of many units, particularly those in more westerly areas (where conversion is more likely due to the relative abundance of remaining grasslands compared to the already converted landscape eastward in the western subspecies' range).

Western Subspecies Future Scenario 2 Resiliency

The condition categories of the habitat and demographic needs of the western subspecies AUs, and their overall resiliency level as anticipated under Future Scenario 2, are provided below in Table 22.

Table 22. Future conditions and resiliency of each analytical unit for the western subspecies of regal fritillary anticipated under Future Scenario 2 using the habitat and demographic factors outlined in the condition category table.

WESTERN SUBSPECIES – FUTURE CONDITIONS UNDER FUTURE SCENARIO 2

REPRESENTATION UNIT NAME	ANALYTICAL UNIT NAME	HABITAT FACTORS							DEMOGRAPHIC FACTORS		RESILIENCY
		NATIVE GRASSLANDS (Quantity and Quality)		RIPARIAN/ WETLAND AREAS (Refugia)	AMBIENT TEMPERATURE	PRECIPITATION (Available Moisture)	LARGE, CONTIGUOUS BLOCKS OF NATIVE GRASSLANDS		ABUNDANCE	GROWTH TREND	
		Percent of AU that is native grasslands	Evaluation of existing native grass types, violets, diverse floral resources, shrubby/tall vegetation, vegetative litter, and grass tussocks	Percent of AU that is potentially suitable habitat	Temperatures relative to key thermal tolerance of larval and pupal stages	Relative moisture supporting floral resources, individual health	Patch size	Connectivity			
MIDWEST	Central Corn Belt Plains	0.53 - X	Low	1.33 - Very Low	16.64 - High	Bloomington, IL: spring 333 (13.1); summer 229 (9.1); 1.33 droughts - High	X	Very Low	Very Low	Low	VERY LOW (1.667)
	Central Irregular Plains - A	0.53 - X	Medium	7.22 - Medium	50.72 - Low	Bethany, MO: spring 368 (14.5); summer 302 (11.9); 0.66 drought - High	X	Low	Medium	Medium	LOW (2.222)
	Driftless Area	1.23 - Very Low	Very Low	5.61 - Medium	18.22 - High	La Crosse, WI: spring 248 (11.2); summer 295 (11.6); 2 droughts - High	Very Low	Low	Very Low	Very Low	LOW (2.000)
	Interior River Valleys and Hills	0.50 - X	Very Low	4.39 - Medium	33.85 - Medium	Carbondale, IL: spring 422 (16.6); summer 241 (9.5); 0.33 drought - High	X	Very Low	Very Low	Low	VERY LOW (1.667)
	North Central Hardwood Forests - A	2.24 - Very Low	Very Low	8.84 - High	24.73 - Medium	Steven's Point, WI: spring 267 (10.5); summer 264 (10.4); 1.33 drought - High	Very Low	Very Low	Low	Low	LOW (2.111)
	North Central Hardwood Forests - B	0.86 - X	Low	14.08 - High	10.84 - High	St. Cloud, MN: spring 236 (9.3); summer 262 (10.3); 1 drought - High	X	Very Low	Very Low	Very Low	LOW (1.889)
	Southeastern Wisconsin Till Plains	0.43 - X	Very low	8.53 - High	13.89 - High	Union Grove, WI: spring 300 (11.8); summer 244 (9.6); 0.33 drought - High	Very Low	Very Low	Very Low	Very Low	LOW (1.889)
	Western Corn Belt Plains	3.75 - Very Low	Very Low	4.33 - Medium	23.19 -Medium	Spencer, IA: spring 264 (10.4); summer 262 (10.3); 1.67 droughts - High	X	Very Low	Low	Low	LOW (1.889)
NORTHERN GREAT PLAINS	Lake Agassiz Plain	1.25 - Very Low	Low	6.57 - Medium	22.77 - Medium	Grand Forks, ND: spring 122 (4.8); summer 196 (7.7); 1.67 droughts - Low	X	Very Low	Very Low	Low	VERY LOW (1.667)
	Middle Rockies	13.61- Medium	Low	9.01 - High	9.48 - High	Custer, SD: spring 211 (8.3); summer 170 (6.7); 2 droughts- Low	Low	Low	Very Low	Very Low	LOW (2.333)
	Northern Glaciated Plains	7.52 - Low	Low	12.26 - High	20.18- High	Mobridge, SD: spring 178 (7.0); summer 224 (8.8); 2 droughts - Medium	Very Low	Low	Low	Low	LOW (2.444)
	Northwestern Glaciated Plains	30.47 - High	Low	13.35 - High	27.15 - Medium	Lake City, SD: spring 155 (6.1); summer 157 (6.2); 1.67 droughts - Medium	Low	Low	Medium	Medium	MEDIUM (2.889)
	Northwestern Great Plains	44.77 - High	Low	18.10 - Very High	34.71 - Medium	Buffalo, SD: spring 183 (7.2); summer 135 (5.3); 1 drought - Low	High	High	Low	Medium	MEDIUM (3.222)
CENTRAL GREAT PLAINS	Central Great Plains	35.74 - High	Very Low	12.30 - High	60.41 - Low	Lebanon, KS: spring 246 (9.7); summer 249 (9.8); 1.67 drought - Medium	Low	Low	Very Low	Very Low	LOW (2.222)
	Central Irregular Plains - B	5.09 - Very Low	Low	10.16 - High	63.22- Very Low	Pittsburg, KS: spring 419 (16.5); summer 307 (12.1); 1 drought - High	Very Low	Medium	Medium	Medium	LOW (2.444)
	Cross Timbers	26.71 - High	Very Low	6.48- Medium	73.46 - Very Low	Ponca City, OK: spring 353 (13.9); summer 267 (10.5); 1.67 drought - High	Very Low	Low	X	X	X (1.778)*
	Flint Hills	58.26 - Very High	Medium	15.86 - High	65.95 - Very Low	Manhattan, KS: spring 330 (13.0); summer 300 (11.8); 1.7 droughts - High	Medium	High	Medium	Medium	MEDIUM (3.333)
	High Plains	37.14 - High	Low	7.21 - Medium	41.12 -Low	Sterling, CO: spring 127 (5.0); summer 147 (5.8); 2.67 droughts - Low	Low	Medium	Very Low	Low	LOW (2.333)
	Nebraska Sand Hills	59.17 - Very High	High	10.81 - High	33.49 - Medium	Hyannis, NE: spring 180 (7.1); summer 173 (6.8); 2 droughts - Low	Very High	Very High	Low	High	HIGH (3.778)
	Ozark Highlands	0.84 - X	Very Low	7.24 - Medium	72.92 - Very Low	Springfield, MO: spring 386 (15.2); summer 259 (10.2); 0.66 droughts - High	Very Low	Medium	Low	Very Low	LOW (1.778)
	Southern Rockies	16.56 - Medium	Very Low	13.90 - High	0.10 - Very High	Laramie, WY: spring 107 (4.2); summer 89 (3.5); 3 droughts - Very Low	High	High	Very Low	Very Low	MEDIUM (2.667)

NOTE: the climate model for Ambient Temperature and Precipitation, Scenario 2, Western Subspecies, is HadGEM2 (NOT same for Eastern Subspecies); *Cross Timbers Average Score is in Low condition category, but the unit is ranked Extirpated due to "X" in Abundance and Growth

Under the moderately worsening conditions of Future Scenario 2, which is between the upper and lower plausible-limit scenarios, the Nebraska Sandhills remains the only AU with high resiliency, and Cross Timbers is still the sole AU in extirpated condition with no resiliency. Compared to current conditions, more than half (11 of 21, or 52 percent) of the western subspecies AUs would drop to the next lower condition category. The Flint Hills and Northern Great Plains drop from their currently high resiliency to medium resiliency. Five AUs decline from medium resiliency to low resiliency: Middle Rockies, Northern Glaciated Plains, Central Great Plains, Central Irregular Plains – B and High Plains.

An AU with very low resiliency appears for the first time under Future Scenario 2 as Central Corn Belt Plains (northern Illinois and northwestern Indiana), Interior River and Valleys and Hills (western Illinois and portions of central Missouri), and Lake Agassiz Plain (far east North Dakota and northwestern Minnesota) decline from their currently low resiliency. The climate and habitat stressors under this scenario would impact relatively more units than in Future Scenario 1. As 30 percent of at-risk lands are converted to agriculture or other land uses, habitat patch sizes become smaller and more isolated, the landscape more fragmented, and appropriate adjustments to disturbance regimes are not necessarily made, ability of areas to support regal fritillaries long term becomes further reduced.

Western Subspecies Future Scenario 3 Resiliency

The condition categories of the habitat and demographic needs of the western subspecies analytical units, and their overall resiliency as anticipated under Future Scenario 3, are provided below in Table 23.

Table 23. Future conditions and resiliency of each analytical unit for the western subspecies of regal fritillary anticipated under Future Scenario 3 using the habitat and demographic factors outlined in the condition category table.

WESTERN SUBSPECIES – FUTURE CONDITIONS UNDER FUTURE SCENARIO 3											
		HABITAT FACTORS							DEMOGRAPHIC FACTORS		
		NATIVE GRASSLANDS (Quantity and Quality)		RIPARIAN/ WETLAND AREAS (Refugia)	AMBIENT TEMPERATURE	PRECIPITATION (Available Moisture)	LARGE, CONTIGUOUS BLOCKS OF NATIVE GRASSLANDS				
REPRESENTATION UNIT NAME	ANALYTICAL UNIT NAME	Percent of AU that is native grasslands	Evaluation of existing native grass types, violets, diverse floral resources, shrubby/tall vegetation, vegetative litter, and grass tussocks	Percent of AU that is potentially suitable habitat	Temperatures relative to key thermal tolerance of larval and pupal stages:	Relative moisture supporting floral resources, individual health:	Patch size:	Connectivity	ABUNDANCE	GROWTH TREND	RESILIENCY
MIDWEST	Central Corn Belt Plains	0.44% - X	Low	1.04% - X	19.64% - High	Bloomington, IL: spring 302 (11.9); summer 251 (9.9); 1 drought - High	X	X	X	X	X (1.111)*
	Central Irregular Plains - A	0.37% - X	Low	3.21% - Low	71.39% - Very Low	Bethany, MO: spring 282 (11.1); summer 297 (11.7); 3 droughts - Medium	X	Low	Low	Low	VERY LOW (1.556)
	Driftless Area	0.72% - X	Very Low	3.46% - Low	21.76% - Medium	La Crosse, WI: spring 267 (10.5); summer 300 (11.8); 2.33 droughts - High	X	Very Low	X	X	X (1.222)*
	Interior River Valleys and Hills	0.38% - X	X	3.06% - Low	45.92% - Low	Carbondale, IL: spring 386 (15.2); summer 290 (11.4); 2 droughts - High	X	X	X	Very Low	X (1.000)*
	North Central Hardwood Forests - A	1.58% - Very Low	Very Low	5.70% - Medium	11.12% - High	Steven's Point, WI: spring 246 (9.7); summer 264 (10.4); 2 droughts - Medium	X	Very Low	Very Low	Very Low	VERY LOW (1.667)
	North Central Hardwood Forests - B	0.62% - X	Very Low	12.88% - High	14.78% - High	St. Cloud, MN: spring 206 (8.1); summer 295 (11.6); 2.33 droughts - Medium	X	Very Low	X	X	X (1.444)*
	Southeastern Wisconsin Till Plains	0.30% - X	X	6.06% - Medium	10.96% - High	Union Grove, WI: spring 259 (10.2); summer 256 (10.1); 1.67 droughts - High	X	X	X	X	X (1.333)*
	Western Corn Belt Plains	2.69% - Very Low	Very Low	3.20% - Low	47.25% - Low	Spencer, IA: spring 206 (8.9); summer 274 (10.8); 3.67 droughts - Medium	X	Very Low	Very Low	Very Low	VERY LOW (1.333)
NORTHERN GREAT PLAINS	Lake Agassiz Plain	1.13% - Very Low	Very Low	5.08% - Medium	18.26% - High	Grand Forks, ND: spring 137 (5.4); summer 216 (8.5); 3.67 droughts - Low	Very Low	Very Low	Very Low	Very Low	VERY LOW (1.667)
	Middle Rockies	3.41% - Very Low	Very Low	5.54% - Medium	28.01% - Medium	Custer, SD: spring 163 (6.4); summer 175 (6.9); 3.67 droughts - Low	Very Low	Very Low	X	X	X (1.333)*
	Northern Glaciated Plains	5.67% - Very Low	Very Low	8.17% - High	23.62% - Medium	Lake City, SD: spring 168 (6.6); summer 249 (9.8); 3 droughts - Low	X	Very Low	Very Low	Very Low	VERY LOW (1.556)
	Northwestern Glaciated Plains	20.76% - Medium	Very Low	9.20% - High	39.13% - Medium	Mobridge, SD: spring 122 (4.8); summer 173 (6.8); 3.33 droughts - Low	Low	Low	Low	Low	LOW (2.333)
	Northwestern Great Plains	30.97% - High	Very Low	12.74% - High	60.35% - Low	Buffalo, SD: spring 127 (5.0); summer 132 (5.2); 4.67 droughts - Very Low	High	High	Low	Very Low	MEDIUM (2.556)
CENTRAL GREAT PLAINS	Central Great Plains	27.89% - High	Very Low	9.90% - High	88.39% - X	Lebanon, KS: spring 201 (7.9); summer 239 (9.4); 5 droughts - Low	Very Low	Very Low	Very Low	Very Low	VERY LOW (1.667)
	Central Irregular Plains - B	3.34% - Very Low	Very Low	6.91% - Medium	86.12% - X	Pittsburg, KS: spring 312 (12.3); summer 330 (13); 2.67 droughts - High	Very Low	Very Low	Very Low	Very Low	VERY LOW (1.444)
	Cross Timbers	22.54% - Medium	Very Low	4.78% - Medium	86.64% - X	Ponca City, OK: spring 267 (10.5); summer 287 (11.3); 2.67 droughts - High	Very Low	Very Low	X	X	X (1.444)*
	Flint Hills	47.52% - High	Low	12.38% - High	86.23% - X	Manhattan, KS: spring 244 (9.6); summer 282 (11.1); 5.7 droughts - Medium	Medium	High	Low	Low	MEDIUM (2.667)
	High Plains	28.43% - High	Very Low	5.52% - Medium	59.64% - Low	Sterling, CO: spring 119 (4.7); summer 178 (7.0); 4.33 droughts - Very Low	Very Low	Very Low	X	X	X (1.444)*
	Nebraska Sand Hills	49.36% - High	Low	6.24% - Medium	63.51% -Very Low	Hyannis, NE: spring 130 (5.1); summer 180 (7.1); 4.33 droughts - Very Low	Very High	Very High	Very Low	Low	MEDIUM (2.667)
	Ozark Highlands	0.68% - X	Very Low	3.98% - Low	49.24% - Low	Springfield, MO: spring 343 (13.5); summer 272 (10.7); 2.67 droughts - High	Very Low	Low	X	X	X (1.333)*
	Southern Rockies	0.30% - X	Very Low	11.01% - High	0.67% - Very High	Laramie, WY: spring 94 (3.7); summer 112 (4.4); 5.33 droughts - X	Very Low	Very Low	X	X	X (1.333)*

NOTE: the climate model for Ambient Temperature and Precipitation, Scenario 3, Western Subspecies is MIROC5 (NOT same for Eastern Subspecies); *Average Scores of these units are in Very Low condition category, but the units are ranked Extirpated due to "X" in Abundance and Growth.

Future Scenario 3 is the lower-plausible limit scenario for the western regal fritillary. Under this scenario, no AUs retain high resiliency; all are medium or lower resiliency. Only one AU retains its current condition category under this scenario: Cross Timbers AU, which remains extirpated with no resiliency

Nine AUs join Cross Timbers AU in extirpated condition under **Future Scenario 3**, including three currently with medium resiliency (Middle Rockies, High Plains, and Southern Rockies) and six currently with low resiliency (Central Corn Belt Plains, Driftless Area, Interior River Valleys and Hills, North Central Hardwood Forests-B, Southeastern Wisconsin Till Plains, and Ozark Highlands). We note that the calculated average scores of these Extirpated AUs were numerically within very low resiliency, per our range of values in Table 23, above; however, because the demographic factors of abundance and growth trend in these AUs were determined to be extirpated condition, the entire AUs themselves cannot be resiliency, so are considered extirpated under this future scenario.

Seven western subspecies AUs decline to very low resiliency under **Future Scenario 3**, including three that are currently medium (Northern Glaciated Plains, Central Great Plains, and Central Irregular Plains-B) and four units that are currently low (Central Irregular Plains-A, North Central Hardwood Forests-A, Western Corn Belt Plains, and Lake Agassiz Plain).

Under this future scenario, we project that a single AU may decline one resiliency level, from medium to low resiliency, the Northwestern Glaciated Plains. Similarly, three AUs decline one resiliency level from high to medium: Northwestern Great Plains, Flint Hills, and Nebraska Sandhills.

Under **Future Scenario 3**, the impacts of climate change become more significant than the other scenarios, with relatively large percentages of AUs impacted by high temperatures and highly frequent severe droughts without adequate reprieve to recover and repopulate areas. Food availability (violets and nectar sources) become unreliable, exacerbated by increased herbicide applications to eliminate forbs in native grasslands and 50 percent increase in invasive plants over current conditions. The majority (70 percent) of at-risk lands are converted, further fragmenting and isolating populations, restricting them to smaller sites that lack appropriate management to retain habitat quality. Extirpations of more AUs occur under Future Scenario 3 than any of the projected scenarios.

Western Subspecies Future Redundancy

Redundancy – the number of AUs with each resiliency category, under each future scenario – is provided below for the western subspecies (Table 24).

Table 24. Future redundancy characterized by the number of analytical units in each condition category and under each future scenario for the western subspecies of regal fritillary.

FUTURE REDUNDANCY - WESTERN SUBSPECIES			
RESILIENCY	**FUTURE SCENARIO 1**	**FUTURE SCENARIO 2**	**FUTURE SCENARIO 3**
Very High			
High	**2**	**1**	
Medium	**6**	**4**	**3**
Low	**12**	**12**	**1**
Very Low		**3**	**7**
Extirpated (X)	**1**	**1**	**10**

A trend among western subspecies AUs toward lower condition categories is observed within all three future scenarios. Currently, 20 western subspecies AUs are considered occupied, and one is extirpated with no resiliency. That remains the case under Future Scenarios 1 and 2 in the future. However, under Future Scenario 3, nearly half (48 percent) of the western subspecies AUs decline to the extirpated condition with no resiliency. The degree of decline in each scenario is dependent upon the level of severity of future stressors and we have high uncertainty regarding the scenarios and potential changes to the AUs.

Western Subspecies Future Representation

Representation, described as the number of AUs with each resiliency category, under each future scenario, and within each representation unit, is provided below for the western subspecies (Table 25).

Table 25. Future representation characterized by the number of analytical units in each condition category in each representation unit and under each future scenario for the western subspecies of regal fritillary

FUTURE REPRESENTATION - WESTERN SUBSPECIES									
CONDITION CATEGORY	MIDWEST			NORTHERN GREAT PLAINS			CENTRAL GREAT PLAINS		
	Future Scenario			Future Scenario			Future Scenario		
	1	2	3	1	2	3	1	2	3
Very High									
High							2	1	
Medium				3	2	1	3	2	2
Low	8	6		2	2	1	2	4	
Very Low		2	3		1	2			2
Extirpated (X)			5			1	1	1	4

All representative units have low-scoring AUs within them, and no AUs have very high resiliency under any of the future scenarios. The lowest condition categories under the future scenarios are exhibited by the most easterly-located AUs in the western subspecies' range, those in the Midwest. The two Great Plains representation units exhibit a greater range of conditions, with only the Central Great Plains predicted to sustain units with high resiliency under Future Scenarios 1 and 2, which have medium resiliency under Future Scenario 3.

The resiliency of some AUs under Future Scenario 1 decline, anticipated in the Northern Great Plains (2 units) and Central Great Plains (1 unit), involving 14 percent of all the 21 western subspecies AUs. In Future Scenario 2, relatively more decline in resiliency among the units is projected as two Midwest units, four Northern Great Plains Units, and four Central Great Plains units (11 of 21 units [52 percent]) drop to their next lowest projected resiliency.

The most significant declines in resiliency are anticipated under Future Scenario 3 when drops in condition categories are predicted for all but Cross Timbers AU of the Central Great Plains representation unit (which is currently already extirpated with no resiliency). Under this lower plausible-limit scenario, 5 (63 percent) of the 8 Midwest AUs would become extirpated, and the remaining 3 units (37 percent) decline to very low resiliency. One (20 percent) of the 5 Northern Great Plains representation units becomes extirpated, and 4 (50 percent) of the Central Great Plains AUs decline to that category under Future Scenario 3. The remaining AUs in the two Great Plains representation units would be distributed with medium resiliency (two in Central Great Plains and one in Northern Great Plains), low (one in Northern Great Plains) and very low (two each in Northern and Central Great Plains) resiliency.

Habitat considerations among these units are relevant to the overall representation of the western subspecies. The two Great Plains units, compared to the Midwest, are drier, typically exhibit reduced composition of tallgrass species, and may be more susceptible to the future impacts of climate, particularly high temperatures and associated increased frequency of drought. For regal fritillaries to survive drought, adequate refugia is required that can support regal fritillaries at the small, localized level during dry years. If adequate connectivity among habitats is maintained, subsequent repopulation of extirpated areas can occur as better conditions return, and populations expand. In the future, particularly under Future Scenario 3, drought in the Great Plains could become a primary limiting factor, as drought severity and frequency increase to the point where inadequate drought reprieve exists, refugia is reduced, and suitable habitat does not rebound due to the lack of more favorable conditions. Local extirpations may then become permanent in some areas, despite the presence of intact grasslands in these western portions of the range.

Midwestern populations of regal fritillaries are not immune to the effects of drought, and have recently been impacted in this matter, although regal fritillary recolonizations in the Midwest may be affected by something besides a lack of more favorable habitat conditions. Severe drought occurred in portions of the Midwest during 2012–2015, and several years have passed with relatively better conditions, yet without a corresponding recolonization of previously occupied sites (P. Hammond, personal communication, 2021). In the Midwest, fragmentation and isolation of habitats presents an additional hurdle to repopulating drought-extirpated habitats. Furthermore, the Midwest's fragmentation and isolation appear to be contributing to reduced gene flow and lower genetic diversity among Midwest populations (Williams et al. 2003, p. 16), potentially further reducing the adaptive capacity of individuals in the Midwest representative unit compared to those in the Northern Great Plains or Central Great Plains. Under all future scenarios, habitat stressors and genetic issues for the western subspecies increase, but the effect is most severe under **Future Scenario 3**.

Chapter 7 – Synthesis of Viability

In this SSA report, we evaluated the current and future conditions in terms of resiliency, redundancy, and representation for the eastern and western subspecies of regal fritillary. Resiliency reflects risk associated with stochastic events, redundancy with catastrophic events, and representation with long-term environmental change. We used future scenarios to capture a range of plausible futures and associated uncertainty for each subspecies. This chapter briefly synthesizes the results of our analysis of current conditions and anticipated future conditions in terms of risk to the subspecies and their viability over time.

The degree to which the 3Rs, and hence the viability for the eastern and western subspecies, may change from their current conditions in the future will depend on the severity of stressors and the adaptive capacity of the subspecies. Although our future scenarios attempt to reduce uncertainty by describing the plausible range of outcomes for stressors and conservation efforts, there is high uncertainty regarding the true effects of these stressors on the subspecies, particularly regarding the potential effects to habitats and demographic associated with global climate change. In general, a changing climate may reduce the quality and quantity of habitats, exacerbate other stressors, and may reduce the viability of both subspecies, although the mechanisms and outcomes may be unclear. Additionally, the regal fritillary uses habitats on a landscape-level scale, and depends on large, generally well-connected, diverse, native grassland habitats that are periodically disturbed, resulting in a shifting mosaic of habitats. The subspecies exhibits high fecundity, which may be offset by low larvae survivorship in poor or unsuitable habitats, and colonies, populations, and AUs, may be resilient to stochastic change, so may rebound and flourish when suitable habitat conditions return. Adults are very mobile, so can avoid localized stressors. Habitat loss and fragmentation may isolate colonies, reduce the resiliency of AUs, and generally increase risk to the subspecies from stochastic events, catastrophes, and environmental change.

Currently, the eastern subspecies resides in a single AU with low resiliency that provides the eastern subspecies' redundancy and representation. The single population in the one AU of the eastern subspecies is found on FTIG military base in Pennsylvania where ongoing management to benefit the subspecies via an INRMP occurs on the five SCAs. The eastern subspecies also exists on an impact area of the base that is not monitored, between SCAs, where active military exercises and management occur without consideration for the species. By the year 2075, the 3Rs of the eastern subspecies do not increase, may remain the same (Future Scenario 1), drop to very low resiliency (Future Scenario 2), or may become extirpated under the most pessimistic future scenario (Future Scenario 3).

Our biological risk assessment for the eastern subspecies concludes that the eastern subspecies is inherently more at risk from stochastic events, catastrophic events, and environmental change, given its small size and distribution across one AU. Its viability is tied to the condition of its remaining habitats and the reduction of its primary stressor, woody encroachment, though management and other activities. We projected that risk to the eastern subspecies' either stays the same or increases into the future. Therefore, viability of the eastern subspecies may remain the same, decrease, or decrease such that the subspecies no longer be viable under the lowest plausible-limit future scenario.

Currently, the western subspecies is distributed within 21 AUs within 3 representation units: Midwest (8 AUs), Northern Great Plains (5 AUs) and Central Great Plains (8 AUs). None of these are currently ranked as very high or very low resiliency, but one is currently considered extirpated. The 3Rs exhibit a decline among all western subspecies analytical units under three future scenarios, resulting in the reduction in the number of AUs by the year 2075, particularly under the most pessimistic of conditions predicted under Future Scenario 3.

Our biological risk assessment for the western subspecies concludes that risk to the subspecies increases into the future to year 2075. The viability of the western subspecies remains largely the same under two out of the three future scenarios (Future Scenarios 1 and 2), although both experience reductions in resiliency across some AUs. Viability declines the most under one future scenario (Future Scenario 3), when stressors are projected to be the most extreme. Therefore, viability of the western subspecies may remain the same, with reductions in resiliency, or decrease more substantially if conditions reach their lower plausible-limit.

Our synthesis of viability, in terms of the 3 Rs for the eastern and western subspecies of regal fritillary is summarized further in the following Tables 26, 27, and 28, and Figure 24, below.

Table 26. Current and future resiliency of the eastern and western subspecies of regal fritillary.

CURRENT AND FUTURE RESILIENCY - EASTERN AND WESTERN SUBSPECIES						
SUBSPECIES	**REPRESENTATION UNIT NAME**	**ANALYTICAL UNIT NAME**	**CURRENT RESILIENCY**	**FUTURE SCENARIO 1 RESILIENCY**	**FUTURE SCENARIO 2 RESILIENCY**	**FUTURE SCENARIO 3 RESILIENCY**
EASTERN	East	Ridge and Valley	LOW	LOW	VERY LOW	X
WESTERN	MIDWEST	Central Corn Belt Plains	LOW	LOW	VERY LOW	X
		Central Irregular Plains - A	LOW	LOW	LOW	VERY LOW
		Driftless Area	LOW	LOW	LOW	X
		Interior River Valleys and Hills	LOW	LOW	VERY LOW	X
		North Central Hardwood Forests - A	LOW	LOW	LOW	VERY LOW
		North Central Hardwood Forests - B	LOW	LOW	LOW	X
		Southeastern Wisconsin Till Plains	LOW	LOW	LOW	X
		Western Corn Belt Plains	LOW	LOW	LOW	VERY LOW
	NORTHERN GREAT PLAINS	Lake Agassiz Plain	LOW	LOW	VERY LOW	VERY LOW
		Middle Rockies	MEDIUM	LOW	LOW	X
		Northern Glaciated Plains	MEDIUM	MEDIUM	LOW	VERY LOW
		Northwestern Glaciated Plains	MEDIUM	MEDIUM	MEDIUM	LOW
		Northwestern Great Plains	HIGH	MEDIUM	MEDIUM	MEDIUM
	CENTRAL GREAT PLAINS	Central Great Plains	MEDIUM	MEDIUM	LOW	VERY LOW
		Central Irregular Plains - B	MEDIUM	MEDIUM	LOW	VERY LOW
		Cross Timbers	X	X	X	X
		Flint Hills	HIGH	HIGH	MEDIUM	MEDIUM
		High Plains	MEDIUM	LOW	LOW	X
		Nebraska Sand Hills	HIGH	HIGH	HIGH	MEDIUM
		Ozark Highlands	LOW	LOW	LOW	X
		Southern Rockies	MEDIUM	MEDIUM	MEDIUM	X

Table 27. Current and future redundancy for the eastern and western subspecies of regal fritillary. Numbers indicate the number of analytical units in a condition category.

CURRENT AND FUTURE REDUNDANCY - EASTERN AND WESTERN SUBSPECIES								
RESILIENCY	**EASTERN SUBSPECIES**				**WESTERN SUBSPECIES**			
	CURRENT CONDITION	**FUTURE SCENARIO 1**	**FUTURE SCENARIO 2**	**FUTURE SCENARIO 3**	**CURRENT CONDITION**	**FUTURE SCENARIO 1**	**FUTURE SCENARIO 2**	**FUTURE SCENARIO 3**
Very High								
High					3	2	1	
Medium					7	6	4	3
Low	1	1			10	12	12	1
Very Low			1				3	7
Extirpated (X)				1	1	1	1	10

Table 28. Current and future representation for the eastern and western subspecies of regal fritillary. Numbers indicate the number of analytical units in a condition category.

CURRENT AND FUTURE REPRESENTATION - EASTERN AND WESTERN SUBSPECIES																
CONDITION CATEGORY	EASTERN SUBSPECIES				WESTERN SUBSPECIES											
	EAST				MIDWEST				NORTHERN GREAT PLAINS				CENTRAL GREAT PLAINS			
	CURRENT CONDITION	FUTURE SCENARIO 1	FUTURE SCENARIO 2	FUTURE SCENARIO 3	CURRENT CONDITION	FUTURE SCENARIO 1	FUTURE SCENARIO 2	FUTURE SCENARIO 3	CURRENT CONDITION	FUTURE SCENARIO 1	FUTURE SCENARIO 2	FUTURE SCENARIO 3	CURRENT CONDITION	FUTURE SCENARIO 1	FUTURE SCENARIO 2	FUTURE SCENARIO 3
Very High																
High									1				2	2	1	
Medium									3	3	2	1	4	3	2	2
Low	1	1			8	8	6		1	2	2	1	1	2	4	
Very Low			1				2	3			1	2				2
Extirpated (X)				1				5				1	1	1	1	4

Figure 24 below provides geographical context by depicting the distribution of the analytical units for both subspecies and their associated current and future conditions as labeled below.

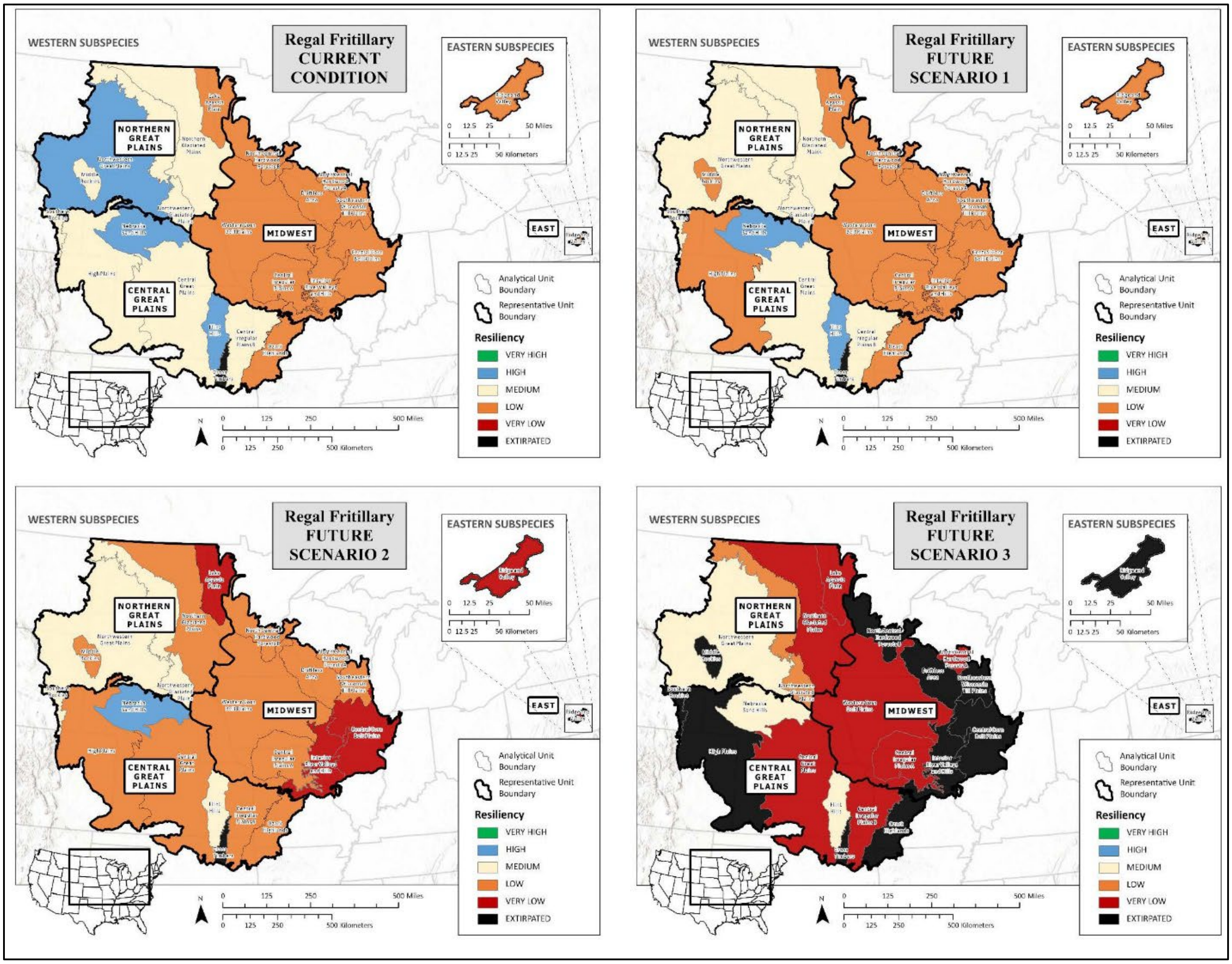

Figure 24. Geographical distribution and resiliency of analytical units of the eastern and western subspecies of regal fritillary currently (upper left) and as predicted by the year 2075 under Future Scenario 1(upper right), Future Scenario 2 (lower left) and Future Scenario 3 (lower right).

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Appendix A – Regulatory History

In this appendix, we summarize the Federal and state regulatory history for the regal fritillary. We provide this summary for informational purposes only.

Previous Federal Actions

In 1984, the U.S. Fish and Wildlife Service (Service), in response to an observed reduction in the number and distribution of the regal fritillary, designated the species as a Category 2 Species, a possible candidate for listing under the Endangered Species Act (Act). This designation identified the regal fritillary as a species for which the best available scientific information at the time indicated to the Service that proposing to list the regal fritillary as endangered or threatened was possibly appropriate; however, we lacked sufficient data on biological vulnerability and threat at the time to support a proposed listing rule. In 1996, to avoid confusion of Category 2 species with species that clearly met the definition of a Candidate Species (for which listing was warranted), the Service eliminated the Category 2 species designation (61 FR 7596; February 28, 1996).

Petition and 90-Day Finding

On April 19, 2013, WildEarth Guardians petitioned the U.S. Fish and Wildlife Service (Service) to list the regal fritillary (formerly known as *Speyeria idalia;* currently known as *Argynnis (Speyeria) idalia*; see Taxonomy section) as threatened or endangered and to designate critical habitat, under the Endangered Species Act of 1973 (Endangered Species Act (Act); 16 U.S.C. § 1531 *et seq.*) (WildEarth Guardians 2013, entire). The petition briefly noted the existence of two subspecies, (*S. i. idalia* (eastern) and *S. i. occidentalis* (western) but the requested action was to list the entire species. The petition identified several factors as threats to the regal fritillary:

- Impacts to its habitat including "crop agriculture, urban and residential development, road construction and maintenance, herbicide and pesticide use, and ill-timed controlled burns";
- "Overutilization for commercial or recreational purposes due to its higher potential commercial value than most other prairie specialist butterflies";
- Inadequate regulatory mechanisms not protective of the species/habitat factors related to the species' "reproductive characteristics";
- "Genetic isolation as a result of habitat fragmentation", which may make the species vulnerable to other factors; and
- "Human population growth", exacerbating the above factors.

On September 18, 2015, the Service found that the petition presented substantial information that the petitioned action may be warranted (80 FR 56423). The Service evaluates species for listing under the Act via our five-factor threats analysis. Specifically, based on the petition's claims regarding the impact of host plant availability and dispersal abilities between suitable habitat patches on the success of regal fritillary populations, we found substantial information indicating that listing may be warranted due to the fragmentation of tallgrass prairie habitat (Factor A) and the potential of synergistic effects of habitat loss and fragmentation and small population dynamics, to render the species at risk of local extinctions caused by stochastic biological and environmental events (Factor E).

Other Federal Designations

The U.S. Forest Service recognizes the regal fritillary as a Sensitive Species in its Regions 1, 2, 8 and 9. A U.S. Forest Service Sensitive Species is a "a plant or animal whose population viability is identified as a concern by a Regional Forester because of significant current or predicted downward trends in abundance and/or in habitat capability that would reduce its distribution" (Selby 2007, p. 8). That recognition means that the species receives management attention; it is considered in most biological evaluations conducted on projects on U.S. Forest Service units to ensure the projects do not lead to a loss of a viability for the species and highlights the species for survey attention. The Sensitive Species status of the regal fritillary is an important conservation tool the U.S. Forest Service has used to help ensure continued viability and persistence of declining species on the units they manage and avoid contributing to the need for future listing under the Act (P. McDonald, personal communication, 2020).

State Designations

State Threatened, Endangered, and Species of Concern

The regal fritillary has been assigned state-level protective status in some states. Five states (Indiana, Michigan, New York, Ohio and Wisconsin) recognize the regal fritillary as endangered under their State laws while the State of Illinois recognizes it as threatened. Of those five states today, the species is found in Indiana, Illinois and Wisconsin. Five additional States (Arkansas, Connecticut, Iowa, Minnesota, and Wyoming) have noted the butterfly as a species of concern in their jurisdictions.

State Species of Greatest Conservation Need

The Service requires that states receiving state wildlife grants for conservation of nongame species develop a Wildlife Action Plan, which serves as a blueprint for their state species conservation efforts. These plans must identify and focus on "species in greatest need of conservation," also called Species of Greatest Conservation Need (SGCN), within their state boundaries. Although the regal fritillary is known/potentially extirpated in some states, the regal fritillary may still be identified as a SGCN in these state plans; currently 19 states do so. The criteria used to establish the regal fritillary as a SGCN vary by state; additional information is provided in the individual *State Wildlife Action Plans*.

Natural Heritage Rankings

The regal fritillary has been assigned national and state conservation rankings (Table 1, Figure 1). The District of Columbia and 16 of the 32 historically occupied states (Connecticut, Delaware, Kentucky, Massachusetts, Maryland, Maine, Michigan, New Hampshire, New Jersey, New York, North Carolina, Ohio, Rhode Island, Virginia, Vermont and West Virginia) have no recent records and are not known to be currently occupied by the regal fritillary, while 15 states still harbor the species (Arkansas, Colorado, Iowa, Illinois, Indiana, Kansas, Minnesota, Missouri, North Dakota, Nebraska, Oklahoma, Pennsylvania, South Dakota, Wisconsin and Wyoming). NatureServe's conservation status ranking for the regal fritillary in subnational categories range from "presumed extirpated" (in numerous, particularly eastern U.S. states) to "apparently secure" (in Kansas), including "not ranked" (in New Jersey) or "Not Applicable" (in Manitoba) (NatureServe 2021). For definitions of each of these conservation status ranks see: https://explorer.natureserve.org/AboutTheData/Statuses.

One state with confirmed historical records, Montana, currently does not have a conservation status assigned to the regal fritillary; the state has very few records of the species which have been described as "strays" on the periphery of the species' range (Selby 2007, p. 10). The same description has been applied to three Canadian Provinces with historical regal fritillary records (New Brunswick, Ontario, and Saskatchewan [Selby 2007, p. 10] with Nova Scotia no longer considered part of historical range [J. Calhoun, personal communication, 2023]). An individual also deemed a stray was identified in Alberta in 2015 (Pohl et al. 2015, p. 7-8). Manitoba is the only Canadian Province with an assigned rank for this species, but its "SNA - Not Applicable" rank indicates it is not considered a suitable target for conservation activities (NatureServe 2021).

Table 1. NatureServe Conservation Rankings for the regal fritillary

	NatureServe Conservation Status Rank*	State Protection Status	Species of Greatest Conservation Need**
GLOBAL	G3-Vulnerable	-	-
UNITED STATES	N3-Vulnerable	-	-
Arkansas	S1-critically imperiled	INV-inventory element	No
Colorado	S1-critically imperiled	-	Yes
Connecticut	SX-presumed extirpated	SCX-special concern extirpated	No
Delaware	SX-presumed extirpated	-	Yes
Dist. of Columbia	SX-presumed extirpated	-	Yes
Illinois	S2S3-imperiled, vulnerable	T-threatened	Yes
Indiana	S1S2-critically imperiled, imperiled	E-endangered	Yes
Iowa	S2-imperiled	SC-special concern	Yes
Kansas	S4-apparently secure	-	Yes
Kentucky	SX-presumed extirpated	-	No
Maine	SX-presumed extirpated	-	No
Maryland	SH-possibly extirpated	-	No
Massachusetts	SH-possibly extirpated	-	No
Michigan	SH-possibly extirpated	E-endangered	No
Minnesota	S3-vulnerable	SC-special concern	Yes
Missouri	S3-vulnerable	-	Yes
Montana	-	-	No
Nebraska	S3-vulnerable	-	Yes
New Hampshire	SX-presumed extirpated	-	No
New Jersey	SNR-unranked	-	No
New York	SH-possibly extirpated	E-endangered	No
North Carolina	SX-presumed extirpated	-	No
North Dakota	S2-imperiled	-	Yes
Ohio	SH-possibly extirpated	E-endangered	Yes

Oklahoma	S1S2-critically imperiled, Imperiled	-	Yes
Pennsylvania[1]	S1-critically imperiled	-	Yes
Rhode Island	SX-presumed extirpated	-	No
South Dakota	S3-vulnerable	-	Yes
Vermont	SX-presumed extirpated	-	Yes
Virginia	SH-possibly extirpated	-	Yes
West Virginia	SH-possibly extirpated	-	Yes
Wisconsin	S1-critically imperiled	E-endangered	Yes
Wyoming	S3-vulnerable	SOC-species of concern	No
CANADA[2]	NHB, N1M-Possibly Extirpated Breeding, Critically Imperiled Migrant	-	-
Alberta	-	-	-
Manitoba	SNA-Not Applicable	-	-
New Brunswick	-	-	-
Ontario	-	-	-
Saskatchewan	-	-	-

[1] No state agency has jurisdiction over this species in Pennsylvania.
* Source: NatureServe; https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.114908/Argynnis_idalia; accessed April 21, 2021. Definitions of the ranks may be accessed at: https://explorer.natureserve.org/AboutTheData/Statuses#Global
**Per individual State Wildlife Action Plans

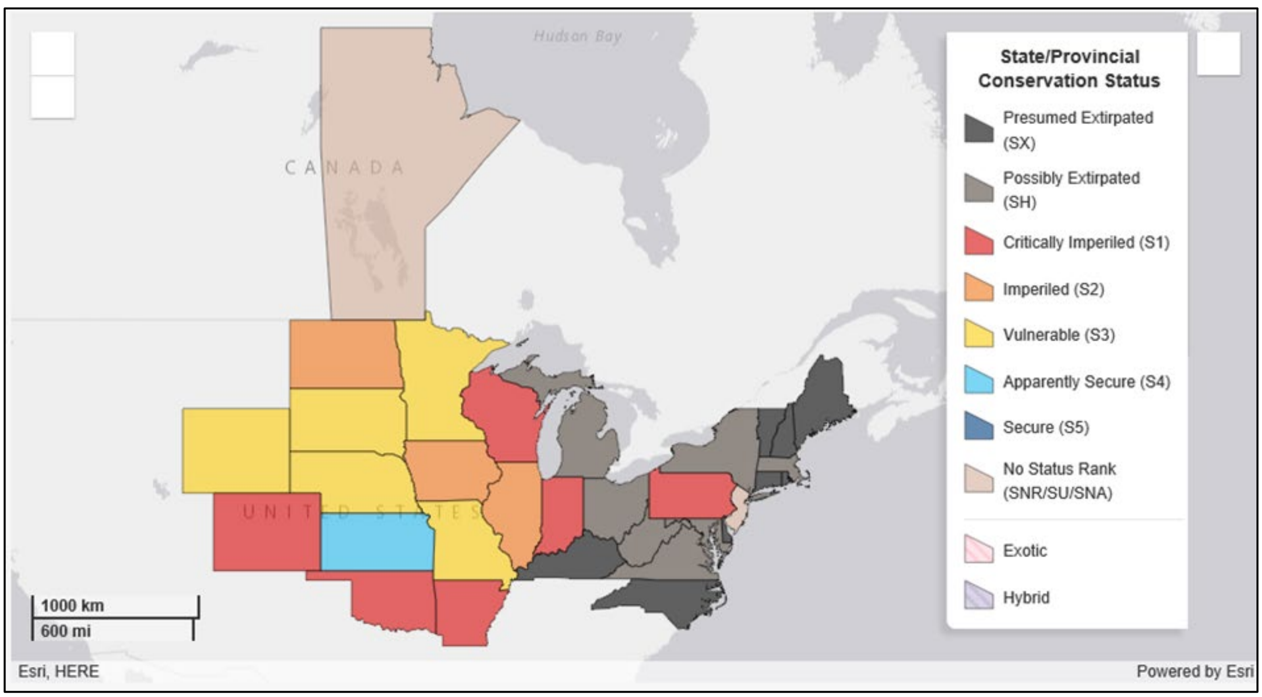

Figure 1. Map of regal fritillary distribution and conservation rank by state/province. NatureServe Explorer Copyright © 2021 NatureServe, 2550 South Clark Street, Suite 930, Arlington Virginia 22202, U.S.A. All Rights Reserved.

Appendix B – Additional Taxonomic Information

In this appendix, we provide additional background on the taxonomy of the regal fritillary, particularly support for the recognition of its two subspecies, the eastern and western subspecies.

Taxonomic History of the Species

The regal fritillary was originally named *Nymphales idalia* in 1773 by the English entomologist Dru Drury based on specimens from New York (which were lost and replaced by a neotype specimen from New York City, New York; dos Passos and Grey 1947, p. 9). Subsequent authors placed *idalia* in the genus *Argynnis*, which was generally used for all larger fritillaries in both the Old (Europe) and New Worlds. Members of this group, often collectively called "silverspots" due to the metallic silver markings beneath their wings, are also popularly known as the greater fritillaries to differentiate them from the smaller species of so-called lesser fritillaries of the genus Boloria (Klots 1951, p. 85, 88). Scudder (1872, p. 44) believed the regal fritillary was unique among greater fritillaries and proposed that it be placed in a separate genus, which he named *Speyeria*, thus making *idalia* the type species of that genus. Later, dos Passos and Grey (1945, p. 12) placed all New World greater fritillaries in the genus *Speyeria*. However, genetic studies by Simonsen et al. (2006, p. 412) suggested that New World species should be returned to the genus *Argynnis*. This concept was supported by recent genomics research of Zhang et al. (2020, p. 17), who concluded that New and Old World species are genetically comparable and *Speyeria* is best treated as a subgenus of *Argynnis*. In January 2021, the online checklist The Butterflies of North America, A Catalog of the Butterflies of the United States and Canada (Pelham 2021, entire; Pelham 2023, entire) reverted the genus name of the greater fritillaries to *Argynnis* once again. Pelham's checklist is generally considered by lepidopterists to be an authoritative source that reflects current nomenclature for butterflies in the U.S. and Canada which cites relevant descriptions and includes references to original research (J. Calhoun, personal communication, 2020). Thus, *Argynnis* is recognized herein as the genus of the regal fritillary, with *Speyeria* as its subgenus (Zhang et al. 2020, p. 17; Pelham 2021, entire; Pelham 2023, entire). Since the nomenclature change is recent, and sources of information on this species conducted work prior to the name correction, we note that *Speyeria* is occasionally mentioned as a genus (e.g., website addresses, literature cited) in this document.

Taxonomic History of the Eastern and Western Subspecies

There are now two recognized subspecies of regal fritillary: a "western" form (*A. i. occidentalis)* and an "eastern" (the nominotypical, or original) form (*A. i. idalia).* No formal process or entity has been established to officially accept or deny proposed taxonomic changes to Lepidopteran species. When additional information is obtained, it may be published and made available for further scrutiny and research for validation. In the case of the regal fritillary, the species was thought to be monotypic (no subspecies) (Dunford 2009, p. 7) until 2001, when the western subspecies (type specimens from near Crete, Illinois) was described based on mitochondrial DNA analysis and supported by morphological differences between specimens in eastern versus western portions of the regal fritillary's range (Williams 2001b, entire).

The mitochondrial analysis for this subspecies designation was performed on 114 specimens from extant populations in nine states: Iowa, Illinois, Kansas, Minnesota, Missouri, Nebraska, Pennsylvania, South Dakota and Wisconsin (Williams 2001b, p. 148). Twenty-two unique haplotypes were identified among the populations sampled across the Midwest and Great Plains, with no apparent geographical associations detected among them (although some Wisconsin haplotypes were possible exceptions) (Williams 2001b, p. 146). This broad distribution of populations was designated a new “western” subspecies (*occidentalis*) of the regal fritillary (Williams 2001b, p. 146).

The exception to this group was an isolated population in Pennsylvania at the Fort Indiantown Gap (FTIG) National Guard Training Center (northeast of Harrisburg), which was found to harbor unique genetic components, fixed in the population, thus presenting a distinct evolutionary lineage distinguished from the other sampled extant populations (Williams 2001b, p. 147). Subsequent use of four microsatellite loci (Williams et al. 2002, p. 88) to examine the effects of fragmentation on genetic diversity of regal fritillaries supported a long history of isolation for the Pennsylvania population from all other extant populations studied (Williams et al. 2003, p. 17). The neotype specimen of *idalia*, designated by dos Passos and Grey (1947, p. 9), was assumed to apply to the Pennsylvania population, thus these were classified as the “eastern” subspecies (*idalia*) of the regal fritillary by Williams (2001b, p. 148). Nuclear microsatellite alleles were recently determined for 198 individuals from the Pennsylvania population (Rutins et al. 2022, p. 4). Phylogenetic analysis of mitochondrial loci (COI+II and ND4) showed very little intraspecific variation within *Argynnis* samples; for the COI+II locus, all individuals were identical to the Pennsylvania haplotype identified by Williams (2002) (Rutins et al. 2022, p. 4).

The morphological differences supporting the subspecies designations were based on measurements of 183 museum specimens (males and females) from six states: Iowa, Illinois, Nebraska, New Jersey, New York, and Pennsylvania (Williams 2001b, p. 145), as well as a larger sample of 369 museum specimens (males and females) from 17 states: Connecticut, Iowa, Illinois, Indiana, Kansas, Massachusetts, Maryland, Maine, Nebraska, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Virginia, and West Virginia (Williams 2001a, p. 240). The eastern specimens (Figure 1) exhibited relatively fewer and smaller number of spots on the ventral hindwing than the western specimens (Williams 2001a, p. 241; 2001b, p. 146) (Figure 2).

*Figure 1. Eastern subspecies regal fritillary (*Argynnis idalia idalia*), male, Nantucket Island, Massachusetts, , USA. Source: Butterflies of America; https://www.butterfliesofamerica.com/t/Speyeria_idalia_a.htm*

*Figure 2. Western subspecies regal fritillary (*Argynnis idalia occidentalis*), male, North Dakota, USA. Source: Butterflies of America; https://www.butterfliesofamerica.com/t/Speyeria_idalia_a.htm*

Notably, although numerous eastern and western samples were obtained from museums for morphological analysis (Williams 2001a, p. 240; Williams 2001b, p. 145), the genetic analysis was conducted only on specimens collected from extant populations which included several samples from western areas, but only one site in the east: the Pennsylvania population (Williams 2001b, p. 144, 148). The majority of eastern populations had been extirpated by that time. In 2001, when the work was published, Virginia also had a remnant regal fritillary population (discovered in 1997 at the Radford Army Ammunition Plant, north of Radford), but its specimens were not included in this mitochondrial study. Although the subspecific designation of the Virginia population was unknown in 2001, it was theorized at that time to align with the eastern taxon based on its eastern locality and morphological comparisons (see below) (Williams 2001b, p. 148; Williams 2001a, p. 240). The mitochondrial study was later repeated with genetic sequences from 230 specimens, this time including the Virginia population (Keyghobadi et al. 2013, p. 235). Information from Williams et al. (2002) was used for the new study, along with numerous museum specimens and samples from some extant populations, culminating in a tally of samples from 20 states and one Canadian Province, including: Connecticut, Illinois, Iowa, Kansas, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Nebraska, New Jersey, New York, Ohio , Pennsylvania, Rhode Island, South Dakota, Virginia, West Virginia, and Ontario (Keyghobadi et al. 2013, p. 236). At least three additional haplotypes from museum specimens were identified (Keyghobadi et al. 2013, p. 238), and the updated findings supported the existence of two separate subspecies.

However, the assumption that the isolated Virginia population would align with the eastern subspecies (Williams 2001b, p. 148) was unsupported; the Virginia population was confirmed to be genetically aligned with the western subspecies (Keyghobadi et al. 2013, p. 240), despite its eastern location. Additionally, although museum specimens from Virginia were observed by Williams (2001a, p. 240) to exhibit eastern subspecific characteristics, examination of wing morphology of individuals from the isolated population in Virginia were also later noted to more closely agree with the western subspecies (Ferster, personal communication, 2005a and Hovis, personal communication, 2005 *in* Selby 2007, p. 13). Anne Chazal of the Virginia Department of Conservation and Recreation notes that regal fritillaries of the Virginia population have not been detected in Virginia since 2009 and the location is currently considered “historical” by the State of Virginia (Chazal 2014, p. 8; A. Chazal, personal communication, 2020).

A geographical transition zone was detected between the eastern and western subspecies. Williams (2001a, p. 240) observed that west-central Pennsylvania museum specimens (not the extant Pennsylvania population) exhibited wing-markings intermediate between the eastern and western subspecies within a “central” zone, defined as western Pennsylvania (distanced from the eastern extant Pennsylvania population), Indiana, Ohio, and West Virginia – areas where the regal fritillary has been extirpated. This region represents a geographical gap between extant western populations and the extant eastern Pennsylvania population (Williams 2001a, p. 242). Keyghobadi et al. (2013, p. 239) provided additional genetic support for that zone with the detection of a mixture of western and eastern haplotypes in specimens from Ohio, western Pennsylvania, Virginia and West Virginia. The exact geographic boundaries between the eastern and western subspecies are not precisely known. An eastern subspecies haplotype was identified in a Kentucky regal fritillary, while only western haplotypes were found in the Virginia population (Keyghobadi et al. 2013, p. 240), despite Virginia’s location, east of Kentucky. This prompted Keyghobadi et al. (2013, p. 240) to note: “…in the region spanning the western Ohio border to Pennsylvania west of the main Appalachian chain, neither geographical location nor wing-pattern (Williams, 2001[a]) appears to predict mitochondrial haplotype.” The eastern haplotype was also detected in specimens from northeastern states including Connecticut, Maryland, Massachusetts, New York and West Virginia (Keyghobadi et al. 2013, p. 235).

The eastern Pennsylvania population was theorized to have historically been connected to Midwestern regal fritillaries by a continuum of intermediate populations across the central states, later separated by habitat fragmentation (Williams 2002, p. 153). Alternatively, Williams (2002, p. 150) suggested it may have been isolated during the Pleistocene as glaciers separated refugia for eastern and western populations and subsequently became further isolated by fragmentation. However, Keyghobadi et al. (2013, p. 240) did not support either of these concepts, instead suggesting that the different haplotype frequencies between eastern and western populations, with a narrow transition zone in between, may reflect selection pressures related to occupation of different habitats based on xeric versus mesic habitats and violet food plant preferences (a difference also noted by Williams (2001b, p. 148)). A study of museum specimens from additional populations formerly occurring in the East (especially southward) would help address the uncertainty regarding the taxonomic status of population within the interface between the eastern and western subspecies (Selby 2007, p. 13), perhaps better defining their geographical boundaries.

As noted above, taxonomic changes to butterfly species are essentially determined over time via peer review by the scientific community of lepidopterists. John Calhoun of the McGuire Center for Lepidoptera and Biodiversity at the Florida Museum of Natural History, and Greg Pohl of Natural Resources Canada (Canadian Forest Service) note that despite the uncertainty regarding their exact distributional boundaries, the subspecies *A. i. occidentalis* and *A. i. idalia* have generally been accepted by the lepidopterist community (J. Calhoun, personal communication, 2020; G. Pohl, personal communication, 2020). These subspecies designations have been supported, not disputed, in the literature; they are recognized in a taxonomic overview of the genus *Speyeria* (now *Argynnis;* Pelham 2021, entire; Pelham 2023, entire) (Dunford 2009, p. 8) and a recent genomics study (Zhang et al. 2020, p. 17), and the two were also listed in a comprehensive checklist last published in 2008 (Pelham 2008, entire), now updated online: https://www.butterfliesofamerica.com/US-Can-Cat.htm (Pelham 2021, entire; Pelham 2023, entire).

Appendix C – Detailed Life Stage Life History Summaries

In this appendix, we provide additional detail regarding the regal fritillary's four life stages: eggs, larvae, pupae, and adults. Unless noted otherwise, these summaries for the species, apply to both the eastern and western subspecies.

Eggs

Figure 1. A mature (gray) regal fritillary egg. Photo credit: Erika McKinney.

Regal fritillary eggs are initially white or cream colored but turn a frosted gray color as they age (Wagner et al. 1997, p. 266) (Figure 1). Females place single eggs in grassland landscapes, usually in shaded microsites or the underside of senesced vegetation (McCullough et al. 2017 p. 149; Kopper et al. 2000, p. 657). Females may also lay eggs on the ground or on rocks, primarily in September, with some egg-laying occurring in late August and October (Wagner et al. 1997, pp. 262, 266). The eggs are not typically laid on live vegetation and not necessarily on, or near, the larval host plants (*Viola* spp.) (Kopper et al. 2000, p. 663) which are senesced at that time of year, albeit recent information indicates the dried stems and leaves of the plants emit olfactory stimuli that induce the females to oviposit nearby (P. Hammond, personal communication, 2023). Females lay hundreds to thousands of eggs in their chosen microsites, likely maximizing reproductive potential (Mason 2001, p. 21). In laboratory settings, incubation of the eggs typically averages approximately 3 weeks with some occurring earlier, such as 18 days (K. McCullough, personal communication, 2020) or later, such as 23 to 25 days (Edwards 1879, p. 219). In general, incubation may last from 10 days (Wagner 1995, p. 2) to 53 days (E. McKinney, personal communication, 2021). Local climatic conditions likely play a role in even greater variability in incubation times for wild populations, and the specific conditions necessary for hatching in terms of weather or sheltering needs are not well known.

Hatching rates of captive-reared regal fritillaries can be highly variable, with hatch rates as low as 19 percent (Wagner et al. 1997, p. 269) or as high as 78 percent, (averaging 64.3 percent) in a laboratory setting. In Pennsylvania, hatching success rates range from 3.3 to 97.7 percent (Becker 2016, p. 5). The upper values of these ranges measured in the laboratory are likely higher than hatching rates of regal fritillary eggs in the wild due to additional environmental risks, such as climatic variation and predation. Hatch rates in natural populations are unknown.

Larvae

The larval stage is the longest of a regal fritillary's life; the bulk of which is spent as the first instar, spanning fall, winter, and early spring months. Newly hatched regal fritillary larvae (caterpillars) are pale yellow-brown, translucent, and approximately 0.08 inch (in.) (2 millimeters (mm)) long (Edwards 1879, p. 217) (Figure 2). Their bodies are segmented with each segment exhibiting eight dark spots from which long black hairs grow, forming longitudinal rows on the body (Edwards 1879, p. 217). The larvae exhibit six instars, undergoing five molts eventually developing into approximately 45-mm (1.8-in) individuals that are "black, banded and striped with ochreous and orange-red, and adorned with six rows of fleshy spines surmounted by several black bristles" (Edwards 1879, p. 217–218). The head is "black, orangish on top rear" (Scott 1986, p. 327).

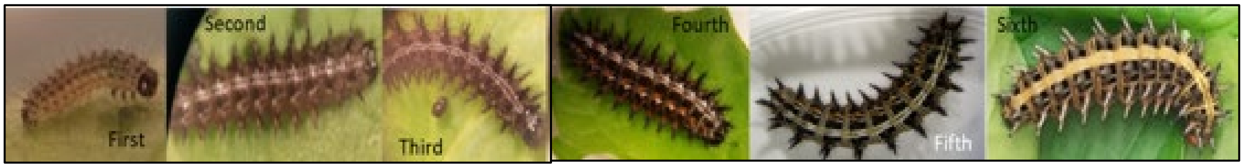

Figure 2. Regal fritillary instars. Photo credit: FTIG Wildlife Staff and ZooAmerica.

Development timeframes of the first-sixth instars after emergence from winter diapause varies from less than a week to more than 3 weeks (Edwards 1879, p. 218; Wagner et al. 1997, p. 270), with these stages of larval development cumulatively lasting as little as 6–7 weeks (McCollough et al. 2017, p 146) to more than 14 weeks (Edwards 1879, p. 217–218).

The tiny first instar larvae emerge from their eggs by consuming some or all of the egg chorion (outer shell) (Figure 3) before finding shelter in the leaf litter and entering winter diapause without additional feeding (Edwards 1879, p. 219; Wagner et al. 1999, p. 269). Exact larval behavior of how and where they find a suitable site and what exactly occurs during this diapause, however, is unknown (Kopper et al. 2000, p. 663).

Figure 3. Newly hatched captive regal fritillary larva among unhatched eggs, consuming its own empty eggshell. Photo credit: Erika McKinney.

The first instars may emerge from winter diapause in April or May (Shepherd and Debinski 2005, p. 245; NatureServe 2021, entire), but perhaps as early as March in southern parts of the range based on observed phenology of subsequent life stages and availability of emerging violets (K. McCullough, personal communication, 2020). Emergence likely occurs after some established energy expenditure level, which fluctuates with temperature (higher temperatures equate to reduced time to break diapause; Mattoon 1971, p. 251) among other factors. In captive rearing situations, light, heat, humidity, and mechanical stimulation have been used to forgo larval diapause, shortening the time needed to raise the butterflies (Mattoon et al. 1971, p. 252; Grey, Moeck and Evans 1963 *in* Mattoon et al. 1971, p. 247; Wagner et al. 1997, p. 265).

Violets (*Viola spp.*) are the larvae's sole source of food. First instar larvae emerging from winter diapause must seek out these important host plants. The distance that the first instars can move in the spring to find young violets is likely no further than that required to encounter the first suitable host plant in their path, but it is likely most individuals perish before doing so. Violets are rarely plentiful and occur in clumps; regal fritillary larvae appear unable to detect them even when they are within 1 to 2 cm (0.4 to 0.8 in) (Kopper et al. 2000, p. 661). Larval behavior or strategies to find these plants are unknown and the tiny larvae must navigate many obstacles on the grassland floor, which increases the difficulty in detecting violets (Kopper et al. 2000, p. 661, 663).

Additionally, the tiny larvae cannot survive on mature violet leaves, perhaps due to the physical barrier presented by hairs that develop on the leaf surface of some violet species (Wagner 1995, p. 3). Tougher and thicker leaves presented by mature violet plants may be more difficult for young instar larvae to consume (P. Hammond, personal communication, 2021). The potential inability of the first instar to locate violets, and other risk factors such as harsh winter or spring conditions or disease encountered during its lengthy 6 to 7-month timeframe make this stage likely the most vulnerable of the regal fritillary's lifecycle and mortality of larvae is typically very high (Mattoon et al. 1971, p. 248; Wagner et al. 1997, p 269; Selby 2007, p. 25; Sims 2017, p. 1). Mold, freezing, and desiccation all cause larval death (Mattoon et al. 1971, p. 251). Even when provided young violet leaves in a laboratory setting, survival of first instars may be only 50 percent (Wagner et al. 1997, p 269).

Figure 4. Late instar regal fritillary larva, feeding on a violet leaf. Photo credit: Kelsey McCullough.

If the larvae find a violet patch, they continue to feed on concurrently maturing violet plants, systematically stripping the stems of their leaves, a characteristic feeding pattern observed in the field and laboratory settings that promotes detection of these notoriously cryptic larvae (Mooreside et al. 2006, p. 176; McCullough et al. 2017, p. 149) (Figure 4). Larvae will move between host plants, or from the host plants to the grassland vegetation, presumably to find shelter or reach additional violets as needed, but information as to the extent of such movements is lacking. In the laboratory, mature larvae are known to eat about four to five leaves per day of a violet species with large heart-shaped leaves (*Viola glabella*) numbering six to eight leaves on a large plant (P. Hammond, personal communication, 2021). The number of leaves consumed in the wild likely varies due to several factors, such as the larvae's size and the size of available violet leaves. Without adequate violets, larvae may take longer to develop, perhaps become stunted, or die of starvation.

While some local food preferences by regal fritillary larvae in various parts of the range have been noted, it has been suggested that most local native violet species would likely suffice (Royer and Marrone 1992, p. 21). However, ornamental violet species originating in Europe have been found to be unsuitable, resulting in death of larvae, delayed development, and dwarfed adults (Mattoon et al. 1971, p. 253). In Pennsylvania, laboratory experiments with three *Viola* species (wood violet (*viola odorata*), arrowleaf violet (*viola sagittata*), and primrose violet (*viola primulifolia*)) appeared to indicate that primrose violets may not yield as many, or as robust, regal fritillary specimens (E. McKinney, personal communication, 2021).

Females spend more time in the larval stage than males (Nagel et al. 1991, p. 149; M. Swartz, personal communication, 2020); perhaps 1 to 2 weeks longer (P. Hammond, personal communication, 2021, Selby 2007, p. 25). The larval stage may end as soon as early May in the southern part of the range based on observations of adult male butterflies by May 21 in northeastern Oklahoma (R. Moranz, personal communication, 2020), while late May and into June are more typical timeframes for central parts of the range (Wagner et al. 1997, p. 262) and later still in northern, cooler regions based on emergence of females observed in mid to late July (Royer and Marrone, 1992, p.25).

Larvae were once thought to be nocturnal, feeding at night and seeking shelter during the day (e.g., Royer and Marrone 1992, p. 26). In contrast, later observations documented larvae to be more active during the day while inactive throughout most of the night (Kopper et al. 96), and more recent work found them to be active during the day, at twilight, and at night (McCullough et al. 2017, p. 149). Schweitzer (1992a, p. 8) indicated late instar larvae tend to be active at night. Since late instar larvae are more likely to encounter warmer temperatures as seasons progress, perhaps timing of activity is a function of temperature, prompting the older larvae to seek shelter during the day as days become warmer. Larvae may find shelter in leaf curls of young violets or at the base of violet plants, in the folds of leaf litter, and in warm season grass tussocks which may provide protection from predators or offer some microclimate conditions (Kopper et al. 2001, p. 96; McCullough et al. 2017, p. 149; Ferster and Vulinec 2010, p. 39).

Pupae

Figure 5. A Regal fritillary pupa. Photo Credit: Dave Zapotok.

Regal fritillary pupae (chrysalises) (Figure 5) are approximately 27.94 mm (1.10 in) long (Edwards 1879, p. 218), and they are "light mottled brown tinged with pink, with small black spots on the wings and thorax, short dorsal cones, and yellow transverse bands on the abdomen" (Edwards 1879, p. 218; Scott 1986, entire).

Mature larvae pupate in the leaf litter of warm season grasses (Selby 2007, p. 32; Ferster and Vulinec 2010, p. 7), which provides shade, cover, and potentially camouflage as the tan coloration resembles senesced vegetation. They move to their chosen location, spin webbing to which they attach themselves and transform into pupae within about 3 days (Edwards 1879, p. 219). At FTIG in Pennsylvania, a pupa was observed in the tussock of a warm season bunchgrass, approximately 1 cm above the ground, perhaps benefitting from protection from the sun and other environmental elements (Ferster and Vulinec 2010, p. 38). Distances moved from larval host plants to a pupation site is not known, and information is lacking regarding characteristics of such sites.

As noted above, pupation may occur in early May in southern parts of the species' range, but more often occurs in late May or June, and this stage may extend into July (Edwards 1879, p. 219). Regal fritillaries remain in the pupal stage for approximately 2.5 to 4 weeks (Wagner et al. 1997, p. 262), with environmental conditions playing a role in development. In captivity under constant temperatures, most (but not all) individuals may spend about 17 days as pupa, but in the wild, this development could be either faster or slower, potentially extending to a month under unusually cold conditions (D. Wagner, personal communication, 2020). The pupal stage has been documented to persist as late as the final days of July (Edwards 1879, p. 219). As with the larval stage, the females also spend more time in the pupal stage than the males (M. Swartz, personal communication, 2020), but it may be only 2 to 3 days longer (the bulk of the delay by females is in the larval stage) (P. Hammond, personal communication, 2021).

Adults

Figure 6. An adult regal fritillary on a milkweed. Photo credit: Jill Haukos

Regal fritillary adults (Figure 6) are relatively large butterflies. Exact wingspan measurements vary, ranging from 6.8 to 9.3 cm (2.69 to 3.63 in.) in the west and 7.9 to 10.5 cm (3.13 to 4.13 in.) in the east (Selby 2007, p. 14). Adults have orange forewings and dark hindwings that feature black bars, fine white markings, and two rows of large spots at the base (Figure 6). Females may be more reddish orange compared to the orange of males.

Females are typically slightly larger than males (Royer and Marrone 1992, p. 3); male forewing measurements may be in the range of 3.8 to 4.7 cm (1.5 to 1.85 in.), while female forewing lengths may be 4.4 to 5.5 cm (1.73 to 2.17 in); male forewing measurements may be in the range of 3.8 to 4.7 cm (1.5 to 1.85 in), while female forewing lengths may be 4.4 to 5.5 cm (1.73 to 2.17 in). Larger adult specimens generally occur in more southern parts of the overall range, presumably due to warmer and longer spring and summer seasons that allow for better development (P. Hammond, personal communication, 2021). A good description of adults is provided in Scudder (1889, p. 535) and summarized herein as follows: the adult head and thorax are covered with tawny-orange to chocolate-brown scales and hairs with a slightly darker abdomen. The dorsal forewing is orange with primarily black bars and spots, edged in black with some small white markings on the periphery.

Adult Flight Period

The regal fritillary flight period typically spans several months from late spring to mid-autumn, varying annually, by location and by sex. The adult stage is relatively long; individuals have been documented to survive up to 90 days in the wild (Barton 1993 *in* Wagner et al. 1997, p. 262). Regal fritillaries are protandrous; males mature and emerge as adults about 2 to 3 weeks earlier than females (Nagel et al. 1991, p. 149; Wagner et al. 1997, p. 262), appearing as early as the end of May in southern areas (Powell et al. 2007, p. 300; R. Moranz, personal communication, 2020; K. McCullough, personal communication, 2020). The species is more typically first observed in June, becoming more common in July as adults continue to emerge and numbers of both sexes peak (Wagner et al. 1997, p. 262; Kopper et al. 2001b, p. 174; Caven et al. 2017, p. 188). Males spend much of their time "patrolling" in search of females (i.e., flying fast, steady, and close to the grass) (Kopper et al. 2001b, p. 174; Selby 2007, p. 29) and begin to die off in mid-July, becoming relatively scarce in late July and August, although some worn individual males may survive into September (Kopper et al. 2001b, p. 174-5; K. McCullough, personal communication, 2020). Some females may disperse after mating or eventually exhibit a summer aestivation period (see *Reproductive Strategy*) becoming more reclusive in August, spending their time feeding and sheltering in vegetation clumps as well as small trees and shrubs (Kopper et al. 2001a, p. 428;

Kopper et al. 2001b, p. 175; Ferster and Vulinec 2010, p. 40). They become more active in late August, approximately a week prior to oviposition (egg-laying) (Kopper et al. 2001a, pp. 428-429), and some survive into late October before expiring, usually within 10 days after laying their last egg (Wagner et al. 1997, p. 266).

Adult Nutrition

Adults rely on nectar sources to sustain them and may also require moisture and nutrients from soils (Wagner et al. 1997, p 268; Selby 2007, p. 33). Observations of regal fritillaries sipping water and obtaining minerals from trails has been reported (Boggs and Ross 1993, entire; Schennum 2017, p. 6). Adults feed on a variety of plants and abundant high-quality resources improves female fecundity and fertility, increasing individual reproductive output (Wagner et al. 1997, p. 266).

Nutritionally adequate sources include those with amino acids or other nitrogenous compounds for laying females (Wagner 1995, p. 4). Regal fritillary females have large bodies, and body mass coincides with internal egg development, so continued nutrition is vital prior to/during egg-laying. Late season nectar sources are needed to sustain females during the 4 to 6-week timeframe individuals lay eggs (Wagner et al. 1997, p 266; Kopper et al. 2001, p. 430). Females emerge from the chrysalis with a fixed number of oocytes, and these may be resorbed by the body (when energy expenditures exceed energy intake) or will develop into eggs that are either laid or remain in body at death (Boggs 1986 and unpublished data *In* Boggs and Ross 1993, p. 437). Supplemented female nutrition (addition of egg albumen [protein]) has coincided with both increased fecundity (number of eggs produced) and improved fertility (proportion of eggs hatched) in a laboratory setting (Wagner et al. 1997, p. 266). Similar improvements in reproductive output, and even increased longevity, have been documented in other butterfly species that received boosted nutrition (e.g., Hill and Pierce 1989, p. 255; Boggs and Ross 1993, p. 438).

Adult Movement

Regal fritillaries are not migratory, but are capable of strong and rapid flight, and individuals have been documented to move long distances from breeding colonies (Selby 2007, p. 26). The species has been described as “very dispersive”; individuals move widely and locations where they are found are typically favored nectaring sites that are abandoned when those resources dry up (Schweitzer 1989, p. 135). Individuals may be capable of moving more than 161 km (100 mi) (P. Hammond, personal communication, 2021). In some instances, “strays” (Selby 2007, p. 10, 20, 23, 37), perhaps displaced by weather events, may be documented in locations with no known breeding colonies nearby as evidenced by an Alberta, Canada, male regal fritillary collected in 2015 (Pohl et al. 2015, pp. 7–8). The distance traveled by that individual is unknown but significant as there are no known populations in adjacent Montana nor Manitoba. Mark-recapture studies have revealed regal fritillary movements of 15.9 km (9.9 mi) in Pennsylvania (Barton 1993/1994 *in* Selby 2007 p. 26). Indiana regal fritillaries occurred in a generally small area until restorations expanded the habitat and the population responded positively, resulting in colonization of adjacent Illinois counties and a new population in Indiana located 40 km (25 mi) from the population source (J. Shuey, personal communication, 2015). Observations of regal fritillaries have been reported at sites in Nebraska located 55 and 129 km (34 and 80 mi) from the nearest known breeding population (Spomer et al. 2020, pp. 206–207) (although with potentially suitable habitats in between the source and the new sites, it may be possible that undocumented populations exist that may have sourced the new populations). Regal fritillaries in Colorado appear to perhaps temporarily move westward toward the foothills of the Rocky Mountains where more moisture is available for improved nectar resource availability (P. Opler, personal communication, 2021).

Despite this documented ability to move significant distances, some observers have noted more limited movements by regal fritillaries, reporting they occur on a more local scale measured in meters or a few kilometers within or between habitat patches (e.g., Nagel et al. 1991; p. 148; Selby 2007, p. 26; Ferster and Vulinec 2010, p. 38). A behavioral tendency for the butterflies to turn back from edges particularly in habitat patches with relatively high conspecific densities was reported in Iowa, although because sexes were not identified in that study, the observation may have been of patrolling males that tend to stay in the natal area as they search for females (Nagel et al. 1991, p. 149, Ries and Debinski 2001, p. 847; P. Hammond, personal communication, 2021). The regal fritillary may fall into an "intermediate mobility category" whereby small local populations and migration failure (dispersers that fail to find suitable habitat or other populations) together increase local extinctions in a highly fragmented landscape, which favors survival of sedentary individuals and may lead to rapid evolution toward less mobile tendencies in the species (Mason 2001, p. 16). Nectar availability, habitat isolation, and varying levels of permeability of habitat edge types may play a role in dispersal. The surrounding matrix can significantly influence the "effective isolation" of isolated suitable habitat patches (Ricketts 2001, p. 87). Trees appear to be more restrictive to passage by regal fritillaries than more open unsuitable habitats such as cropland. Proportions of regal fritillaries that cross various barriers as follows: trees – 8 percent, crops – 25 percent, fields – 29 percent, and roads – 43 percent (Ries and Debinski 2001, p. 845). Trees as relatively more effective barriers to individual emigration compared to more open unsuitable habitats like roads and crops, which may limit dispersal ability (Caven et al. 2017, p. 200).

Another factor explaining the conflicting dispersal characterizations of the regal fritillary is likely sex-specific behavior: females are longer-lived and known to be more prone to dispersal than males, particularly when ovipositing (Schweitzer 1989, p. 135; Schweitzer 1992a, p. 8). Males sometimes do move long distances, perhaps when nectar availability changes or when the habitat patches are expansive with fewer barriers (Schweitzer 1992a, p. 20). Males have also been documented moving among suitable habitat patches; e.g., a mark-recapture study in Missouri documented movements by males of at least 4.7 km and 5.5 km (2.9 mi. and 3.4 mi.) between suitable habitat patches (one of which involved crossing of a four-lane highway) (Marschalek 2020, p. 894). Male recaptures in that study were relatively steady, but the lack of female recaptures (due to dispersal) was a hindrance (Marschalek 2020, p. 894). Generally, males tend to adhere more to the natal patch, patrolling back and forth to find newly emerged females with which to mate (Nagel et al. 1991, p. 149; Selby 2007, pp. 25–26). Notably, in Iowa where regal fritillaries were observed turning back from habitat edges, the sex of individuals was not easily determinable in flight, thus was not reported (Ries and Debinski, 2001, p. 842). Males also do not live as long as females, resulting in less opportunity to move over time. In contrast to the low patrolling flight patterns of males, female regal fritillaries may often be observed flying high (Figure 7) and are capable of cruising multiple kilometers a day.

Figure 7. A late season regal fritillary flying high off the ground in western South Dakota. Photo credit: Rebecca Newton.

Females must acquire adequate nutritional sources to survive into September, which is when they lay most of their eggs. Local nectar sources change as seasons progress and greater mobility allows access to these resources. Additionally, while dispersal during egg-laying has been documented (Schweitzer 1989, p. 135), an initial post-mating dispersal phase has also been observed (Kopper et al. 2001b, p. 174; P. Hammond, personal communication, 2021). As daily temperatures increase (generally approaching 90 degrees Fahrenheit), adults appear to fly less and females generally become more sedentary, tending to hide in the shade of clumps of grass or shrubby vegetation during the heat of the day (summer aestivation) (Kopper 2001a, p. 428; Kopper et al. 2001b, p. 174; P. Hammond, personal communication, 2021). Then in late August, stimulated by a rise in hormone levels coinciding with egg development about a week before laying eggs, females disperse again in search of areas to oviposit (Kopper et al. 2001b, pp. 174, 176).

In short, two dispersal periods and longevity of three months allows females to move significant distances. This dispersal ability allows for recolonization of sites that may become extirpated when conditions impacting required resources (e.g., drought reduces violet or nectar availability). Recolonization can immediately if source populations exist adjacent to extirpated sites, or it may take years if populations contract significantly and the species is reduced to survival only in small suitable habitat patches at low densities (P. Hammond, personal communication, 2021).

Adult Reproduction

Sex Ratios

Although sex ratios of adults are presumed to be even, male versus female abundance depends partially on timing of observations (Powell et al. 2007, p 306). At the beginning of the flight period, the sex ratio is 100% male; however, when females eclose (emerge as adults from the pupa) the ratio then likely approximates 50:50 (Nagel et al. 1991, pp. 145,159; Kopper et al. 2001b, p. 174). As the males die off in mid to late summer, females dominate the ratio into the fall (Kopper et al. 2001b, p. 174). Behavioral differences between the sexes can also skew observed sex ratios. When not resting or feeding, the males spend much of their time patrolling thus are more visible and easily counted. In contrast, the females in summer aestivation are reclusive, spend a lot of time nectaring or resting in clumps of prairie vegetation (Kopper et al. 2001a, p. 428; Kopper et al. 2001b, p. 175), and are not as easily observed as the males.

Mating

Upon emergence from the chrysalis, males tend to stay close to the natal area, near the still-pupating females, which allows for mating to begin almost immediately after adult females appear (Figure 8) (Nagel et al. 1991, p. 149).

Figure 8. Mating regal fritillaries in Illinois, June 2021. Photo credit: Daniel Kim.

Species-specific sex pheromones in both males and females used during courtship and mating appear to ensure hybridization is nearly always avoided, although natural incidences have been noted (e.g., a regal fritillary female cross with a male great spangled fritillary (*A. cybele*) in South Dakota, yielding infertile offspring) (Hammond et al. 2013, pp. 265, 272–273). Females mate only once, sometimes immediately, and generally within the first several days (Kopper et al. 2001a, p. 429). Mating occurs during flight (Kopper et al. 2001b, p. 175). Most females have mated by mid-July, and male numbers start to decline at that time (Kopper et al. 2001b, p. 175). Unfavorable weather conditions (e.g., cold, rain, or high winds [perhaps above 10 kph (6.2 mph); Ries and Debinski 2001, p. 850]) have the potential to temporarily hinder regal fritillary mating activities as these conditions may force individuals to seek shelter or possibly slow emergence from the chrysalis (Selby 2007, p. 42).

Reproductive Strategy

The regal fritillary reproductive strategy is characterized as a “sweepstakes” method by (Wagner 1995, p. 3), whereby the female lays a very high number of eggs across the landscape, expending no post-laying maternal effort, resulting in a high number of hatched larvae on the ground to face the poor odds of surviving the winter and finding young violets in the spring. Kopper et al. (2000, p. 661) concurred with the “sweepstakes” strategy, but further explained that it is somewhat unique and does not fit neatly into existing reproductive strategy categories observed in Lepidoptera based on characteristics such as the life stage at which egg development occurs, which gonadotropic hormones activate egg development, whether adults feed and whether females mate with more than one male (Ramaswamy et al. 1997, entire). Instead, the regal fritillary exhibits aspects of several strategies. The males emerge first, females mate a single time soon after emergence, females are long-lived and feed throughout their lives, egg-development is delayed until late in adult female lifespan and egg maturation appears to be controlled by juvenile hormones (see Oviposition section). This combination of characteristics may represent a new reproductive strategy category (Kopper et al. 2001a, p. 430).

Regal fritillary females exhibit reproductive diapause; while mating occurs early in adult life, egg-development in females is delayed for 6 to 8 weeks, through mid-to-late summer, generally without males present (Kopper et al. 2001a, p. 430). This may be an adaptation to the phenology of the *Viola* host plants; hot summers, sometimes with drought, result in violet senescence in late summer so these plants are not available to larvae (Kopper et al. 2001a, pp. 429–430). Reproductive diapause, combined with larval diapause in winter, results in availability of young violet leaves to young larvae in the spring (Kopper et al. 2001a, p. 431). Delay of oviposition until late summer and early fall can also reduce egg and larval exposure to desiccating heat, parasitioids and predators (Kopper et al. 2001a, p. 431).

Oviposition

In late summer, rising juvenile hormone levels occur within females, stimulating egg development, spurring the females to become more active, and begin oviposition – perhaps within a week of this increased activity (Kopper et al. 2000, p. 653; Kopper et al. 2001a, p. 429). The females will fly about 0.5 to 2 m (1.6 to 6.6 ft) above the vegetation, intermittently drop down, walk among the plants, select a site to lay a single egg, and fly off to repeat the process elsewhere (Kopper et al. 2000, p. 653). Individual female regal fritillaries can lay a very high number of eggs. A range of eggs laid per female in a lab setting was recorded as 277 to 2,494 (mean: 1,447), with the upper value being more than twice the number of eggs known to be laid by any butterfly species at that time (Wagner et al. 1997, p. 266). Since then, even higher numbers have been documented. In Nebraska, a female laid 2,596 eggs in her lifetime (Spomer et al. 2020, p. 206). Prior to that, a captive female in Pennsylvania was documented to have laid 2,969 eggs (Becker 2016, p. 5) and later another captive female in Pennsylvania laid 3,737 eggs (E. McKinney, personal communication, 2021).

Appendix D – Regal Fritillary Species Distribution Model Report

Report for *Argynnis (Speyeria) idalia* (Regal fritillary)

Prepared by U.S. Fish and Wildlife Service, Ecological Services,
HQ07 – October 2021

Summary

We developed a species distribution model (SDM) of potential regal fritillary habitat to help us better understand its current distribution and to help identify population-level analytical units (AUs) for our SSA analysis. The SDM effort used an ensemble of five different algorithms projected over a defined extent to predict suitable habitat and species occurrence. The occurrence data used for the SDM process was a compilation of 6,521 regal fritillary occurrence points from 2010 to 2020 from various federal and local government agencies, state heritage programs, and publicly available databases. All models were built at 1-km (0.6-mi) resolution using only one occurrence point per pixel. Environmental covariates were selected by species biologists in the Service and species experts outside of the agency. Covariates used included National Land Cover Database (NLCD) (Yang et al. 2018) 2016 percent herbaceous, NLCD 2016 percent hay and pasture, NLCD 2016 percent cultivated crops, 20-year average soil moisture (Abatzoglou et al. 2018), topographic wetness index (U.S. Geological Survey 2016), and 30-year average (June-August) temperature (PRISM 2021).

Detailed environmental covariate data capturing required resources for the species such as violets, nectar sources, warm season bunchgrasses, litter, and shrubby/tall vegetation were not available for the model extent. NLCD data, resampled to a 1-km (0.6-mi) resolution, was used as a surrogate for these requirements by using landcover types, including cultivated crops, which has a strong negative relationship with regal fritillary abundance and occupancy. Soil moisture and a topographic wetness index were also used to capture the moisture needs of the species. Finally, climate data were incorporated using a 30-year average temperature layer for June-August, when adult flight occurs.

The mean suitability model was reviewed by species experts both internally and outside FWS. Species experts reviewed several thresholds of the SDM output to address under and over prediction. Thresholds were set at various percentages where a 1 percent (%) threshold indicates that 1% of the occurrence data used to create the model was left out of the map, 2% threshold indicates that 2% of the occurrence data used was left out, and so on. Upon review, species experts identified that a 2% threshold accurately depicted the range; however, it was also clear that certain parts of the range required a different threshold. To address ecological differences across the range, SDM thresholds were selected at individual Regal Fritillary SSA analytical units (see SDM Model Report). After individual thresholds were applied to each analytical unit, the layers with the applied thresholds were merged together and clipped to SSA analytical units to create regal fritillary current range dataset.

The SDM and current range may be revised in the future if we obtain additional data important to the species that can improve the SDM accuracy. Below, we provide the full report on the development of the SDM for regal fritillary.

Occurrence Data

There were **6521** occurrence data locations available for model creation. All models were built at **1 km (**0.6 mi) resolution, and only one occurrence point per pixel was used to prevent pseudo-replication. This reduced the total number of occurrence points to **812** for model building. Additionally, five-fold cross-validation was used to evaluate the performance of all models, so only 80% of the occurrence data were used for each iteration.

Model Algorithms

Model outputs are an ensemble of five different algorithms, each run for 10 iterations. The five algorithms used were: boosted regression trees (BRT), generalized additive models (GAM), generalized linear models (GLM), maximum-entropy (MAXENT), and random forest (RF). The results fromthese algorithms were then projected over a custom model extent. The model extent was primarily based on EPA Level IV Ecoregions. Ecoregions included in the extent cover all current (2010-2020) regal fritillary occurrence data provided by state agencies, state naturalheritage programs, other government agencies, areas of occurrence referenced by Selby (2007), and important areas noted by species experts. The extent was further refined basedon information from species experts and included trimming some ecoregions that extendedoutside of the known range for regal fritillary. Specifically, areas of mixed habitat and non- habitat in Oklahoma, Arkansas, and Missouri were removed by using HUC 8 watersheds. Outside the Midwest, regal fritillary only occurs in Lebanon County, PA. To capture the extentof this population, HUC10 units were used. All HUC10 units that intersected Lebanon Countywere extracted and dissolved. This resulting polygon was merged with the Midwest polygonto form the model extent.

Absence/Pseudo-absence Data

For BRT and RF, the same number of pseudo-absence points were used, however, for the remaining algorithms (GAM, GLM, and MAXENT) the number of occurrence data used was 10x. No true absence data were supplied by species biologists. Therefore, pseudo-absences were drawn randomly from the background (i.e., same as the projection extent). The actual versus expected number of pseudo-absence data for this species are shown in Table 1.

Table 1: Actual versus expected number of pseudo-absence data

	Equal	10x
Expected	650	6500
Actual	650	6500

Environmental Covariates

The environmental covariates included in this model were selected by biologists based on their knowledge of the species. A ranking of each covariate's importance by algorithm is provided in Table 2. These relative importance values are calculated by first making a prediction with all covariates. Then, a single covariate is randomized and a new model constructed. The two models are then compared by calculating a correlation score, which estimates a covariate's importance in the full model. Higher correlation scores indicate higher importance. This process is repeated for each covariate until all have been evaluated.

Table 2: The relative importance of each covariate by algorithm

Covariate	BRT	GAM	GLM	MAX	RF
NLCD 2016 Herbaceous, percent	0.01	0.29	0.29	0.39	0.17
NLCD 2016 Hay/pasture, percent	0.00	0.07	0.14	0.23	0.11
NLCD 2016 Cultivated crops, percent	0.55	0.38	0.55	0.36	0.27
Soil moisture in mm, 20-year average	0.52	0.58	0.59	0.64	0.44
Topographic wetness index (TWI)	0.00	0.02	0.05	0.04	0.04
June-Aug temperature, 30-year average	0.00	0.17	0.15	0.20	0.13

Evaluation Metrics

Sensitivity, also known as the true positive rate, measures the proportion of occurrence datain the test sample that were correctly identified as positives. Conversely, specificity is a measure of the true negative rate or the proportion of grid cells in the test sample that werecorrectly identified as negatives. Both Cohen's kappa and Area Under the Curve (AUC) are metrics used to assess model accuracy by taking into account both sensitivity and specificity. Cohen's kappa is threshold dependent, meaning a threshold has to be chosen to calculate it. In this case, the chosen threshold maximizes its value. Conversely, AUC can be calculated without applying a threshold. Table 3 shows a range of values for each metric and how that relates to model accuracy, although both have limitations and these values should beinterpreted with extreme caution.

Table 3: Range of values for Cohen's kappa and AUC

Cohen's kappa		Area Under the Curve	
0.75 - 1.00	Excellent	0.90 - 1.00	Outstanding
0.40 - 0.75	Fair to Good	0.80 - 0.90	Excellent
0.00 - 0.40	Poor	0.70 - 0.80	Acceptable

For all iterations, five-fold cross-validation was used to test all models. The average values (across all 10 iterations) of sensitivity, specificity, Cohen's kappa and AUC, are shown in Table 4.

Table 4: Mean values of sensitivity, specificity, Cohen's kappa and AUC across all iterations from each model algorithm

Algorithm	Sensitivity	Specificity	Cohen's kappa	AUC
BRT	82.9	67.0	0.51	0.82
GAM	70.1	82.2	0.40	0.86
GLM	70.2	82.9	0.41	0.86
MAX	69.2	86.2	0.47	0.87
RF	81.4	86.8	0.68	0.91

Table 5 shows the average metrics across all algorithms.

Table 5: Average Metrics

Sensitivity	Specificity	Cohen's Kappa	Area Under the Curve
74.8	81	0.49	0.86

Ensemble and Threshold Maps

The 50 model outputs were averaged to obtain a mean suitability map for this species (Figure 1). In addition to this map, a threshold was applied to each individual model output and then summed to obtain a model concordance map (Figure 2), yielding the number of models that predict occurrence. The value that maximized Cohen's kappa for each was used to threshold the outputs.

To obtain a simple presence/absence output map, three thresholds were applied to both theaverage suitability and concordance outputs, yielding six outputs. The thresholds applied tothe average suitability output were one that included all occurrence data and two that excluded grid cells that contained occurrence data in the lowest 5th and 10th percentile of suitability values. For the model concordance output, thresholds were applied by removing grid cells that did not have at least one, five, and 10 model(s) that predict presence.

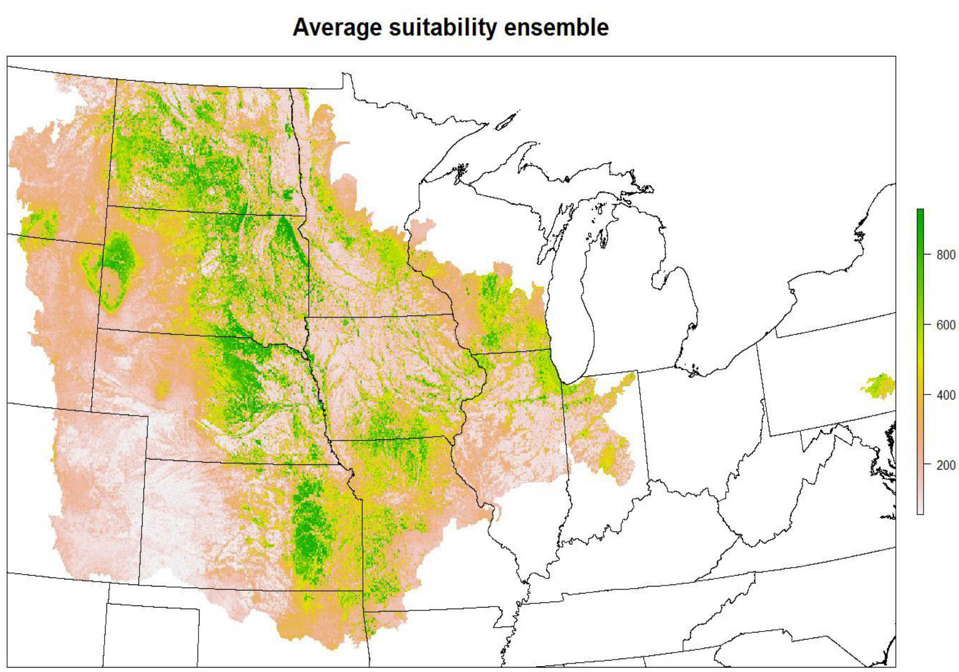

Figure 1. A map showing average habitat suitability as an ensemble of the models used across the range of the regal fritillary.

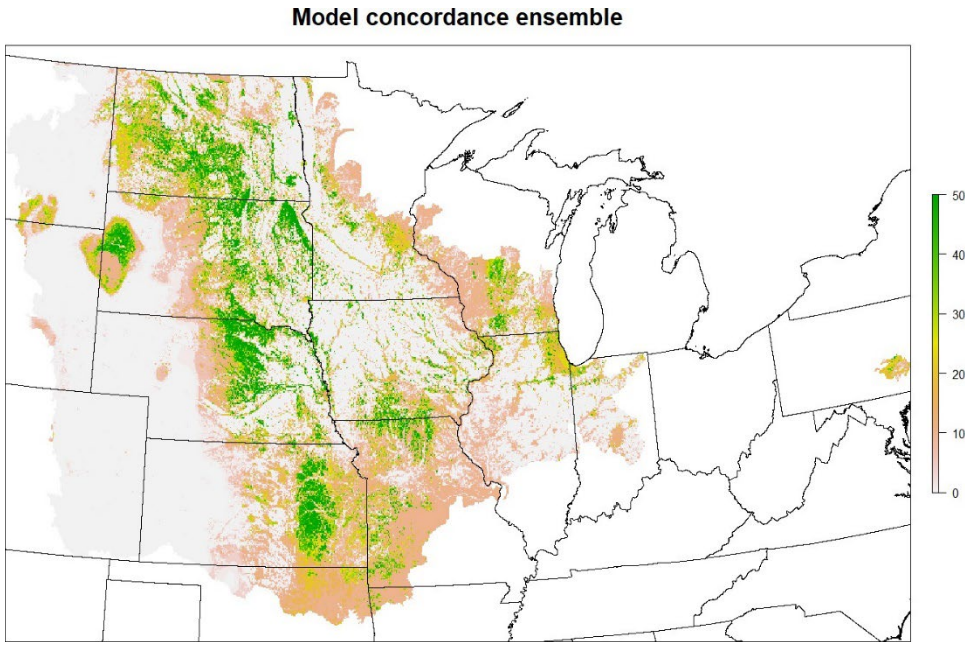

Figure 2. Map showing model concordance across the range of the regal fritillary.

For each of these thresholds, sensitivity was calculated (Table 6), which is the number and percent of occurrence data that falls within the predicted range of the species after the threshold is applied. Note: anything less than 100% indicates some occurrence data falling outside the threshold outputs.

Table 6: Number and percent of occurrence data included in threshold outputs

	Number	Percent
0 percentile	6520	100.0
5 percentile	6214	95.3
10 percentile	5868	90.0
KAPPA 1 model	6499	99.7
KAPPA 5 model	6473	99.3
KAPPA 10 model	6389	98.0

Post-processing and Final Map

Species biologists/experts reviewed several thresholds of the SDM output. Upon review, species biologists/experts identified that a 2% threshold accurately captured the range formost of the species extent, however, it was also clear that certain parts of the extent required a different threshold. To address ecological differences across the extent and accurately capture the range, SDM thresholds were addressed by Regal Fritillary SSA Analytical Units (AUs). Note that Regal Fritillary SSA AUs were based off EPA Level III Ecoregions that matched theoriginal modeling extent, however, there were some refinements and modifications to AUs that changed the overall extent. Refinements and modifications include the following:

Montana (MT) – Species biologists/experts identified that the extent and AUs extended toofar into MT and included non-habitat areas. AUs covering MT were refined by selecting HUC10 units that covered non-habitat areas and removed them from the AUs.

HUC10s include: 1004010410, 1004010414, 1004010417, 1004010418, 1004010420,

1004010421, 1004010422, 1004010423, 1004010424, 1004010425, 1004010426,

1004010427, 1004010501, 1004010502, 1004010503, 1004010504, 1004010505,

1004010506, 1004010507, 1004010508, 1004010509, 1004010601, 1004010602,

1004010603, 1004010604, 1004010605, 1004010606, 1004010607, 1004020205,

1004020206, 1004020209, 1004020210, 1004020305, 1004020501, 1004020502,

1004020505, 1004020508, 1005001501, 1005001501, 1005001508, 1005001601,

1005001602, 1005001604, 1006000102, 1006000104, 1006000105, 1006000106,

1006000107, 1006000108, 1006000112, 1006000113, 1006000114, 1006000201,

1006000202, 1006000203, 1006000204, 1006000205, 1006000206, 1006000207,

1006000208, 1006000209, 1006000210, 1006000211, 1006000212, 1006000213,

1006000214, 1006000215, 1006000216, 1006000301, 1006000302, 1006000303,

1006000306, 1006000307, 1006000308, 1006000309, 1006000310, 1006000401,

1006000402, 1006000403, 1006000404, 1006000405, 1006000406, 1006000407,

1006000502, 1006000602, 1006000603, 1006000605, 1006000606, 1006000607,

1006000608, 1006000609, 1006000610, 1006000611, 1006000612, 1006000613,

1006000614, 1006000618, 1006000619, 1006000620, 1008001507, 1008001508,

1008001510, 1009010207, 1009010208, 1009010209, 1009010212, 1009010213,

1009020907, 1009020908, 1009020909, 1009020910, 1009020911, 1009020912,

1009020913, 1009021002, 1009021003, 1010000101, 1010000102, 1010000103,

1010000104, 1010000105, 1010000106, 1010000107, 1010000108, 1010000109,

1010000110, 1010000111, 1010000112, 1010000113, 1010000114, 1010000115,

1010000116, 1010000117, 1010000118, 1010000119, 1010000120, 1010000121, 1010000122, 1010000123, 1010000124, 1010000125, 1010000126, 1010000127,

1010000128, 1010000129, 1010000201, 1010000202, 1010000203, 1010000204,

1010000205, 1010000306, 1010000401, 1010000402, 1010000403, 1010000404,

1010000405, 1010000406, 1010000407, 1010000408, 1010000409, 1010000410,

1010000411, 1010000412, 1010000413, 1010000414, 1010000415, 1010000501,

1010000502, 1010000503, 1010000504, 1010000506, 1010000507, 1010000508,

1010000509, 1010000510.

Remnants of the Northwestern Glaciated Plains and Northwest Great Plains ecoregionswere deleted.

Colorado (CO) – Species biologists/experts identified that the Southwestern Tablelands Ecoregion/AU in southern CO covered areas of poor habitat. Based on species biologists/expert information and lack of current occurrence data, the Tablelands Ecoregion/AU was removed.

Kansas (KS) – Like Colorado, based on poor habitat quality and lack of current occurrence data, species biologists/experts identified that the Southwestern Tablelands Ecoregion/AUin Kansas should be removed.

Oklahoma (OK) – Based on poor habitat quality and lack of current occurrence data, species biologists/experts identified that the Cross Timbers Ecoregion/AU in Kansas should be removed.

Indiana (IN) – Species biologists/experts identified that the extent and AUs covering IN nolonger represent regal fritillary current range. Although there are historic records, the species has been extirpated from most of IN.

Southern Michigan/Northern Indiana Drift Plains and Eastern Corn Belt PlainsEcoregions/AUs were removed.

Pennsylvania (PA) - Species biologists/experts identified that the extent and AUs for PAwere too large and that regal fritillary only occurs in a small area in and around FTIG military installation.

HUC10 units that intersected the FTIG installation (0205030506, 0205030509, 0205030510) were selected and used to clip the L3 Ridge and Valley ecoregion.

Sand Hills Ecoregion Polygons

The Sand Hills ecoregion included three separate polygons including one large polygon andtwo smaller polygons. For SSA analysis purposes it was simpler to incorporate the two smaller polygons into other larger AUs.

Northern small polygon incorporated into the Northwestern Glaciated Plains AU.
Southern small polygon incorporated into the High Plains AU.

SDM Thresholds by AU
Majority of the Midwest Range

2% threshold used for the majority of the regal fritillary range. This includes the following ecoregions/AUs:

Central Great Plains, Central Irregular Plains, Cross Timbers, Driftless Area, Flint Hills, Lake Agassiz Plain, Middle Rockies, North Central Hardwood Forests, Northern Glaciated Plains, Northwestern Glaciated Plains, Ozark Highlands, Southeastern Wisconsin Till Plains, Western Corn Belt Plains.

2% threshold clipped to selected AUs.

Northwest Range – Northwestern Great Plains and Southern Rockies Ecoregion/AU

FWS biologists and outside species experts identified that the 2% threshold was under predicting and leaving out areas of regal fritillary habitat along the western edge of the range. To address this, the 1% threshold was used in the Northwestern Great Plains andSouthern Rockies Ecoregion/AU.

The 1% threshold was used in the Northwester Great Plains and Southern Rockies Ecoregion/AU. This was used to capture the range except for areas of Montana as noted inthe Montana section.

Montana

USFWS biologists and outside species experts identified that the 2% and 1% thresholds over predicted and included too much area in Montana. Biologists and species experts identified that this was unrealistic and captured areas outside the regal fritillary range. Toaddress this, the 3% threshold was used for portions of Montana.

The Montana portion of AUs identified using HUC8 units: 10060005, 10060006, 10060007, 10080016, 10090101, 10090102, 10090207, 10090209, 10090210, 10100003, 10100004,

10100005, 10110201, and 10110202.

The 3% threshold was clipped to the Montana HUC8 units.

The Montana HUC8 units from the 1% Northwestern Great Plains Ecoregion/AU data were erased.

The MT HUC8 units from the 2% Northwestern Glaciated Plains Ecoregion/AU data were erased.

Southwest Range – Nebraska Sand Hills and High Plains Ecoregions/AUs

FWS biologists and outside species experts identified that the 2% threshold was under predicting and leaving out areas of regal fritillary range along the western edge of the range. To accurately capture regal fritillary range in the southwest portion of the range, thetop 75% of values from the SDM mean suitability model were used (roughly 0.5% threshold).

The top 75% of values were extracted from the SDM mean suitability model and clipped tothe Nebraska Sand Hills and High Plains Ecoregions/AUs.

Illinois – Central Corn Belt Plains and Interior River Valleys and Hills Ecoregions/AUs

FWS biologists and outside species experts identified that the 2% threshold was under predicting and leaving out areas of regal fritillary range covering Illinois. To accurately capture regal fritillary range in Illinois, the top 75% of values from the SDM mean suitability model were used (roughly 0.5% threshold).

The top 75% of values were extracted from the SDM mean suitability model and clipped tothe Central Corn Belt Plains and Interior River Valleys and Hills Ecoregions/AUs.

Pennsylvania

FWS biologists and outside species experts identified that the 2% threshold over predicted and included too much area in Pennsylvania. SCAs and other polygon datasets thatidentify where regal fritillary are known to occur were checked against different SDM threshold datasets. The data comparisons and information from species biologists/expertsidentified that the 3% threshold accurately identified regal fritillary range for Pennsylvania.

A 3% threshold was applied to the Pennsylvania AU.

Final Range Processing Steps

Species biologists/experts identified that highly developed urban areas should not be included in the species range. To address this, the High and Medium Urban Developmentcategories from NLCD 2016 were used to remove these landcover types from the regal fritillary range.

Literature Cited for Appendix D

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Appendix E – Analytical Units (AUs) and Representation Units

In this appendix, we provide additional information on how we refined the boundaries of the AUs for our analysis. We also provide additional information on the representation units.

Refinement of AU Boundaries

To improve the AUs for our SSA analysis, we modified the boundaries for some of our AUs. Specifically, we modified 11 of the Level III ecoregion boundaries (https://www.epa.gov/eco-research/level-iii-and-iv-ecoregions-continental-united-states) on the southern periphery of the regal fritillary's current range to reflect the extent of the overall range. These 11 AUs are: Central Great Plains, Cross Timbers, High Plains, Interior River Valleys and Hills, North Central Hardwoods, Northwestern Glaciated Plains, Northwestern Great Plains, Ozark Highlands, Southeastern Wisconsin Till Plains, Southern Rockies, and Ridge and Valley. As a result, these AUs are smaller than their corresponding Level III ecoregions. Reducing the size of the North Central Hardwoods ecoregion also resulted in splitting it into two units, which were defined and analyzed separately as units A and B, herein. The Central Irregular Plains ecoregion is mapped by the EPA as two geographically separate units with the same name, so we evaluated these as separate individual analytical units as well. The Nebraska Sand Hills are split into three separate units per EPA's Level III ecoregion maps: a large unit and two satellite units. However, the satellite units were considered too small to be evaluated individually and were incorporated into their surrounding ecoregions for our SSA evaluation.

Further, some modifications to EPA Level III ecoregion boundaries were made for the purposes of this SSA. Our modeling extent (see SDM section above) was based on EPA Level IV ecoregions with some modifications using hydrologic unit codes (HUCs), which are sequences of numbers or letters that identify hydrological features like rivers, or areas like drainage basins (see: https://nas.er.usgs.gov/hucs.aspx), to remove areas of non-habitat outside the range of the regal fritillary with exception of the unit in Pennsylvania (more on that below). The modeling extent was then used to define the analytical units by dissolving, or generalizing, the Level IV ecoregions to their Level III ecoregion name. From that point, further modifications were made (e.g., some very small ecoregions were too small to be analyzed alone while others with multi-polygon ecoregions were analyzed separately). We considered using Level IV ecoregions to evaluate the 3Rs for the regal fritillary but determined that we lacked information to evaluate the species at that finer scale, and the relatively high number of units in the extent would also have been unwieldy. Thus, our SSA analytical units are based on Level III ecoregions but do not exactly match their boundaries.

Uncertainties/assumptions exist with using this analytical unit scale. There are unique differences in habitats at a local scale that influence resiliency beyond what can be measured at the Level III Ecoregion scale; we have attempted to capture such factors in our assessment of condition within the analytical units, but this evaluation is limited primarily to qualitative assessments of habitat suitability. EPA's Level III ecoregion may divide areas of occupancy by the regal fritillary; actual connectivity and movements among/between colonies, populations and metapopulations are difficult to define.

The eastern subspecies has one AU, Ridge and Valley, which is the smallest of all the AUs. This unit is defined primarily by occupied HUC10 watersheds with regal fritillary occurrences, clipped to the Level III Ridge and Valley ecoregion, on the military base where the sole remaining eastern subspecies population exists today. Yet it is a stand-alone unit, on par with western subspecies analytical units that are orders of magnitude larger. If the entire EPA Level III Ridge and Valley unit were used in the SSA, it would extend across numerous states including some where the species has never occurred; thus, the results of current and future conditions could potentially be significantly different. Therefore, we reduced the AU size to reflect current occupancy and considered other factors unique to this unit (e.g., genetics, isolation, management, habitat) are additional relevant considerations for this unit beyond its size.

Representation Units

Three distinct regions supporting regal fritillaries had previously been identified by Williams (1999, p. 3): East, Midwest, and Great Plains. These regions were based initially on morphological differences of the butterflies and observed habitat differences and their conservation implications (Williams 1999, p. 3). Genetic analysis was subsequently performed, and the western subspecies (*A. i. occidentalis*) was identified and described in 2001, with the previously described neotype specimen from New York (dos Passos and Grey 1947, p. 9) defaulting to the eastern subspecies, *A. i. idalia* (Williams 2001b, p. 148) (see also Taxonomy section).

After the two subspecies were identified, additional genetic studies were conducted that examined levels of differentiation to determine if effects of isolation and fragmentation could be detected in regal fritillary populations (Williams et al. 2003, entire). Williams et al. (2003, pp. 11-13) used four microsatellite loci to genotype over 300 individuals: five populations in continuous habitat from Great Plains states, five populations in recently fragmented habitat from Midwestern states, and specimens from the single isolated population in the East atFTIG in Pennsylvania. Consistent with long-term fragmentation and isolation, the FTIG population showed the most allelic differentiation from all others (Williams et al. 2003, p. 14). Analysis showed increased differentiation among Midwestern population samples when compared to Great Plains samples, which is also consistent with the effects of habitat fragmentation (Williams et al. 2003, p. 14). The least amount of allelic differentiation was detected among Great Plains samples (Williams et al. 2003, p. 14).

General boundaries of these three regional areas, East, Midwest, and Great Plains have been mapped (Williams et al. 2003, p. 12). The three regions also differ by vegetative types, habitat conditions, and environmental conditions. Herein, we recognize the validity of Williams et al.'s (2003) general regions and use the EPA Level III Ecoregions (U.S. Environmental Protection Agency 2013) occurring within them to refine their borders for our representation analysis. Further, we recognize that climatic differences exist between northern occupied regal fritillary sites in the Great Plains and those in more southerly portions of this region that are relevant to both the phenology and future climate impacting the regal fritillary.

Appendix F – Resource Needs of Individuals

Individual needs of the four life-history stages in terms of breeding, feeding and sheltering are summarized in this section below and described in Chapter 3, Table 8.

- Breeding:
  - For mating to occur, no specific conditions are known to be required beyond timing, initiation/reception, survival, opportunity, and perhaps favorable early to mid-summer weather to promote butterfly activity (e.g., warm sunny days, relatively low winds, lack of precipitation). This assumes the natal habitat remains intact and both sexes are present. For reproduction, females must have access to adequate resources to survive through egg development and egg-laying; adequate nutrition improves fecundity and fertility
- Feeding:
  - *Egg.* The developing larvae within the eggs rely on the nutritional content present when the eggs were laid, likely dependent at least partly on female nutrition.
  - *Larva.* First instars consume a portion of the egg chorion and enter winter diapause; in spring they require young violet plants within proximity (accessible) to their overwintering site and adequate availability and abundance of violet plants thereafter to complete larval development. Inadequate nutrition can stunt caterpillar growth or cause mortality and potentially reduce adult longevity.
  - *Pupa.* Pupae are reliant on internal stores for continued development, dependent upon adequate nutrition during the larval stage.
  - *Adult.* Male regal fritillary butterflies require adequate quantity and quality of nectar and mineral resources for nutrition and hydration for the few weeks they await female emergence and the opportunity to mate. Females require the same resources upon emergence, but the need extends into the fall season to survive until egg-laying stage; higher quality and quantity nutrition appears to promote improved female fecundity and fertility.
- Sheltering:
  - *Egg.* Specific sheltering requirements that promote successful egg development and hatching are not well known. Assuming grassland habitat deemed suitable by gravid regal fritillaries exists, shaded microsites on the underside of senesced vegetation and appropriate local ambient conditions (parameters unknown) may be all that is necessary.
  - *Larva.* Litter buildup and tussocks of warm season bunchgrasses, such as big bluestem and little bluestem, provide shelter during winter diapause and throughout spring larval development. Shelter at this stage must be adequate for

the larvae to endure adverse conditions such as harsh winter weather, late hard frosts following spring thaws, severe storms, severe solar radiation or excessive heat, and cool, damp conditions.

- *Pupa.* Structure within the leaf litter of the late spring or early summer grassland vegetation (e.g., bunchgrass tussocks) provides a point of attachment and shelter for regal fritillary pupa from adverse local climatic factors.

Adult. Adult regal fritillaries find shelter from local climatic factors such as wind, precipitation, and excessive heat in vegetation clumps, including warm season bunchgrasses, shrubs, or shrub-like vegetation.

Appendix G – Precipitation and Drought Climate Data

REPRESENTATION UNIT	ANALYTICAL UNIT NAME	CITY	HISTORIC (BASELINE/CURRENT CONDITIONS) DATA				SCENARIO 1 - Climate model INMCM4.0; warm & wet with normal to dry summer				SCENARIO 2 - Climate model MRICGCM3; warm spring and dry summer				SCENARIO 3 - Climate model HadGEM2; hot and dry summer			
			Spring Precip. (inches)	Summer Precip. (inches)	*SPEI Drought/decade	CONDITION CATEGORY	Spring Precip. (% change over historic)	Summer Precip. (% change over historic)	*SPEI Drought/Decade (difference from historic)	CONDITION CATEGORY	Spring Precip. (% change over historic)	Summer Precip. (% change over historic)	*SPEI Drought/Decade (difference from historic)	CONDITION CATEGORY	Spring Precip. (% change over historic)	Summer Precip. (% change over historic)	*SPEI Drought/Decade (difference from historic)	CONDITION CATEGORY
EAST	**Ridge and Valley**	Harrisburg, PA	11.6	11.4	0.8	High	11.9 (2.586)	12.2 (7.017)	0.7 (-0.1)	High	11.8 (1.7241)	11.3 (-0.88)	2 (1.92)	High	12.8 (10.344)	10.8 (-05.263)	2.3 (2.22)	High
REPRESENTATION UNIT	**ANALYTICAL UNIT**	**CITY**	**HISTORIC (BASELINE/CURRENT CONDITIONS) DATA**				**SCENARIO 1 - Climate model INMCM4.0; warm & wet with normal to dry summer**				**SCENARIO 2 - Climate model HadGEM2; hot and dry summer**				**SCENARIO 3 - Climate model MIROC5; hot and dry all year**			
			Spring Precip. (inches)	Summer Precip. (inches)	*SPEI Drought/decade	CONDITION CATEGORY	Spring Precip. (% change over historic)	Summer Precip. (% change over historic)	*SPEI Drought/Decade (difference from historic)	CONDITION CATEGORY	Spring Precip. (% change over historic)	Summer Precip. (% change over historic)	*SPEI Drought/Decade (difference from historic)	CONDITION CATEGORY	Spring Precip. (% change over historic)	Summer Precip. (% change over historic)	*SPEI Drought/Decade (difference from historic)	CONDITION CATEGORY
MIDWEST	**Central Corn Belt Plains**	Bloomington, IL	10.7	11.3	0.33	Very High	11.8 (10.280)	11.2 (-0.885)	0.333 (0.0)	Very High	13.1 (22.4299)	9.1 (-19.469)	1.333 (1.003)	High	11.9 (11.215)	9.9 (-12.389)	1 (0.67)	High
	Central Irregular Plains - A	Bethany, MO	11	14	0.66	High	12.4 (12.7273)	13.4 (-4.286)	0.66 (0.0.)	High	14.5 (31.8182)	11.9 (-15.0)	0.66 (0)	High	11.1 (0.9091)	11.7 (-16.429)	3 (2.34)	Medium
	Driftless Area	La Crosse, WI	9.3	13.3	0.667	High	12.8 (37.63)	10.6 (-20.301)	1.667 (1.0)	High	11.2 (20.4301)	11.6 (-12.782)	2 (1.33)	High	10.5 (12.9032)	11.8 (-11.278)	2.333 (1.666)	High
	Interior River Valleys and Hills	Carbondale, IL	14.2	11.5	0.667	High	15.9 (11.972)	12.7 (10.434)	0.333 (-0.333)	Very High	16.6 (16.9014)	9.5 (-17.391)	0.333 (-0.334)	High	15.2 (7.0423)	11.4 (-0.87)	2 (1.333)	High
	North Central Hardwood Forests - B	St. Cloud, MN	7.5	11.8	0.667	High	8.8 (17.333)	11.1 (-5.932)	1.333 (0.667)	High	9.3 (24.00)	10.3 (-12.713)	1 (0.333)	High	8.1 (8.00)	11.6 (-1.695)	2.333 (1.666)	Medium
	North Central Hardwood Forests - A	Steven's Point, WI	8.5	11.9	0.667	High	9.8 (15.294)	11.1 (-6.723)	1 (0.333)	High	10.5 (23.5294)	10.4 (-12.605)	1.333 (0.666)	High	9.7 (14.1176)	10.4 (-12.605)	2 (1.333)	Medium
	Southeastern Wisconsin Till Plains	Union Grove, WI	9.2	11.5	0.333	High	10.1 (9.782)	11.9 (3.478)	1 (0.667)	High	11.8 (28.2609)	9.6 (-16.522)	0.333 (0)	High	10.2 (10.8696)	10.1 (-12.174)	1.667 (1.334)	High
	Western Corn Belt Plains	Spencer, IA	9	12.2	1	High	9.8 (8.889)	12.4 (1.639)	2.333 (1.333)	Medium	10.4 (15.5556)	10.3 (-15.5556)	1.667 (0.667)	High	8.9 (-1.111)	10.8 (-11.475)	3.6667 (2.667)	Medium
NORTHERN GREAT PLAINS	***Lake Agassiz Plain**	Grand Forks, ND	4.7	9.3	1	Medium	5.3 (12.766)	8.9 (-4.301)	1.333 (0.333)	Medium	4.8 (02.1277)	7.7 (-17.204)	1.667 (0.667)	Low	5.4 (14.89)	8.5 (-8.602)	3.667 (2.667)	Low
	***Middle Rockies**	Custer, SD	6.6	8	0.667	Medium	7 (6.061)	7.4 (-7.500)	1.333 (0.667)	Medium	8.3 (25.7576)	6.7 (-16.25)	2 (1.333)	Low	6.4 (-3.03)	6.9 (-13.75)	3.667 (3)	Low

	***Northern Glaciated Plains**	Lake City, SD	6.2	9.9	0.667	Medium	7 (12.903)	9.3 (-6.061)	2.333 (1.667)	Medium	7 (29.032)	8.8 (-11.11)	2 (1.333)	Medium	6.6 (6.45)	9.8 (-1.01)	3 (2.333)	Low
	***Northwestern Glaciated Plains**	Mobridge, SD	5	7.2	1	Low	5.1 (2.00)	6.5 (-9.722)	2 (1)	Low	6.1 (22.00)	6.2 (-13.889)	1.667 (0.667)	Medium	4.8 (-4.0)	6.8 (-5.556)	3.333 (2.333)	Low
	***Northwestern Great Plains**	Buffalo, SD	5	6.3	0.667	Medium	5.5 (10.00)	5.6 (-11.111)	1.67 (1)	Low	7.2 (44.00)	5.3 (-15.873)	1 (0.333)	Low	5 (0)	5.2 (-17.46)	4.667 (4)	Very Low
CENTRAL GREAT PLAINS	**Central Great Plains**	Lebanon, KS	8.1	10.6	0.667	High	9.7 (19.753)	10.1 (-4.717)	0.667(0.00)	High	9.7 (19.7531)	9.8 (-07.547)	1.667 (1)	Medium	7.9 (-02.469)	9.4 (-11.321)	5 (4.333)	Low
	Central Irregular Plains - B	Pittsburg, KS	14	13	0.667	High	16.2 (15.714)	13 (0.00)	0.6677 (0.0)	High	16.5 (17.8531)	12.1 (-06.923)	1 (0.33)	High	12.3 (-12.143)	13 (0.0)	2.667 (2)	High
	Cross Timbers	Ponca City, OK	11.6	11.4	0.333	Very High	13.3 (14.655)	10.9 (-4.386)	0.667 (0.33)	High	13.9 (19.8276)	10.5 (-07.895)	1.667 (1.334)	High	10.5 (-9.48)	11.3 (-0.877)	2.667 (2.334)	High
	Flint Hills	Manhattan, KS	10.1	12.9	0.667	Very High	11.6 (14.852)	11.9 (-7.752)	1 (0.333)	High	13 (28.7129)	11.8 (-08.527)	1.7 (1.3)	High	9.6 (-4.95)	11.1 (-13.953)	5.7 (5.3)	Medium
	High Plains	Sterling, CO	4.9	7.2	1.33	Low	5.4 (10.204)	6.5 (-9.722)	0.667 (-0.667)	Medium	5 (02.0408)	5.8 (-19.444)	2.667 (1.337)	Low	4.7 (-4.082)	7 (-2.778)	4.333 (3.003)	Very Low
	Nebraska Sand Hills	Hyannis, NE	6.2	8.2	1	Medium	6.7 (8.0645)	7.6 (-7.317)	1 (0.0)	Medium	7.1 (14.5161)	6.8 (-17.073)	2 (1)	Low	5.1 (-17.74)	7.1 (-13.415)	4.333 (3.333)	Very Low
	Ozark Highlands	Springfield, MO	13.1	11.8	0.333	Very High	14.6 (11.450)	12.2 (3.3898)	0.666 (0.333)	High	15.2 (16.0305)	10.2 (-13.559)	0.66 (0.327)	High	13.5 (3.05)	10.7 (-09.322)	2.667 (2.334)	High
	Southern Rockies	Laramie, WY	3.7	4.2	0.667	Low	3.9 (5.405)	3.8 (-9.524)	1.333 (0.667)	Low	4.2 (13.5135)	3.5 (-16.667)	3 (2.33)	Very Low	3.7 (0)	4.4 (4.7619)	5.333 (4.666)	X

CRITERIA TO RANK PRECIPITATION CONDITIONS			
Condition Category/Points	**Spring Precip. range: 94-361 millimeters (mm) 3.7-14.2 inches (in)**	**Summer Precip. range: 107-356 mm (4.2 -14.0 in)**	**Droughts per Decade (range: 0.3-1)**
Very High/5	>=254 mm (10 in)	>=254 mm (10 in)	<0.5
High/4	216-254 mm (8.50-9.99 in)	216-254 mm (8.50-9.99 in)	0.6-0.9
Med/3	152-216 mm (6.0-8.49 in)	216-254 mm (6.0-8.49 in)	1.0-1.9
Low/2	114-152 mm (4.50-5.99 in)	114-152 (4.50-5.99 in)	2.0-2.9
Very Low/1	76-114 mm (3.0-4.49 in)	76-114 mm (3.0-4.49 in)	3.0-3.9
Extirpated/0	<76 mm (3 in)	<76 mm (3 in)	≥4.0

Appendix H – Cause-and-Effect Tables

These Cause-and Effect tables have questions to help us understand the relationship between a **source** and its **effects** on the **species**. The Key:

ESA Factors	Analysis
SOURCE(S)	*What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*
Activity(ies)	*What is actually happening on the ground as a result of the action? Be specific here.*
STRESSOR(S)	*What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*
Affected Resource(s)	*What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*
Exposure of Stressor(s)	*Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.*
Immediacy of Stressor(s)	*What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*
Changes in Resource(s)	*Specifically, how has(is) the resource changed(ing)?*
Response to Stressors: INDIVIDUALS	*What are the effects on individuals of the species to the stressor? (May be by life stage)*
Effects of Stressors: POPULATIONS [RESILIENCY]	*What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc)?*
GEOGRAPHIC SCOPE	*What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*
MAGNITUDE	*How large of an effect do you expect it to have on the populations and species overall?*
SPECIES LEVEL: SUMMARY	*What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects? **Past, Current, Future.***

Terminologies for characterizing confidence levels in the CE tables.

Confidence Terminology	Explanation
Highly Confident	We are more than **90% sure** that this relationship or assumption accurately reflects the reality in the wild as supported by documented accounts or research and/or strongly consistent with accepted conservation biology principles.
Moderately Confident	We are **70 to 90% sure** that this relationship or assumption accurately reflects the reality in the wild as supported by some available information and/or consistent with accepted conservation biology principles.
Somewhat Confident	We are **50 to 70% sure** that this relationship or assumption accurately reflects the reality in the wild as supported by some available information and/or consistent with accepted conservation biology principles.
Low Confidence	We are less than **50% sure** that this relationship or assumption accurately reflects the reality in the wild, as there is little or no supporting available information and/or uncertainty consistency with accepted conservation biology principles. Indicates areas of high uncertainty.

a) DISEASE and PARASITISM

[ESA Factor(s): C]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Parasites and pathogens (bacteria, protozoans, viruses, fungi)	Highly confident	Myers and Cory 2015, p. 231
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	Pathogens or parasites are present in the environment, individuals become infected via contact or ingestion, or infected individuals may pass pathogens to others; transmission may be vertical (pathogens passed from adult to young), or horizontal (ingested, or contact with infected individuals/excrement/dead bodies)	Highly confident	Wagner et al. 1997, p. 271; Kopper 2001c, p. 96; Selby 2007, p. 32-34; Ferster and Vulinec 2010, p. 34-35
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	Pathogens/parasites negatively affect the health of individuals; some environmental conditions may make individuals more vulnerable to pathogens (i.e., cool damp springs equates to more susceptible to fungal infection)	Highly confident	Schweitzer 1992a, p. 5; Myers and Cory 2015, p. 239
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	Direct effects to individuals: reduced fitness or death	Highly confident	Schweitzer 1992a, p. 5; Wagner et al. 1997, p. 271; Kopper 2001c, p. 96; Selby 2007, p. 32-34; Ferster and Vulinec 2010, p. 34-35; Myers and Cory 2015, p. 234

- Exposure of Stressor(s) *Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.*	Potentially occurs year-round in any occupied habitat, but may be less prevalent during winter diapause (particularly in northern areas where cold suppresses vectors), although fungal infection may occur under warm winter conditions	Somewhat confident	Documented pathogens/parasites in regal fritillaries have typically been in seasons other than winter, but overwintering first instar larvae are not well-studied
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Pathogens/parasites are present naturally in the environment, and insects support a wide diversity of pathogens. Individuals and populations may be affected at any time (albeit may be less likely during cold time of year); occurs past/present/future	Highly confident	Schweitzer 1992a, p. 5; Wagner et al. 1997, p. 271; Kopper 2001c, p. 96; Selby 2007, p. 32-34; Ferster and Vulinec 2010, p. 34-35, Myers and Cory 2015, p. 231
Changes in Resource(s) *Specifically, how has(is) the resource changed(ing)?*	Pathogens and parasites may reduce the number of regal fritillaries that survive to reproduce and/or reduce their fertility; effects can range from slightly debilitating to lethal	Highly confident	Schweitzer 1992a, p. 5; Wagner et al. 1997, p. 271; Kopper 2001c, p. 96; Selby 2007, p. 32-34; Ferster and Vulinec 2010, p. 34-35, Myers and Cory 2015, p. 232

EFFECTS TO INDIVIDUALS			
<u>Response to Stressors:</u> **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage)* . The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	May be most likely to occur in more vulnerable early life stages than adults. Could be non-symptomatic or exhibit altered behavior, reduced feeding success, reduced growth, depressed autoimmune response, reduced fecundity, reproductive failure, death	Moderately confident	Wagner et al. 1997, p. 271; Selby 2007, p. 32-34; Ferster and Vulinec 2010, p. 34-35; Myers and Cory 2015, p. 234;
EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) – RESILIENCY			
<u>Effects of Stressors:</u> **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc)?*	Potential for reduced survival rates, lower reproductive rate, reduced population growth, but data is limited	Moderately confident	Schweitzer 1992a, p. 5; Wagner et al. 1997, p. 271; Selby 2007, p. 32-34; Ferster and Vulinec 2010, p. 34-35; Myers and Cory 2015, p. 234;

EFFECTS TO THE SPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	Data lacking, but not likely geographically restricted; potential impacts all AUs to some extent annually. Some observations of disease in the eastern subspecies; may lead to eastern subspecies being more vulnerable to fungus as a result of decreased genetic diversity.	Moderately confident	Pathogens/parasites have been documented in west e.g., Kopper 2001c, p. 96 and east (e.g., Ferster and Vulinec 2010, p. 34-35
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Pathogens/parasites likely exert some control on the species annually as natural sources of mortality, lowering survival rates and decreasing resiliency. Significant local-level outbreaks likely occur periodically but are not well documented. Unlikely to have catastrophic effect (barring unforeseen new pathogen/parasite) on entire western subspecies due to current distribution and size of range (i.e., unlikely to impact widely separated AUs at same level of severity); however, one or more significant outbreaks has potential to severely weaken or directly extirpate the eastern subspecies due to small population size in restricted location.	Moderately confident	Ferster and Vulinec 2010, p. 34-35; M. Swartz, personal communication, 2021
<u>Effects of Stressors:</u> **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Severe outbreaks potentially could reduce the number of populations in AUs, lowering overall redundancy levels of western subspecies; redundancy/representation of eastern subspecies could be eliminated entirely. Note that to our knowledge, such effects have not yet been documented in wild populations	Moderately confident	Ferster and Vulinec 2010, p. 34-35; M. Swartz, personal communication, 2021

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	Disease and parasites are known to occur in regal fritillaries, as with all Lepidoptera, and are likely a common annual occurrence that limits, at least to some degree, regal fritillary abundance. Disease has been theorized to contribute to the collapse of populations in the eastern portion of the range, but this is not verified with field studies. Could become more of an issue for AUs in the future if climate change results in winters that are warmer, and springs are damper and cooler. The eastern subspecies is significantly more vulnerable than the western subspecies due to the former's small size and isolation.	Moderately confident	Wagner et al. 1997, p. 271; Selby, 2007, p. 32-34; NatureServe 2021; Regal Fritillary SSA Expert Workshop participants, personal communications, September 2021

b) PREDATION

[ESA Factor(s): C]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Predators that feed on insects	Highly Confident	Barton 1995, p. 5; Selby 2007, p. 32-34
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	A variety of vertebrate and invertebrate predators consume individual regal fritillaries; no known predator is specific to *Argynnis* or *A. idalia*	Highly Confident	Royer and Marrone 1992, p. 28; Barton 1995, p. 5; Selby 2007, p. 32-34
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	Reduction in number of regal fritillaries	Highly Confident	Barton 1995, p. 5; Selby 2007, p. 32-24
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	Direct mortality of individuals	Highly Confident	Barton 1995, p. 5; Selby 2007, p. 32-34
- Exposure of Stressor(s) *Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to*	Predation is possible during all seasons, all life stages and in all locations throughout the range, however, winter risk may be relatively lower unless temperatures are high enough to rouse predators such as spiders. Mobile adults may be	Highly Confident	M. Swartz, personal communication, 2021

describe where geographically it is occurring, but where in terms of habitat.	relatively less susceptible to predation than the early life stages that are restricted to the ground		
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Likely common and ongoing - past/present/future	Highly Confident	Selby 2007, p. 32-34
Changes in Resource(s) *Specifically, how has(is) the resource changed(ing)?*	No information available	N/A	N/A

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage)* . The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	Effects may range from behavioral (e.g. hiding to avoid predation) or sublethal (e.g. reduced feeding when predators are present), to death	Somewhat Confident	Not Available
EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) – RESILIENCY			
Effects of Stressors: **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc)?*	Survival rates are reduced. With exception of adult mortality that occurs after egg-laying, predation at all other life stages leads to fewer individuals available to produce next annual generation. Information on population-level impacts is lacking.	Somewhat Confident	Selby 2007, p. 32-34

EFFECTS TO THE SPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	Occurs in all populations rangewide.	Highly Confident	Selby 2007, p. 32-34
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	May have outsized impact on small populations, but no data available. Not apparent that species is any more susceptible to predation than other Lepidoptera	Highly Confident	Royer and Marrone 1992
Effects of Stressors: **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Not reported to be a population-limiting factor anywhere in the species range; not known to impact redundancy or representation	Highly Confident	Selby 2007, p. 32-24; Participants in Regal Fritillary SSA Expert Workshops, personal communications, September 2021
SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	Predation is known to occur (past, current, future) but no information is available to suggest it occurs at levels that would limit regal fritillary populations. No predators specific to regal fritillaries are known, and regal fritillaries not known to be differentially targeted or more vulnerable than any other Lepidopteran.	Highly Confident	Royer and Marrone 1992, p. 28; participants in Regal Fritillary SSA Expert Workshops, September 2021

c) SYMPATRIC FRITILLARIES

[ESA Factor(s): E]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Sympatric fritillary species	Highly Confident	Royer and Marrone 1992, p. 24; . Barton 1995, p. 12; Selby 2007, p. 33; P. Hammond, personal communication, 2021
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	Other grassland fritillary species occur in the same area as the regal fritillary and compete for the same resources (species: Aphrodite fritillary, Edwards fritillary, great-spangled fritillary the meadow fritillary (*Boloria bellona*) and the silver-bordered fritillary (*B. selene*).)	Highly Confident	Royer and Marrone 1992, p. 24; . Barton 1995, p. 12; Selby 2007, p. 33; P. Hammond, personal communication, 2021
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	Reduction in food resources	Highly Confident	Royer and Marrone 1992, p. 24; . Barton 1995, p. 12; Selby 2007, p. 33; P. Hammond, personal communication, 2021

- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	Violet plants (larval food)	Highly Confident	. Barton 1995, p. 12; Selby 2007, p. 33; P. Hammond, personal communication, 2021
- Exposure of Stressor(s) *Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.*	Spring to early summer when violets are available and larvae are feeding	Highly Confident	Barton 1995, p. 12; P. Hammond, personal communication, 2021
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Annual occurrence, each spring to early summer when larvae are feeding/developing; has occurred in the past, is ongoing, and is expected to continue	Highly Confident	Barton 1995, p. 12; P. Hammond, personal communication, 2021
Changes in Resource(s) *Specifically, how has(is) the resource changed(ing)?*	Other species may consume violets, competing for the same resource and could have an advantage if they develop faster than regal fritillaries; if violets are scarce, the plants become less available to regal fritillary larvae	Moderately Confident	Barton 1995, p. 12; P. Hammond, personal communication, 2021

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage).* The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	Reduced feeding success and possible risk of starvation by regal fritillaries (death), or sublethal effects such as reduced growth rates and reduced fecundity of surviving adults	Somewhat confident	P. Hammond, personal communication, 2021
EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) – RESILIENCY			
Effects of Stressors: **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc)?*	Varies depending on sympatric species density and/or violet availability; population resiliency could be reduced via lower survival of regal fritillary larvae or reduced fecundity of adults due to sublethal effects	Highly Confident	Selby 2007, p. 33; P. Hammond, personal communication, 2021;

EFFECTS TO THE SPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	Has the potential to occur throughout range, but is not necessarily known to occur, or be problematic in all areas Eastern Subspecies: observational information at FTIG indicates that regal fritillary have mated with Aphrodite and Great spangled butterfly males	Highly Confident	Royer and Marrone 1992 p. 24; Barton 1995, p. 5; Participants in Regal Fritillary SSA Expert Workshops, personal communications, September 2021
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Not known to be significant on all populations, although occurrences of sympatric fritillary larvae occurring in higher numbers than regal fritillary larvae have been reported, particularly in eastern populations	Moderately confident	Barton 1995, p. 12; participants in Regal Fritillary SSA Expert Workshops, personal communications, September 2021; P. Hammond, personal communication, 2021
Effects of Stressors: **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Potential to impact populations, but not clearly documented & requires further study	Somewhat confident	Barton 1995, p. 12; Selby 2007, p. 33; P. Hammond, personal communication, 2021

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	Observations are anecdotal, effects are less clear. Unknown whether this has, is, or will have any affects beyond local scale; past/current/future issue	Low Confidence	Selby 2007, p. 33; participants in Regal Fritillary SSA Expert Workshops, personal communications, September 2021

d) COLLECTION

[ESA Factor(s): B]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Human collecting of regal fritillary specimens	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	Permanent removal of individual regal fritillaries from the wild	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	Collection may occur prior to breeding, precluding annual reproduction by collected individuals	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	Direct loss of individual in the population and subsequent loss of their potential reproduction to establish next annual generation	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36
- Exposure of Stressor(s) *Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to describe*	Collectors typically target adult butterflies in suitable grassland habitats, potentially interrupting mating, resting, summer diapause, feeding, or reproducing activities	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36

where geographically it is occurring, but where in terms of habitat.			
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Random and likely infrequent; typically occurs when adults are present in best form (June/July) but may occur anytime adults are present June-October; past/present/future	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36
Changes in Resource(s) *Specifically, how has(is) the resource changed(ing)?*	Removal of individual results in fewer individuals to reproduce; but may be less prevalent today than historically as some states require permits and have assigned protective status to the species	Moderately confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** • *What are the effects on individuals of the species to the stressor? (May be by life stage)* . The spectrum tables may help. • Spectrum of Adverse Animal Responses and Effects • Spectrum of Beneficial Animal Responses and Effects • Spectrum of Adverse Plant Responses and Effects • Spectrum of Beneficial Plant Responses and Effects	Possible sublethal effects in response to capture attempts: alarm, altered behavior, reduced feeding, displacement, avoidance. In the case of capture: mortality and likely prevents reproduction unless collected after September	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36
EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) - RESILIENCY			
Effects of Stressors: **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc)?*	Reduced population size and reduced population growth rate, albeit imperceptible in most cases as collection typically occurs at low levels	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36

EFFECTS TO THE SPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	May occur anywhere, rangewide	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Largely anecdotal information suggests the magnitude can rise to local population level only in rare cases where collection is high in populations already vulnerable due to small population size or other factors	Moderately confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36
Effects of Stressors: **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Not known to cause lowered redundancy or representation	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	Local effects only, and likely in rare cases	Highly confident	Schweitzer 1992a, p. 13; Selby 2007, p. 36

e) PESTICIDE USE AND DRIFT – HERBICIDES

[ESA Factor(s): A]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Agricultural use on crops or to improve range condition. For Glysophate, use pre-plant, during growth and pre-harvest. For pasture the use of 2, 4 D or Dicamba. Application may be aerial or ground-based, ranging from local application to landscape-level use.	Highly Confident	Bork et al. 2007, p. 1554; Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 531; Johnson et al. 2020, p. 2
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	Loss of nectar plants to glysophate Drift from spraying soybean aphids Spraying broadleaf weeds in grasslands	Moderately Confident	Bork et al. 2007, p. 1554; Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p.531; Johnson et al. 2020, p. 2
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	Loss of host and nectar plants up 1312 feett (400 meters) from field edges. Reduced survivorship and growth rates with sublethal doses of herbicides	Moderately Confident	Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 532; Cordova et al. 2020, p. 4
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	Both the loss of nectar and violet resources, as well as effects on individuals	Moderately Confident	Bork et al. 2007, p. 1554; Selby 2007, p. 36; Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 531; Sánchez-Bayo 2021, p. 5

- Exposure of Stressor(s) *Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.*	The stressor has multiple points of contact. Glysophate maybe applied as many as 4 times during the growing season, corresponding closely to larvae emerging from diapause, late instar larvae, newly emerged adults, and the female egg laying period.	Highly Confident	Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 531
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Stressor are varied. In parts of the range, cleaning grasslands of weeds to improve forage for cattle is uncommon to common. Applications of herbicides is an ongoing phenomenon occurring 4-5 times each growing season	Highly Confident	Bork et al. 2007, p. 1554; Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 531-533; Johnson et al. 2020, p. 2
Changes in Resource(s) *Specifically, how has (is) the resource changed(ing)?*	Reduces nectar and host plant availability up 400 yards from ag fields. Grassland weed treatments have and may affect entire pastures.	Highly Confident	Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 531-533; Johnson et al. 2020, p. 2

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage).* The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	Indirectly kills individuals through toxins (surfactants and adjuvants) or loss of host or nectar plants. At a minimum, reduces available habitat through drift from spraying. Labels suggest 33 meters (108 feet), but studies find reductions in insect biomass and diversity up to 400 meters (1312 feet).	Highly confident	Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 531-533; Cordova et al. 2020, p. 4; Sánchez-Bayo 2021, p. 5
EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) - RESILIENCY			
Effects of Stressors: **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc)?*	Control of weeds at the pasture scale to improve hay crops or livestock forage results in much lower forb diversity, killing larval host and adult nectar plants	Highly confident	Bork et. al. 2007, p. 1554; Sánchez-Bayo 2021, p. 5

EFFECTS TO THE SPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	For pastures, complete spraying rarely occurs annually, but the use of herbicides at the pasture scale is more common in eastern SD. Rarely used in Western SD. Pesticide drift due to agriculture is rare in western parts of the Northern Great Plains. Much more likely in Eastern of the following states ND, SD, NE and KS, as well as throughout MN, IA, IL, and WI.	Moderately Confident	Bork et al. 2007, p. 1554; Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 531-533; Johnson et al. 2020, p. 2; Sánchez-Bayo 2021, p. 5
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Pesticides could have a profound effect on the population, especially those in more agricultural matrices. The western edge of the range should remain relatively unaffected as long as row crop agriculture does not move in	Moderately Confident	Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 531
<u>Effects of Stressors:</u> **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Pesticides could have a large effect on Redundancy and Representation, especially in the Great Lakes region with smaller patches occurring close to agriculture	Moderately Confident	Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 531-533; Sánchez-Bayo 2021, p. 5

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	This stressor is important at all three levels. More so for populations occurring in areas with greater agricultural intensities	Moderately Confident	Thogmartin et al. 2017, p. 8; Stenoien et al. 2018, p. 531-533; Cordova et al. 2020, p. 4; Sánchez-Bayo 2021, p. 5

f) PESTICIDE USE AND DRIFT – INSECTICIDES

[ESA Factor(s): E]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Agricultural use on crops (corn, soy, wheat) at multiple stages. Seed coats, direct spaying, and genetically modified (neonicotinoids, chlorpyrophos, pyrethroids, BT) and mosquito control, and roadside herbicides	Highly Confident	Schweitzer 1993, p. 9; Thogmartin et al. 2017, p. 8; Krishnan et al. 2021a, p.1762; Krishnan et al. 2021b, p. 3
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	Spraying for spongy moths Drift from spraying soybean aphids or insects in corn	Moderately Confident	Schweitzer 1993, p. 9; Thogmartin et al. 2017, p. 8; Grant et al. 2021 p. 991; Krishnan et al. 2021a, p. 1762; Krishnan et al. 2021b, p. 3; Goebel et al. 2022, p. 151745-2
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	Direct mortality of larvae and adults from contact with insecticides. Reduced survivorship and growth rates with sublethal doses of insecticides. Grant et al. (2021) modeled drift from insecticides to establish monarch safe zones for milkweed in agricultural areas	Moderately Confident	Schweitzer 1993, p. 9; Thogmartin et al. 2017, p. 8; Grant et al. 2021, p. 991; Krishnan et al. 2021a, p. 1762; Krishnan et al. 2021b, p. 3
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	The direct and indirect toxicity from drift and systemic uptake of neonicotinoids in plant tissues/nectar	Moderately Confident	Schweitzer 1993, p. 9; Thogmartin et al. 2017, p. 8; Krishnan et al. 2020, p. 930; Krishnan et al. 2021a, p. 1762; Krishnan et al. 2021b, p. 3
- Exposure of Stressor(s)	Past use of neonicotinoids corresponded to early larval stages. Use of Chlopyrophos corresponds to female	Highly Confident	Thogmartin et al. 2017, p. 8; Krishnan et al. 2021a, p. 1762;

Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.	diapause and egg laying. Drift could occur to nectar flowers or grasses used for thermal refugia		Krishnan et al. 2021b, p. 1-3; Goebel et al. 2022, p. 6
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Applications of herbicides and insecticides is an ongoingphenomena occurring at least twice but as many as 4-5 times each growing season	Highly Confident	Thogmartin et al. 2017, p. 8; Krishnan et al. 2020, p. 930; Grant et al. 2021, p. 991; Krishnan et al. 2021a, p. 1762
Changes in Resource(s) *Specifically, how has(is) the resource changed(ing)?*	Insecticides kills larvae and adults on contact from 33 yards to 400 yards from ag field.	Highly Confident	Thogmartin et al. 2017, p. 8; Grant et al. 2021, p. 991; Krishnan et al. 2021a, p. 1762; Krishnan et al. 2021b, p. 3

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage)*. The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	Either directly or indirectly kills individuals through toxins Labels suggest 33 meters (108 feet), but studies find reductions in insect biomass and diversity up to 1312 feet (400 meters).	Moderately Confident	Grant et al. 2021 p. 991; Goebel et al. 2022, p. 6
EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) - RESILIENCY			
Effects of Stressors: **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc)?*	Control of spongy moth in PA resulted in the loss of an entire Regal Population at Gettysburg National Park	Highly confident	Schweitzer 1993, p. 9

EFFECTS TO THE SPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	Pesticide drift due to agriculture is rare in western parts of the Northern Great Plains. Much more likely in Eastern of the following states ND, SD, NE and KS, as well as throughout MN, IA, IL, and WI.	Moderately Confident	Thogmartin et al. 2017, p. 8; Grant et al. 2021, p. 991; Krishnan et al. 2021a, p. 1762; Krishnan et al. 2021b, p. 3; Goebel et al. 2022, p.
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Insecticides could have a profound effect on the population, especially those in more agricultural intensive matrices. The western edge of the range should remain relatively unaffected as long as row crop agriculture does not move in	Moderately Confident	Thogmartin et al. 2017, p. 8; Grant et al. 2021, p. 991; Krishnan et al. 2021a, p. 1762; Krishnan et al. 2021b, p. 3
<u>Effects of Stressors:</u> **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Pesticides could have a large effect on Redundancy and Representation, especially if the Great Lakes region.	Highly Confident	Thogmartin et al. 2017, p. 8; Grant et al. 2021, p. 991; Krishnan et al. 2021a, p. 1762

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	This stressor is important at all three levels, but at varying intensities throughout the western edge of the range	Moderately Confident	Thogmartin et al. 2017, p. 8; Grant et al. 2021, p. 991; Krishnan et al. 2021a, p. 1762; Krisnhan et al. 2021b, p. 3; Goebel et al. 2022, p. 6

g) GRASSLAND CONVERSION – AGRICULTURE

[ESA Factor(s): A]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Conversion of native grasslands to planted crops or other non-native vegetation	Highly Confident	Schweitzer 1992a, p. 12; Selby 2007, p. 32-34
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	Native grasslands are converted to other uses, resulting in removal of habitat and/or reduction in patch size, increased fragmentation and isolation.	Highly Confident	Selby 2007, p. 33; NatureServe 2021; Niemuth et al. 2021
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	Removal of shelter (exposure to elements, predators) for all life stages, of food for larvae (violets) and adults (nectar sources), and of breeding habitat for adults; reduced connectivity among suitable habitats (fragmentation, isolation), reduced vegetative diversity (monocultures of invasive plants)	Highly Confident	Selby 2007, p. 3, 32-34; NatureServe 2021
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	All required resources are affected: large, contiguous, diverse, connected native grasslands with warm season bunchgrasses/tussocks/litter, violets, nectar sources, shrubby/tall vegetation are removed or degraded Direct impacts to individuals in early life stages will occur if the species is present on the ground during development/conversion activities; required resources and conditions (large, connected patches, diverse floral resources) are lost immediately or later in time due to subsequent associated degradation	Highly Confident	Selby 2007, p. 33; NatureServe 2021

- Exposure of Stressor(s) *Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.*	Conversion activities typically occur spring-fall, coinciding with all life stages except overwintering 1st instar larvae. Any development in native grasslands has the potential to impact individuals present during the activity and affect (reduce or preclude) future generations after the activity.	Highly Confident	Selby 2007, p. 33; NatureServe 2021
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Past, present, & future. Native prairie conversion to agricultural began in the 1800s, is ongoing (at variable rates often driven by economics) and is anticipated to continue with exception of legally protected remnants.	Highly Confident	Selby 2007, p. 33; NatureServe 2021
Changes in Resource(s) *Specifically, how has(is) the resource changed(ing)?*	Resource availability has been significantly reduced from originally vast expanses of native prairie and continues to decline; quality of most areas is degraded to some degree with inappropriate disturbance regimes, invasive plants, fragmentation and isolation	Highly Confident	Selby 2007, p. 33

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage)*. The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	Mortality is highly likely for individuals present in early life stages within footprint of development activities Eventual mortality or stunting of any surviving individuals may occur due to loss of resources (shelter, food) during and after conversion activities Activities conducted during adult life phase will disturb/displace individuals, forcing dispersal to find resources elsewhere, possibly reducing individual fitness, or increasing individual mortality risk Mortality of adults remaining in/near disturbance area may occur due to related factors (e.g., pesticide use)	Highly Confident	Removal of habitat with regal fritillaries present will either directly kill individuals or will reduce resources available to individuals nearby.

EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) - RESILIENCY			
<u>**Effects of Stressors:**</u> **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc.)?*	Population sizes are reduced, or entire population(s) eliminated Reduced connectivity among/within populations and metapopulations, lowering genetic diversity and population health Reduced landscape-level access to shifting mosaic of resources, interrupting recolonization rates resources on landscape scale	Highly Confident	Williams et al. 2003, p. 11-12

EFFECTS TO THE SUBSPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	Western subspecies: risk to native grasslands is greatest in the Great Plains primarily because relatively more unprotected grasslands remain there. Less risk exists in the Midwest because less prairie remains to be impacted and many remnants are legally preserved. Eastern subspecies: range is completely within military base on SCAs not subject to typical conversion factors; woody encroachment and occasional small-scale disturbances may reduce habitat availability temporarily, but habitat loss via agriculture is not a significant factor for this population.	Highly Confident	M. Swartz,, personal communication, 2020-21; FTIG National Guard Training Center Integrated Natural Resource Management Plan 2021, p. F-10-F-16
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Depending on future economics or advances in agricultural practices, significant amounts of privately owned Great Plains grasslands currently harboring the western subspecies could be lost over time. Midwest and East are highly isolated and currently experiencing reduced genetic variability and vulnerability to stochastic/catastrophic events; if habitat loss in Great Plains increases to similar proportions, no portion of the range would be able to support the species on a landscape level scale.	Highly Confident	Royer and Marrone 1992, p. 26; Williams et al. 2003, p. 14; Selby 2007, p. 31, 33; NatureServe 2021
Effects of Stressors: **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Redundancy could be reduced significantly if habitat loss results in extirpation rates that outpace recolonization rates. Reduced representation would occur with lowered genetic and ecological diversity as populations become smaller, more isolated, and occupy fewer areas.	Highly Confident	Selby 2007, p. 31; NatureServe 2021

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	Habitat loss has likely been the most significant stressor to the regal fritillary at the species and subspecies level since the 1800s; it continues today and is anticipated to continue in the future. The species is a grassland specialist reliant on access to high quality native grasslands on a large scale – a resource that continues to be permanently lost, fragmented, and isolated. This precludes the species from accessing the resources it needs on the landscape level at which it functions, resulting in extirpations outpacing recolonizations and overall species decline.	Highly Confident	Selby 2007, p. 33; NatureServe 2021

h) GRASSLAND CONVERSION – DEVELOPMENT

[ESA Factor(s): A]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Urban development, energy projects, transportation projects, gravel mining	Highly confident	Selby 2007, p. 3
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	Grassland habitats are converted to other uses	Highly confident	Size/type of activities vary, sometimes incurs disturbance impacts to adjacent areas (e.g., road rights of way), but primary result the is the same: permanent removal of grassland habitat.
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	Direct and permanent loss of all required resources, preluding occupancy, potentially fragmenting habitat	Highly confident	Size/type of activities vary, sometimes incurring disturbance impacts to adjacent areas (e.g., road rights of way), but primary result the is the same: permanent removal of grassland habitat.
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	All required resources in the footprint of the activity - as well as any early life stages present on the ground - are eliminated. Connectivity between occupied areas may be reduced (fragmentation).	Highly confident	As occupied habitat is removed, all immobile occupants are removed with it.
- Exposure of Stressor(s) *Overlap in time and space. When and where does the stressor overlap with the resource*	Development can overlap in time and space with all resource needs of the species.	Highly confident	Development does not necessarily target grasslands, but can (e.g., wind projects). Not all grasslands are equally vulnerable

need of the species (life history and habitat needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.			to every kind of development, but none are excluded from risk unless grasslands are under complete protective status.
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Past/present/future. Majority of activities, particularly in northern areas, are initiated during warmer construction months, generally spring to fall. Thus, intact overwintering habitat may be least at risk of removal while occupied.	Highly confident	Development in western subspecies range is ongoing, can happen nearly anytime, albeit least likely in winter
- Changes in Resource(s) *Specifically, how has(is) the resource changed(ing)?*	Development reduces habitat availability and connectivity, increases fragmentation and isolation	Highly confident	Any action that removes native grassland has the effect of reducing habitat availability and connectivity

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage).* The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	Egg, larvae, pupa present in development footprint will suffer direct mortality Adults may suffer startle, alarm, displacement, and perhaps reduced feeding success or reduced reproductive success, and potential mortality (e.g., vehicle collision)	Highly confident	Any immobile life stages present when habitat is removed will be destroyed; adults would likely be able to escape
EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) – RESILIENCY			
Effects of Stressors: **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc.)?*	Small local populations could be eliminated. If only portions of populations are impacted, overall survival, growth and reproductive rates may be reduced, particularly the year in which construction occurs due to initial mortality. Subsequent lack of impacted habitat will result in displaced populations	Highly confident	Impacts to populations depends on their site-specific conditions (size of habitat, amount impacted, surrounding matrix, etc.)

EFFECTS TO THE SPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	Eastern subspecies: unlikely to be affected, population is limited to FTIG and managed on SCAs, not open to development Western subspecies: development could theoretically occur anywhere in the range. May be relatively more likely adjacent to existing developments with access (e.g., expansion of cities into countryside or along highways), but not the case with energy development which includes building new roads for access, or with transportation projects	Moderately confident	Local impacts are likely occurring annually throughout the western subspecies range; extent and level of impact vary widely
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Effects are most often at local scale, impacting individuals, not necessarily (but sometimes) populations; reducing overall habitat availability and connectivity, but not likely to significantly reduce resiliency at AU scale unless grasslands are targeted	Moderately confident	Local impacts are likely occurring annually throughout the western subspecies range; extent and level of impact vary widely
Effects of Stressors: **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Redundancy and Representation are not likely to be impacted by development; projects are usually small, at local scale	Moderately confident	Local impacts are likely occurring annually throughout the western subspecies range; extent and level of impact vary widely

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	Development can and does result in individual mortality, loss of habitat, and potential loss of populations – particularly those already small and vulnerable. However, it is not typically of a scale and extent that would impact resiliency and redundancy of either subspecies	Moderately confident	When it overlaps with native grasslands it eliminates habitat, but typically occurs on a local scale.

i) INVASIVE GRASSES AND WOODY PLANT ENCROACHMENT (SUCCESSION)

[ESA Factor(s): A]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Western subspecies: invasion of Eastern Red Cedar (*Juniperus virginiana*) as well as smooth brome (*Bromus inermus*) and Kentucky bluegrass (*Poa patrensis*) Eastern subspecies: woody succession with species from surrounding forested habitats	Highly Confident	Royer and Marrone 1992, p. 28;, Selby 2007, p. 33; M. Swartz, personal communication, 2020-21; Gaskin et al. 2021, p. 236-237
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	The introduction of invasive plants and woody encroachment may shade out violets, decrease bunchgrass cover, disrupt contiguous grasslands, alter disturbance regime, reduced patch size, fragmentation and isolation	Highly Confident	Selby 2007, p. 33; Bahm et al. 2011, p. 190; DeKeyser et al. 2015, p. 259; Swartz et al. 2015, p. 826; NatureServe 2021
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	May replace native bunch grasses with monoculture of turfgrasses. Removal of shelter (exposure to elements, predators) for all life stages, of food for larvae (violets) and adults (nectar sources), and of breeding habitat for adults; reduced connectivity among suitable habitats (fragmentation, isolation), reduced vegetative diversity (monocultures of invasive plants)	Highly Confident	Selby 2007, p. 3, 32-34; Swartz et al. 2015, p. 826; USDA Forest Service 2018, p. 2; Gaskin et al. 2021, p. 236-237; NatureServe 2021U.S.
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	All required resources are affected: large, contiguous, diverse, connected native grasslands with warm season bunchgrasses/tussocks/litter, violets, nectar sources, shrubby/tall vegetation are displaced, suppressed or degraded	Highly Confident	Selby 2007, p. 3; Swartz et al. 2015, p. 826; NatureServe 2021
- Exposure of Stressor(s) *Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat*	Invasive species likely occur in all or nearly all extant regal fritillary habitats; dominant in many; likely to preclude regal fritillary occupancy.	Highly Confident	Royer and Marrone 1992, p. 28; Bahm et al. 2011, p. 191; DeKeyser et al. 2015, pp. 256-

needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.			257; USDAU.S. Forest Service 2018, p. 2
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Past, present, & future; invasive species affect native grasslands throughout species' range	Highly Confident	Selby 2007, p. 33; Swartz et al. 2015, p. 826; NatureServe 2021
Changes in Resource(s) *Specifically, how has (is) the resource changed(ing)?*	Availability of native bunchgrasses, violets, nectar sources is reduced as monocultures of invasive grasses/woody plants displace native vegetation	Highly Confident	Cully et al. 2003, p. 994; Selby 2007, p. 33; Bahm et al. 2011, p. 191; Setter and Lym 2013, p. 158; DeKeyser et al. 2015, pp. 256-257

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage)*. The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	Reduced feeding success, reduced breeding habitat, perhaps reduced growth rates if violets become rare or lowered reproductive rates if nectar sources become rare, displacement	Highly Confident	Kral-Obrien et al. 2019, pp. 304-305
EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) - RESILIENCY			
Effects of Stressors: **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc.)?*	Lower reproductive rates, lower growth rates, change in distribution, population size decrease, extirpation, reduced connectivity among/within populations and metapopulations, contributing to lowered genetic diversity and population health, reduced landscape-level access to shifting mosaic of resources, interrupting recolonization rates resources on landscape scale	Highly Confident	Royer and Marrone 1992, p. 28; Williams et al. 2003, entire; USDAU.S. Forest Service 2018, p. 2

EFFECTS TO THE SUBSPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	Likely entire species range; both subspecies	Highly Confident	Royer and Marrone 1992, p. 28;. Bahm et al. 2011, p. 191; . . DeKeyser et al. 2015, pp. 256-257; USDAU.S. Forest Service 2018, p. 2; M. Swartz, personal communication, 2020-21; PADMVA 2021, p. F-15;
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Significant amounts of grasslands currently harboring the western subspecies may be lost over time, likely lowering resiliency of all or nearly all AUs. Eastern subspecies must be constantly managed to prevent woody encroachment, without it resiliency would be significantly lowered.	Highly Confident	Royer and Marrone 1992, p. 28; Selby 2007, p. 33; M. Swartz, personal communication, 2020-21; NatureServe 2021;PADMVA. 2021., p. F-15
Effects of Stressors: **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	If rates of spread remain unchecked, redundancy potentially could be reduced eventually, particularly in AUs with small, isolated habitats. Reduced representation would occur with lowered genetic and ecological diversity as populations become smaller, more isolated, and occupy fewer areas.	Highly Confident	Williams et al. 2003, p. 16; Selby 2007, p. 33; NatureServe 2021

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	Past, current and future. Invasive plants and woody encroachment are widespread and a constant pressure to native grasslands. Once established they are difficult to remove. In the west, many areas with invasives are entirely unmanaged and spread continues unchecked. In the east, continuous disturbance is required to limit forest succession and subsequent loss of eastern subspecies. Invasives potentially impact nearly all, if not all, habitats in the regal fritillary's range and are known to be increasing. This stressor has the potential to reduce species viability.	Highly Confident	Royer and Marrone, 1992, p. 28; Selby 3, 33; DeKeyser et al. 2015, p. 259; Swartz et al. 2015, p. 826

j) PERIODIC DISTURBANCES

[ESA Factor(s): A]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Natural and anthropogenic actions - primarily fire, haying, and grazing activities - that remove surface vegetation Eastern subspecies: fire (prescribed and from military activities) and physical ground disturbance (e.g., tanks) are the primary activities	Highly Confident	Fuhlendorf and Engle 2001, p. 626; 2004, p. 2; Ferster 2005, p. 11; Fuhlendorf et al. 2009, p. 2; M. Swartz,, personal communication, 2021
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	Grassland succession is forestalled or set back to an earlier stage. Specific responses depend on timing, frequency, duration, intensity of the action(s) as well as site characteristics. Positive results include removal of excessive thatch, impacts to woody vegetation and invasive plants, stimulation of native vegetative growth, and opening of the canopy to allow growth of violet and nectar plants. Negative results (typically coinciding with excessive duration/frequency/intensity) include long-term loss of grassland structure and biodiversity, invasive plant encroachment and loss of required violet and/or nectar resources. Additionally, individuals may suffer burning, trampling, exposure and experience immediate loss of shelter and/or food sources. Lack of disturbance may provide important temporary refugia, but results in succession long-term that renders habitats unsuitable for occupancy	Highly Confident	Royer and Marrone 1992, p. 26; Swengel 1996, p. 80; Swengel 1997, p. 12; Swengel 1998, p. 79; Fuhlendorf and Engle 2001, p. 626; Panzer 2002, p. 1304; 2004, p. 2; Swengel 2004 p. 3; Powell et al. 2007, p. 304; Fuhlendorf et al. 2009, p. 2; Caven et al. 2017, p. 201; McCullough et al. 2019, p. 12; ;
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal*	Complete or partial removal of surface grassland vegetation reduces or eliminates shelter and food resources. Intensity, frequency, timing, duration and site characteristics dictate the specific changes in environmental conditions. Generally, low to moderate activities (often used in combination) that are infrequent, alternately timed, patchily	Highly Confident	Schweitzer 1992a, p. 13; Swengel 2004, p. 3; Selby 2007, p. 33; Swengel and Swengel 2007, p. 273; Caven et al. 2017,

of nesting habitat, increased temperature, loss of flow.	distributed, and/or of short duration will preclude complete removal of resources and create beneficial habitat mosaics with refugia. Alternatively, high intensity, frequent, extensive, long-duration actions that denude the landscape reduce habitat suitability long term by removing/excluding shelter and food resources and precluding occupation. Complete lack of disturbance allows may temporarily provide important refugia, however, grassland succession that eventually results in overgrowth and loss of food sources (violets and nectar sources)		p. 201; Royer and Marrone, p. 26; McCullough et al. 2019, p. 9
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	Affected resources: bunchgrass tussocks, litter, shrubs or tall vegetation for shelter at all life stages; larval food (violets), adult food (nectar sources). Direct mortality may also occur	Highly Confident	Schweitzer 1992a, p. 13; Swengel 1996, p. 80; Swengel 1997, p. 12; Swengel 1998, p. 79; Panzer 2002, p. 1304; Powell et al. 2007, p. 304; Selby 2007, p. 33
- Exposure of Stressor(s) *Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.*	Activities may overlap in time and space with all life stages (and may not occur at all some years), but activities occur primarily (not exclusively) spring-fall, thus overwintering first instar larvae are least likely to be exposed to stressors. The adult phase is mobile and is less likely to suffer direct mortality from periodic disturbance stressors but may be affected by removal of shelter and nectar sources via activities conducted mid-late summer/early fall. Relatively immobile developing larvae and pupae are most vulnerable.	Highly Confident	Schweitzer 1992a, p. 13; Panzer 2002, p. 1304; Selby 2007, p. 32-34
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Past, present and future. Timing and frequency of disturbance events are variable by week, month, year, or may not occur with any regular frequency at all in some unmanaged areas. Fire typically occurs spring through fall due to thunderstorms or prescribed burning management objectives and may occur annually, rotationally, infrequently or not at all. Prescribed burning in Midwest/Northern Great Plains is often conducted in early spring to suppress coolseason grasses. Grazing may occur	Highly Confident	Mason 2001, p. 19; McCullough et al. 2019, p. 12 Many occupied habitats, particularly private lands, are working lands not purposefully managed for native prairie

	year-round, rotationally, or not at all and may vary by duration and intensity based on management objectives (or lack thereof). Summer-fall haying depends on vegetative growth which varies within and between seasons and/or management objectives. Other activities, such as brush removal, weed spraying, vehicular traffic, etc. generally occur randomly, opportunistically or occasionally per management goals. Eastern subspecies: colonies at FTIG are managed by periodic prescribed fire impact zone burns and experiences ground disturbance randomly via ongoing military activities		conservation/diversity; activities (and effects) may also be dictated by annual climate conditions (e.g. drought vs wet conditions may dictate timing/frequency of grazing or haying)
Changes in Resource(s) *Specifically, how has (is) the resource changed(ing)?*	Disturbances remove surface vegetation and litter completely or partially, exposing bare soil, opening grassland canopy, potentially setting back or killing shrubs, and stimulating native plant growth. When this occurs at varying levels at varying times on the landscape, and sometimes in combination (e.g. fire and grazing) a shifting mosaic of resources develops that benefits regal fritillaries. Conservation of native grassland ecosystems occurs via stimulated growth of native vegetation (violets, nectar plants, native grasses), removal of invasive plants; perpetuation of a shifting mosaic of successional stages on the landscape. Alternatively, negative effects may also occur; degradation of native grassland ecosystems via excessive, long-term vegetative removal, suppression of native plants and favorable conditions for invasives (e.g., heavy/season-long grazing)	Highly confident	Nagel et al. 1991, p. 148; Royer and Marrone 1992, p. 28; Selby 2007, p. 35; Fuhlendorf and Engle 2001, p. 626; 2004, p. 2; Fuhlendorf et al. 2009, p. 2; McCullough et al. 2019, p. 2

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage)* . The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	Adverse effects: eggs, larvae, pupae may be burned in fires, trampled by grazing animals, crushed by vehicles/heavy equipment, exposed to elements due to removed shelter, starved due to removal of food resources (violets for larvae), poisoned by herbicides. Adults may be displaced by loss of nectar sources. Disturbances tend to be fewer (but not absent) in winter when first instar larvae are present. Beneficial effects: conservation of native grasslands and perpetuation of violet/nectar sources promote growth of early life stages, survival at all stages, and reproduction by adults.	Highly Confident	Ferster 2005, p. 11; Selby 2007, p. 36-39; McCullough et al. 2019, p. 12

EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) – RESILIENCY			
Effects of Stressors: **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc.)?*	Resiliency may be reduced or improved, depending on frequency/timing/duration/intensity and site characteristics. Generally light/moderate activities, conducted periodically, patchily distributed with adjacent undisturbed sites improve resiliency via improved survival, increased reproduction, improved growth rates and overall perpetuation of a diverse mosaic of grassland patches on a landscape scale. This is most successful on large patches but can be accomplished on numerous small, but proximal patches. Alternatively, intense disturbances conducted long-term, too frequent, and/or over too much of an occupied area – particularly small, isolated patches – reduce resiliency via lowered survival rates, reduced reproductive rates, reduced population growth rates, or extirpations and overall degradation of habitat that does not support the species or makes it more vulnerable to other stressors	Highly Confident	Schweitzer 1992a, p. 12; Selby 2007, p. 36-39; Henderson et al. 2018, p. 45; McCullough et al. 2019, p. 14

EFFECTS TO THE SPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	Disturbances occur on every occupied habitat throughout the species' range; as without periodic disturbance, grasslands become degraded and unsuitable for regal fritillary occupancy. Disturbance activities are an inherent factor in conserving suitable habitat. Eastern subspecies: not currently subjected to haying or grazing; fire and physical disturbance from heavy equipment are primary activities.	Highly confident	Royer and Marrone 1992, p. 28; Ferster 2005, p. 11; Selby 2007, p. 37-39; M. Swartz, personal communication, 2021
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Magnitude is patch-size/isolation dependent: Small/isolated populations - improper timing, severity, intensity and/or frequency of disturbances can significantly reduce populations so they become vulnerable to other stressors (reduced resiliency), or entire populations may be extirpated Large/connected populations (and in some cases small/connected populations) - actions typically impact portions of habitats (not entire area or not all proximal patches) leaving refugia in adjacent sites and allowing recolonization of temporarily impacted areas when more favorable conditions return Lack of disturbance regime can be beneficial in the short term, but eventually results in reduction and eventual extirpation as severely overgrown areas are no longer able to support violets and nectar sources required by larvae and adults	Highly confident	Swengel 1996, p. 80; Swengel 1997, p. 12; Swengel 1998, p. 79; Swengel 2004 p. 3; Powell et al. 2007, p. 304; Helzer 2012, p. 9; Caven et al. 2017, p. 201; Henderson et al. 2018, p. 45; McCullough et al. 2019, p. 12

Effects of Stressors: **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Patch-size/isolation dependent: In areas dominated by small/isolated populations, improper disturbance regime can lower redundancy by lowering the resiliency of populations or eliminating populations entirely In areas with large (sometimes small)/connected populations, redundancy and representation are not likely to be permanently impacted by disturbance regime, even if improperly applied in some areas as temporary negative impacts are offset by beneficial effects to vegetation and recolonization can occur from adjacent areas In areas not subject to any disturbance regime, redundancy can be reduced over time as initial refugia becomes overgrown, eventually lacking required resources and becoming unsuitable for occupation.	Highly confident	Swengel 1996, p. 80; Swengel 1997, p. 12; Swengel 1998, p. 79; Powell et al. 2007, p. 304; Helzer 2012, p. 9; Caven et al. 2017, p. 201; Henderson et al. 2018, p. 45; McCullough et al. 2019, p. 12

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects? **Past, Current, Future.***	Disturbances have been, are, and will continue to be highly influential on grasslands and regal fritillaries, they are essential to maintaining healthy grassland ecosystems that the regal fritillary relies on. However, effects to the species may be positive or negative, temporary or permanent, and are dependent on intensity, duration, frequency, and extent of the disturbances as well as characteristics of the sites to which they are applied. These stressors have the capacity to negatively impact resiliency, redundancy, and representation if conducted without consideration of regal fritillary conservation, as well as the capacity to ensure populations thrive via improvement of the 3Rs.	Highly confident	Selby 2007, p. 37-39; McCullough et al. 2019, p.14

k) DROUGHT

[ESA Factor(s): E]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Climate (atmospheric circulation patterns)	Highly confident	Selby 2007, p. 37-39; Intergovernmental Panel on Climate Change (IPCC) 2022 https://www.ipcc.ch/report/ar6/wg1/downloads /report/IPCC_AR6_WGI_SPM.pdf; Wishart 2011
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	Precipitation levels are periodically reduced below normal levels for extended periods of time, available moisture is reduced, vegetative growth/phenology and development of individuals is negatively affected	Highly confident	Known phenomenon, occurs periodically, particularly in Great Plains, P. Hammond, personal communication, 2021
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	Food, shelter, moisture resources required by regal fritillary are reduced	Highly confident	P. Hammond, personal communication, 2021
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this stressor? Or is it a direct effect on individuals?*	Violets may senesce prematurely depriving larvae of food; nectar sources are reduced or unavailable to adults; grassland vegetation is stunted reducing shelter and litter for all life stages; desiccation risk increases	Highly confident	P. Hammond, personal communication, 2021 M. Swartz, personal communication, 2020-21
- Exposure of Stressor(s)	Could potentially occur in any season, affect all life stages. Extent may vary from local (e.g., county	Highly confident	Wishart 2011

Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.	level) to regional (e.g., Northern Great Plains). May occur on seasonal, annual, or multi-year basis		
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Part of a climate condition that occurs normally in grasslands - variable in frequency, length, severity, and timing. Anticipated to occur in future, likely more severe with warming conditions	Moderately confident	Wishart 2011
Changes in Resource(s) *Specifically, how has(is) the resource changed(ing)?*	Required food, moisture, shelter is reduced or eliminated	Highly confident	P. Hammond, personal communication, 2021

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage) .* The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	Eggs: desiccation, sublethal developmental impacts, mortality Larvae: desiccation, sublethal developmental impacts, starvation, mortality Pupa: desiccation, sublethal developmental impacts, mortality Adult: desiccation, reduced fecundity/fertility, starvation, mortality	Moderately confident	Lack of moisture and/or food pose risks to all life stages
EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) - RESILIENCY			
Effects of Stressors: **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc)?*	Range contraction via extirpations, lower abundance, slower growth rates; some AUs may become sinks (or at least not sources); northward shift in range possible	Moderately confident	Swengel and Swengel 2017, p. 19; NatureServe 2021

EFFECTS TO THE SPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	More likely to affect western subspecies; may be more severe in the western portion of the western subspecies range than in the eastern portion, particularly in southern and western portion of the Great Plains	Moderately confident	Zhang et al. 2021, p. 4-5
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Can significantly reduce resiliency of affected AUs, particularly in southern/westernmost (hotter/drier) portions of the range or in AUs already at risk due to isolation and small population size.	Moderately confident	Local extirpations due to drought have been observed, particularly in small, isolated sites; expected to continue and become more severe over time; P. Hammond, personal communication, 2021
Effects of Stressors: **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Redundancy may be reduced if drought is severe/prolonged enough; representative units may shrink in occupancy	Moderately Confident	Local extirpations due to drought have been observed, particularly in small, isolated sites; expected to continue and become more severe over time; P. Hammond, personal communication, 2021

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	Drought, exacerbated by increasing temperatures in the future, may be more likely, frequent, and lengthy, particularly in southern/western portions of the range. Potential to reduce abundance within AUs, cause AU extirpation, and range contraction. Possible northward range expansion. Sites already vulnerable due to other factors such as isolation are more susceptible to (possibly permanent) extirpation	Moderately Confident	Swengel and Swengel 2017, p. 19; NatureServe 2021; P. Hammond, personal communication, 2021; Zhang et al. 2021, p. 4-5.

l) LOCAL CLIMATE EVENTS AND CLIMATE CHANGE

[ESA Factor(s): E]	Analysis	Confidence / Uncertainty	Supporting Information
SOURCE(S) *What is the ultimate source of the actions causing the stressor? i.e., Urban Development, Oil & Gas Development, Agriculture*	Anthropogenic carbon emissions trapped in the atmosphere warm the planet and alter climatic conditions.	Highly Confident	IPCC 2022, p. 7
- Activity(ies) *What is actually happening on the ground as a result of the action? Be specific here.*	Temperatures are warming, precipitation amounts are changing, heat waves are becoming more severe and temperature extremes are occurring outside normal timeframes, stochastic storm events are becoming more frequent and more severe, reduced length of winter temperatures (earlier springs/later falls) and a potential for mismatch in phenology between host/nectar plants and larvae adult life spans	Highly Confident	Parmesan and Yohe. 2003, p. 38 Root et al. 2003, p. 58 Cohen et al. 2018, p. 225 Zografou et al. 2021, p. 1; IPCC 2022, p. 7
STRESSOR(S) *What are the changes in environmental conditions on the ground that may be affecting the species? For example, removal of nesting habitat, increased temperature, loss of flow.*	Drought-induced lowered availability of shelter and larval and adult food sources; increased temperatures affect individual development and reduce survival; milder winter conditions and early springs followed by cold conditions impact individual development and survival	Moderately Confident	Selby 2007, p. 18, 36; Nail 2016, p. 15; M.. Swartzpersonal communications 2020-21; Leslie Ries, personal communication, 2021
- Affected Resource(s) *What are the resources that are needed by the species that are being affected by this*	Drought induced early senescence of violets required for larval development, reduced or unavailable nectar sources, other vegetation stunted reducing shelter and litter. Direct mortality or sublethal effects may occur (e.g., stunted growth, reduced vigor, slowed development and increased	Moderately Confident	Selby 2007, p. 3;. M. Swartz, personal communications, 2020-21; P. Hammond, personal communication, 2021

stressor? Or is it a direct effect on individuals?	exposure to other mortality factors). Increased storm severity and frequency may cause more mortality via hail/rain (flooding)/wind. Irregular winter weather may interrupt winter diapause causing larvae to expend energy and decrease survival; predators (e.g., spiders) can become active in warmer temperatures and prey on larvae; early spring emergence followed by cold damp weather may cause mortality		
- Exposure of Stressor(s) *Overlap in time and space. When and where does the stressor overlap with the resource need of the species (life history and habitat needs)? This is not the place to describe where geographically it is occurring, but where in terms of habitat.*	All seasons, all life stages, potentially all habitats where each subspecies occurs	Highly Confident	Climate change is occurring globally, affecting all seasons and habitats
- Immediacy of Stressor(s) *What's the timing and frequency of the stressors? Are the stressors happening in the past, present, and/or future?*	Increases in temperature and precipitation variability were detected decades ago, are ongoing, and are likely to become more severe in the future if carbon emissions are not significantly reduced quickly	Highly Confident	Parmesan and Yohe 2003, p. 41 Root et al. 2003, p. 59 Cohen et al. 2018, p. 228
Changes in Resource(s) *Specifically, how has (is) the resource changed(ing)?*	Required environmental conditions and resources are becoming more erratic, less reliable. Temporal shifts are occurring, and severity of conditions is increasing	Highly Confident	; Parmesan and Yohe 2003, p. 38; Root et al. 2003, p. 58 Cohen et al. 2018, p. 225; Zhang et al. 2020, p. 1; Zografou et al. 2021, p. 1; IPCC 2022, p11-12

EFFECTS TO INDIVIDUALS			
Response to Stressors: **- INDIVIDUALS** *What are the effects on individuals of the species to the stressor? (May be by life stage).* The spectrum tables may help. Spectrum of Adverse Animal Responses and Effects Spectrum of Beneficial Animal Responses and Effects Spectrum of Adverse Plant Responses and Effects Spectrum of Beneficial Plant Responses and Effects	Egg: mortality (desiccation in heat/drought; drowning in floods) Larva: mortality (desiccation, drowning, energy expenditure during diapause); delayed development (resulting in increased exposure to other mortality factors); reduced feeding success (may stunt individuals) Pupa: increased mortality (desiccation, drowning slowed development, increased exposure to other mortality factors), stunted development. Adult: increased mortality, reduced feeding success, stunted development, reduced fecundity/fertility	Moderately Confident	Mason 2001, p. 17; Selby 2007, p. 36;; Selby 2007, p. 10, 32, 36; P. Hammond, personal communication, 2021
EFFECTS TO POPULATIONS (or ANALYTICAL UNITS) - RESILIENCY			
Effects of Stressors: **- POPULATIONS [RESILIENCY]** *What are the effects on population characteristics (lower reproductive rates, reduced population growth rate, changes in distribution, etc.)?*	Range contraction for western subspecies, reduced abundance; slower population growth rates; some AUs may become sinks (or at least not sources); northward shift in range is possible, potential extirpation of eastern subspecies	Moderately Confident	M. Swartz, personal communication, 2020-21; Participants in Regal Fritillary SSA Expert Workshops, personal communications, September 2021.

EFFECTS TO THE SPECIES – REDUNDANCY AND REPRESENTATION			
- GEOGRAPHIC SCOPE *What is the geographic extent of the stressor relative to the range of the species/populations? In other words, this stressor effects what proportion of the rangewide populations?*	Entire range affected, both subspecies. Western subspecies: more likely impacted by drought and high temperatures in the Great Plains and Midwet than in East Eastern subspecies: impacted by winter/spring temperature and precipitation changes affecting first instar larvae survival/overwintering success, likely less affected by drought		M. Swartz, personal communications 2020-21; P. Hammond, personal communication, 2021.
- MAGNITUDE *How large of an effect do you expect it to have on the populations and species overall?*	Western subspecies: potential extirpation of AUs possible - particularly in southern/westernmost (hotter/drier) portions of the range or in some Midwest AUs already at risk due to isolation and small population size. Eastern subspecies: could eliminate entire eastern subspecies with a single stochastic environmental event	Moderately Confident	Participants in Regal Fritillary SSA Expert Workshops, personal communications, September 2021
Effects of Stressors: **- SPECIES [REDUNDANCY and REPRESENTATION]** *Given the geographic scope and magnitude above, what is the effect of the stressor on the species, in terms of redundancy and representation?*	Western subspecies: redundancy potentially reduced in the future, representative units may shrink in occupancy Eastern subspecies: redundancy does not exist at population level – one event could result in extirpation of the only occupied AU/representative unit.	Moderately Confident	M. Swartz, personal communications 2020-21; Participants in Regal Fritillary SSA Expert Workshops, personal communications, September 2021.

SYNTHESIS: Individuals, Populations (Resiliency), Species (Redundancy and Representation)			
SUMMARY *What is the bottom line- is this stressor important to carry forward in your analysis, or is it only having local effects, or no effects?* ***Past, Current, Future.***	Climate change has been, is, and will occur, becoming more severe as temperatures increase in the future. Western subspecies: Severe and prolonged drought/heat (more likely in southern/western portions of the range, but possible in Midwest) has the potential to result in reduced abundance in AUs, AU extirpation, possibly northward range expansion and range contraction in the south. Sites already vulnerable due to other factors such as isolation are more susceptible to climate change stochastic events. Eastern subspecies: a stochastic event at the AU scale can be catastrophic for the eastern subspecies – the entire AU/representative unit (and with it, the subspecies) could be lost with a single environmental event (alternatively an event could weaken the population so as to make it vulnerable to extirpation from other factors). Ongoing changes observed regarding mild winters/altered spring conditions are expected to continue to occur.	Moderately Confident	Parmesan and Yohe 2003, entire; Root et al. 2003 entire; Zhang et al. 2020, p. 1; M. Swartz, personal communications 2020-21; participants in Regal Fritillary SSA Expert Workshops, personal communications, September 2021; . IPCC 2022, p. 11-12

Appendix I – Condition Category Table (Categorical Model for Resiliency)

FACTORS	**HABITAT FACTORS**							**DEMOGRAPHIC FACTORS**	
	NATIVE GRASSLANDS (Quantity and Quality)		**RIPARIAN/ WETLAND AREAS (Refugia)**	**AMBIENT TEMPERATURE**	**PRECIPITATION (Available Moisture)**	**LARGE CONNECTED BLOCKS OF NATIVE GRASSLANDS**		**ABUNDANCE**	**GROWTH TREND**
	Percent of AU that is native grasslands (surrogate: NLCD 2019 Herbaceous)	*Evaluation of existing native grass types, violets, diverse floral resources, shrubby/tall vegetation, vegetative litter, and grass tussocks*	*Percent of AU that is potentially suitable habitat (surrogates: NLCD 2019 Herbaceous, Pasture/Hay, Scrub/Shrub, Emergent Herbaceous Wetland) within 100 m (328 ft) buffer of streams and wetlands*	*Temperatures relative to key thermal tolerance of larval and pupal stages: percent of AU with Temperatures over 41 °C (105 °F) for 2+ days*	*Relative moisture supporting floral resources, individual health: spring and summer precipitation in mm (in) and number of droughts per decade*	*Patch size: percent of AU composed of patches sized 1,000+ ha (2,471 ac)*	*Connectivity: percent of AU comprised of grass patches within 5 km (3 mi) of 500+-ha (1,236+-ac) patches or within 3 km (1.9 mi) of 101-to 500-ha (247- to 1,236-ac) patches*		
DESCRIPTION OF THE NEED AND NOTES	Native grasslands provide specific habitat components for breeding, feeding, and sheltering for the two subspecies. The higher the percentage of native grasslands within an Analytical Unit (AU), the more resilient the AU.	Native grasslands with characteristics most closely resembling historical conditions are most resilient, particularly those harboring tallgrass vegetation (tallgrass prairie was historically the core of the western subspecies' range where the subspecies were likely most resilient). Native grasslands should be heterogeneous and include a sufficient quality and quantity of specific resource needs used by individuals including reliable violets, nectar sources, litter/tussocks, with some shrubby/tall vegetation.	Riparian corridors and wetland edges provide moisture that supports necessary violet and nectar sources during dry years and/or in relatively dry locations. These also may provide connectivity among suitable habitat patches, particularly in highly fragmented landscapes. The more of these resources in an AU, the more resilient it is, particularly during drought.	Ambient temperature is known to be important during larval & pupal stages; thermal tolerances are fairly consistent across Lepidopteran species during these stages. Exposure of larvae and pupa to temperatures over 38 °C (100 °F) may slow development, temperatures over 40 °C (104 °F) may increase mortality, and temperatures over 42 °C (108 °F) may result in complete mortality. Multiple days of extreme heat may reduce AU resiliency and limit the species' range.	Adequate and relatively reliable precipitation helps ensure resource availability and greater AU resiliency. Anticipated changes in spring and summer precipitation, as well as drought indices are primary factors considered here as these may impact individuals directly (desiccation) and/or indirectly (availability of violets and nectar resources).	Large patches (1,000+ ha [2,471+ ac]) of native grasslands typically represent the best suitable habitats and tend to support relatively larger populations long-term. AUs dominated with large habitat patches are more resilient than AUs with primarily mid-sized (e.g., 500-hectare ha [1,236-ac]) or small-sized (e.g., 100-ha [247-acre]) habitat patches. Similarly mid-size patch dominance is typically better than AUs dominated by small patches.	Adequate proximity of habitat patches promotes successful dispersal, allowing access to shifting resources, particularly in fragmented landscapes. As patch size decreases, more numerous and closely situated patches are needed to support population needs. Connectivity among suitable habitat patches within an AU may affect genetics and is an important factor in population persistence.	AUs need a sufficient number of individuals in order to withstand stochastic events and adequate abundance buffers against extirpation. This factor measures the contribution of abundance within each AU to the resiliency of the AU.	Resilient AUs exhibit positive growth trends under favorable conditions and some level of stability when conditions decline. This allows populations to persist and maintain ability to rebound when favorable conditions return. This factor is an assessment of abundance trends in each AU.
METRIC OR EVALUATION METHOD	*QUANTITATIVE. Percent of AU composed of NLCD 2019 (Dewitz 2021) Grassland/Herbaceous land cover is the surrogate for quantity of native grasslands. A threat-based model (Smith et al. 2016) is used to evaluate future availability via risk of conversion*	*QUALITATIVE. Species experts evaluated this factor qualitatively due to lack of quantitative means to reliably and consistently measure the quality of native grasslands rangewide and/or the specific habitat needs of individuals within these areas (e.g., number of available violets, presence of diverse floral resources). The quantitative descriptions of habitat components provided below are based on literature information and intended to improve the qualitative evaluation of this habitat factor.*	*QUANTITATIVE. Percent of AU composed of NLCD 2019 (Dewitz 2021) Grassland/Herbaceous, Scrub/Shrub, Pasture/Hay, Emergent Herbaceous Wetlands land covers, quantified within 100 m (328 foot) buffer of streams and wetlands (U.S. Environmental Protection Agency 2021).*	*QUANTITATIVE. Climate data and projections (Collins et al. 2008; Volodin et al. 2010; AORI 2016; Yukimoto et al. 2012) were used to measure portions of AUs that may exhibit at least 2 days of temperatures reaching 41 °C (105 °F) to help assess future conditions for this habitat factor; current temperatures are presumed to be adequate.*	*QUANTITATIVE. Climate models were used (Collins et al. 2008; Volodin et al. 2010; AORI 2016; Yukimoto et al. 2012) to evaluate precipitation availability and drought frequency of AUs, based on a location within each AU. Condition category criteria (below) were established separately for spring precipitation, summer precipitation, and droughts per decade and then applied to the climate model outputs for each AU. The associated condition category point values (0-5) for each precipitation factor were then summed and averaged to determine the overall condition category (same 0-5 point scale) for each AU.*	*QUANTITATIVE. Percent of AU composed of NLCD 2019 (Dewitz 2021) geospatial layers Herbaceous, Pasture/Hay, Scrub/Shrub, Emergent Herbaceous Wetland, that exist as patches sized 1000+ ha (2471+ ac) vs 100-1000 ha (247-2471 ac) vs <100 ha (< 247 ac)*	*QUANTITATIVE. Percent of AU composed NLCD 2019 (Dewitz 2021) Herbaceous, Pasture/Hay, Scrub/Shrub, Emergent Herbaceous Wetland), sized 500 ha (1,236 acres) and above that are within 5 km (3.1 mi) of other patches, and habitat patches sized 100-499 ha (247-1,233 ac) that are within 2 km (1.2 mi) of other similarly small patches.*	*QUALITATIVE. Species experts evaluated whether colonies, populations, or metapopulations are collectively adequate in number and size to be resilient and persist despite order-of-magnitude fluctuations in abundance. Determination of exact abundance required for persistence within an AU is difficult and subjective; data is lacking*	*QUALITATIVE. Species experts evaluated the general capacity of AUs to exhibit population growth. Determination of exact growth trends within an AU is difficult and subjective; data is lacking.*
VERY HIGH	≥51% of AU is composed of native grasslands	75-100% of AU grasslands are high quality, native tallgrass dominant (75% or more), and diverse with a heterogenous mosaic of successional stages. Vegetative litter/tussocks always available within the majority of patches at ideal level (several years buildup). Violets are highly abundant (e.g., 5 or more plants per 1 m^2 (11 ft^2 [50,000+ per 1 ha or 2.5 ac]) throughout AU. Diverse floral resources are always abundant and available. Shrubs/tall vegetation is available (without woody encroachment concerns) in nearly all patches.	Potentially suitable habitat within riparian and wetland 100-m (328-ft) buffer represents 16.1% or more of AU	<1% of area of AU exceeds 41 °C (105 °F) for 2+ days during spring and summer	spring precipitation ≥254 mm (10 in); summer precipitation ≥254 mm (10 in); <0.5 droughts/decade	≥81% of AU is composed of habitat patches sized1000+ ha (2471+ ac)	≥81% of AU is composed of connected habitat patches (within 3-5 km [1.9-3.1 mi])	Adults are abundant in nearly all populations throughout the AU in most years; approximately 25 per 1 ha (2.5 ac) or more and nearly ubiquitous throughout habitat; the AU is a consistent source for satellite areas within the AU or adjacent AUs	All populations in AU consistently exhibit exponential growth in good years and stable trend during poor years.
HIGH	26-50%	50-74% of AU grasslands are high quality (limited degradation), native, tallgrass dominant (~50-74%), and heterogenous with a mosaic of successional stages. Vegetative litter/tussocks are available in most habitats at ideal levels (several years buildup). Violets are generally plentiful (2-4.9 plants/m^2) in most areas. Diverse floral resources are abundant and available annually. Shrubs/tall vegetation is available (without woody encroachment concerns) in most patches.	8.1-16%	1-20%	spring precipitation 216-254 mm (8.50-9.99 in); summer precipitation 216-254 mm (8.50-9.99 in); 0.6-0.9 droughts per decade	61- 80%	61- 80%	Adults are abundant in most populations throughout the AU in most years; ubiquitous with ~10-24 individuals per 2.5 ac (1 ha) in good years; more patchily distributed and less common (5-10 individuals/ha) in poor years; the AU is a consistent source for satellite areas	Most populations in AU exhibit exponential growth in good years, and stable trend in poor years. Some smaller areas may be extirpated in poor years, but repopulation and growth happen quickly.

MEDIUM	11-25%	25-49% of grasslands in the AU are native, diverse, and high-quality mixed grass (25-49% tallgrass composition). On average grasslands are of moderate quality, generally a mix of heterogenous native grasslands and homogenous nonnative grasslands or with woody encroachment. Vegetative litter/tussocks may or may not be available in most patches - about as likely to be present as not (buildup may be limited in many habitats with less than 2 years buildup or excessive with a decade or more of no disturbance). Violets are available, but at relatively lower densities (1-1.9 plants/m^2) in most areas. Diverse floral resources may be widely available some years but limited in others. Shrubs/tall vegetation may/may not be available or woody encroachment (succession) may be occurring in a few areas to the detriment of native grasses and floral resources.	4.1-8%	21-40%	spring precipitation 152-216 mm (6.0-8.49 in); summer precipitation 152-216 mm (6.0-8.49 in); 1.0-1.9 droughts per decade	41-60%	41-60%	Adults are common to locally abundant in populations across some areas of the AU but absent in other areas most years; ~5-10 individuals per 1 ha (2.5 ac) in good years, 1-5 individuals per 1 ha (2.5 ac) or less in poor years - typically not a source for satellite areas	Populations in AU exhibit exponential growth infrequently. May provide some refugia and act as a source, but repopulation and growth of satellite areas is relatively slow.
LOW	6-10%	5-24% of grasslands in the AU are diverse, native, and high quality. Overall, grasslands are low quality, often homogenous, non-native, shortgrass dominant (5-24% tallgrass). Vegetative litter/tussocks are usually not adequate or available in most habitats (e.g., denuded or overgrown and rarely ideal condition). Violet density is low (0.5-0.9 plants/m^2) in most areas. Floral resources are not diverse or abundant and are a limiting factor most years. Shrubs/tall vegetation may either not be available, or woody encroachment (succession) may become dominant over grasslands in some areas.	2.1-4%	41-60%	spring precipitation 114-152 mm (4.50-5.99 in); summer precipitation 114-152 mm (4.50-5.99 in); 2.0-2.9 droughts per decade	21-40%	21-40%	Adults occur in low numbers within populations throughout the AU in most years; very few locally common/abundant populations exist; 1-4 individuals per 1 ha (2.5 ac) in good years, less than 1 individual 1 ha (2.5 ac) or absent in poor years; not a source for adjacent AUs, many areas may be sinks	Populations in AU typically do not exhibit exponential growth, even in good years. The AU does not act as refugia or source – repopulations are reliant on dispersers from adjacent AUs
VERY LOW	1-5%	< 4% of grasslands in the AU are native, diverse, and high quality; the AU is dominated by homogenous nonnative or low-quality habitats; <4% tallgrass composition or shortgrass dominant. Vegetative litter/tussocks are extremely limiting; almost never available at appropriate level (denuded or overgrown). Violet densities are very limiting; 0.9 plants/m^2 or less) in most areas. Floral resources are a limiting factor nearly every year. Shrubs/tall vegetation are either not available or woody encroachment (succession) dominates in many/most areas.	1.1-2%	61-80%	spring precipitation 76-114 mm (3.0-4.49 in); summer precipitation 76-114 (3.0-4.49 in); 3.0-3.9 droughts per decade	1-20%	1-20%	Adults typically occur in very low numbers within populations; uncommon to rare throughout the AU in most years; no locally abundant populations; less than 1 individual per 1 ha (2.5 ac) in good years, no individuals detected in poor years; most areas act as sinks	Populations in AU consistently exhibit little to no growth in most years, many are extirpated in poor years. Repopulation may take years, if it occurs at all. AU may be a population sink or may only harbor dispersing adults occasionally with few to no populations most years.
EXTIRPATED (X)	< 1%	No good quality grasslands present	≤1.0%	81-100%	spring precipitation <76 mm (3 in); summer precipitation <76 mm (3 in); ≥4.0 droughts per decade	<1%	<1%	Absent	Absent

Summary of Metrics used to Evaluate Conditions for each AU

Qualitative Methods for Abundance, Growth Trend, and Native Grass Quality

Lacking rangewide data and/or feasible means of obtaining data for native grass quality and population demographic factors of regal fritillary abundance and growth trends, we reached out to regal fritillary experts - as well as state representatives with expertise in either the butterfly, its habitats, or both - for qualitative evaluations of the habitat factor Native Grassland (Quality) and for demographic factors Abundance and Growth Trend. We held three virtual Regal Fritillary Expert Workshops, September 2, 9, and 16, 2021, and involved participants representing every state in the species current range. We provided background information on the species and our processes (e.g., ESA process, SSA process, and anticipated timelines), plus products developed by that time (e.g., SDM, potential factors affecting the species, condition category criteria). Workshop participants provided feedback on our products, and during the September 16 workshop, four breakout groups composed of experts with experience in each of our four representation units were asked to rank the factors for analytical units within their local area of expertise. An early draft of the condition category table with qualitative descriptors of all habitat and demographic factors was used as a tool for this initial ranking. We ultimately reduced the number of habitat factors in our final version of the condition category table, combining several individually ranked native grassland factors (e.g., violets, nectar sources, litter cover) into a single qualitative rank of native grasslands. The experts were asked to evaluate analytical units as a whole, rather than portions of the units where the species may be present.

Quantitative abundance measures within analytical units are lacking rangewide and are highly variable, thus qualitative descriptions of each condition category were required. Evaluation of abundance is based solely on the number of adults since determining numbers of eggs, larvae, or pupae is not realistic due to low detection probability. Individuals per hectare were garnered from literature resources that provided descriptions of abundance and patch sizes; this information is used as a guide for abundance in “good” years (when populations are relatively high in number) and “poor” years (when populations crash, as they are prone to do occasionally). Additional subjective descriptors include whether the species is usually rare versus ubiquitous within habitats.

As with abundance, quantitative data on growth trends of populations are not available rangewide, and this information is highly variable. Qualitative descriptions of each condition category are based on conditions described in the literature and by observations of populations and boom/bust cycles, such as whether exponential growth occurs, whether populations appear stable, if repopulations happen quickly, and if an area seems to act as a source or a sink.

Native Grasslands – Quantity and Quality

The percentage of NLCD (Dewitz 2021) Grassland/Herbaceous land cover in each analytical unit was the criteria used to rank native grassland quantity. A value of 51 percent or higher assigned to the Very High condition category and less than 1 percent assigned to the Extirpated category with percentages in-between distributed among the remaining ranks. In the absence of a rangewide native prairie geospatial layer, the Grassland/Herbaceous landcover is used as a surrogate with the assumption that this land cover captures the majority of native grasslands and is likely to harbor native violets, although we acknowledge this is not necessarily the case in all areas. Without violets, regal fritillaries cannot persist, regardless of all other factors. This layer may be an underestimate in some ways (e.g., the species may utilize other identified NLCD land covers, such as Scrub/Shrub) or an overestimate in others (e.g., grassland that is not native is likely included). Other NLCD layers were considered for these criteria, but seemed more problematic (e.g., Pasture/Hay data may include native grasslands in some parts of the range but is dominated by planted vegetation not likely to contain violets in other areas) or were specific to criteria we used to rank other habitat factors (i.e., Emergent Herbaceous Wetlands for the riparian/wetland area factor).

Due to these shortcomings, we also added a qualitative measure for this important habitat factor. Descriptors were used to characterize immeasurable variables, including the percentage of grasslands considered high quality, relative dominance of tallgrass species, presence of a shifting mosaic of successional stages, densities of violets, and the availability of diverse nectar sources, litter buildup, grass tussocks and tall/shrubby vegetation. The Very High category describes ideal conditions with more than 75% of grasslands in high quality conditions providing an abundance of food and shelter resources, while the Extirpated category is essentially devoid of them, with a range of descriptors corresponding to the remaining condition categories in between those extremes.

The area of native grasslands for each analytical unit was calculated in ArcPro using a combination of the analytical units shapefile and NLCD 2019 Land Cover (Dewitz 2021). The "summarize within" function in ArcPro was used to calculate the area within each analytical unit specifically for the "grassland/herbaceous" class within the NLCD 2019 data layer. The "grassland/herbaceous" class from NLCD 2019 was the surrogate used to depict "native grassland" expressed as a percentage within each analytical unit as described above.

Riparian/Wetland Areas (Refugia) Method

Moist riparian and wetland buffers often support floral resources more reliably than drier uplands and provide other vegetative types that facilitate adult butterfly dispersals to other habitats. The zone of moisture surrounding streams and wetlands can be highly variable in width, depending on many factors including topography, size of the waterbody, and adjacent land use. We used a geospatial layer developed by the U.S. Environmental Protection Agency delineating a 328-feet (ft), or 100-meter (m), buffer to streams and wetlands (U.S. Environmental Protection Agency 2021) to determine the boundaries of this habitat and identified the amount of NLCD 2019 (Dewitz 2021) land covers (Grassland/Herbaceous, Scrub/Shrub, Pasture/Hay and Emergent Herbaceous Wetland) existing within that zone that could potentially provide some habitat components at various life stages of the regal fritillary. Since this resource occurs in less abundance than uplands, the scale to rank this factor is smaller than for the native grasslands factor. The upper reach of the scale developed for riparian/wetland areas was established at 16.1% or more for the Very High condition category, 1% or less is in the Extirpated category, with the percentages in-between distributed among the remaining ranks.

As noted above, we adopted a geospatial layer developed by the U.S. Environmental Protection Agency: EnviroAtlas - Watershed Index Online Riparian Zone Mask for the Conterminous

United States (U.S. Environmental Protection Agency 2021) to quantify the Riparian/Wetland Area factor. This layer provided a 328-ft (100-m) buffer around streams and wetlands, likely capturing the majority of this relatively moist habitat. From within this buffer, we calculated the quantity, in hectares, of four NLCD 2019 (Dewitz 2021) land cover layers: Grassland/Herbaceous, Scrub/Shrub, Pasture/Hay and Emergent Herbaceous Wetland. The amount of habitat was then converted to a percentage of the larger analytical unit and ranked as

Ambient Temperature

Temperature thresholds specific to the regal fritillary are not known, but with a warming climate, high temperatures are anticipated to occur more often, in more areas, and for longer time periods. Thus, we looked to a heat threshold of 40 degrees Celsius (40 °C), or 104 degrees Fahrenheit (°F), known to cause mortality in a similarly sized species (the monarch; Nail 2016, entire) as a surrogate for regal fritillaries. Larval and pupal stages are most vulnerable to high temperatures, so we focused our analysis on the spring and summer development period. Our general method included examination of output from the following four global circulation models (GCMs): HadGEM2, INMCM4.0, MIROC5, and MRICGCM3 (Collins et al. 2008; Volodin et al. 2010; Atmosphere and Ocean Research Institute [AORI] 2016; Yukimoto et al. 2012). We used an intermediate carbon dioxide emission scenario that represents a continuation (not increase) of current carbon emissions projected to decline starting 2045 (representative concentration pathway 4.5 [RCP 4.5]) to estimate changes to future temperatures in order to count the number of 4.6 km by 4.6 km (2.86 mi by 2.86 mi) raster cells in each analytical unit with projected temperatures exceeding 40 ˚C (104 °F) for more than two days between April 1 and July 15 (during the vulnerable larval and pupal life stages) in a given year. We then calculated the percentage of cells within each analytical unit for which lethal temperatures occurred for at least two days within a given year to get the area over which we anticipate the temperature threshold will be exceeded. In the Very High condition category, fewer than 1% of those cells in an analytical unit meet those conditions, while if 81-100% of cells meet those conditions, the Extirpated condition category applied. The remaining percentages were distributed evenly in condition categories between those extremes.

To calculate the current conditions for this factor, the area of each analytical unit over which temperatures exceed 40˚C for more than two days between April 1 and July 15, we downloaded climate data from the Multivariate Adaptive Constructed Analogs (MACA) Climatology Lab (Abatzoglou and Brown 2012). The MACA Climatology Lab provides downscaled climate data from several Coupled Model Intercomparison Project 5 (CMIP5) climate models (Taylor 2012, entire). For our baseline, we averaged projections of the number of days exceeding 40˚C between April 1 and July 15 from 1971-2010 produced by the climate model INMCM4.0 for two reasons. First, the INMCM4.0 represents the least change to temperature and precipitation over time, and second, there are minimal differences between models estimating past climatic variables, and INMCM4.0 is an accurate reconstruction of the past climatic variables (Volodin et al. 2010). The dataset came in the form of a NetCDF file, which consists of "stacked" raster datasets. Each approximately 4.6 km by 4.6 km (2.86 mi by 2.86 mi) grid cell of the dataset contains the daily "tasmax," or maximum air temperature, 2 m (6.6 ft) above the surface of the Earth for a specific day. We used the raster package in RStudio to import the data as a raster brick, or a stack of the 106 rasters, with each raster representing one day between April 1st and

July 15th (Hijmans 2017; RStudio Team 2015). To calculate the total number of cells in a raster with tasmax values above 40˚C between April 1st and July 15th, we reclassified each raster, assigning all cells with tasmax values 40˚C or below a value of 0, and all remaining cells (i.e., cells with tasmax values above 40˚C) a value of 1. The result was a stack of 106 reclassified rasters, each containing cells with values of 0 or 1 indicating whether the tasmax was above 40˚C at that location. We can refer to the reclassified value of each cell as a “cell day.” Since each individual raster represents a single day, the maximum cell day value for any given cell is 1. We summed the rasters together to get the total number of cell days above 40˚C between April 1 and July 15th in a given year. The final combined raster for each year is the sum of all 106 individual rasters, and therefore, the maximum cell day value for any given cell in the resulting raster is 106, which would mean that every day between April 1st and July 15th has a tasmax above 40˚C at that location. We repeated this process for each year that made up the baseline time frame (1971-2010). For each year, we used the zonal histogram tool in ArcGIS Pro to calculate the number of raster cells within each analytical unit that represented locations with more than two days with maximum temperatures exceeding 40˚C. We calculated this as a spatial proportion by dividing by the total number of cells in the analytical unit and multiplying by 100. For example, if an analytical unit was made up of 100 cells, and the histogram indicated that 10 cells represented locations with two or more days with maximum temperatures exceeding 40˚C, the proportion of area that exceeded our temperature threshold is 10%.

Precipitation (Available Moisture)

Criteria to evaluate precipitation (available moisture) were formulated using the same GCMs applied to ambient temperature (HadGEM2, INMCM4.0, MIROC5, and MRICGCM3; Collins et al. 2008; Volodin et al. 2010; AORI 2016; Yukimoto et al. 2012) under the RCP 4.5. A centrally located city within each analytical unit was selected, and three values for each location were obtained as indices of moisture levels: 1) average spring precipitation, 2) average summer precipitation, and 3) the number of droughts per decade calculated using the standardized precipitation and evapotranspiration index (SPEI) averaged over the summer months (June, July, and August). Any summer month-averaged values at or below -1.5 indicates severe drought for that year, and an average number of droughts per decade was calculated for 1980 to 2019 by dividing all the summers meeting the severe drought definition by 40 years (Hegewisch and Abatzoglou 2021, North Central Climate Adaptation Science Center 2021). Spring and summer precipitation levels are important factors affecting key resources required by regal fritillaries, particularly violets and nectar sources, and droughts are known to negatively impact populations. To evaluate this factor, two different sets of criteria, in alignment with the same condition categories and point values shown in Chapter 5, Table 13 above were developed: one used to rank both spring and summer precipitation and one used to rank the number of droughts per decade. The scales for these criteria were developed on the assumption that precipitation and drought frequency values from midwestern tallgrass prairie localities within the core of the species’ historical range represent the Very High condition category. Progressively lower precipitation values and higher drought frequencies were used to establish criteria for the remaining condition categories. The precipitation (available moisture) factor in the condition category table is measured by combining the individual point values for spring precipitation, summer precipitation, and droughts per decade; calculating their average point value; and determining the appropriate condition category per the range of values shown in Chapter 5,

Table 13. See Appendix G for the precipitation values obtained from the climate models and the system used to rank the analytical units.

To calculate current conditions, we used data generated by 20 CIMP5 Model Mean.0 (Volodin et al. 2010) based on the historic (1971-2010) timeframe and developed condition category rankings based on midwestern locality data as described in section 2.8.2 above. These scales used the same condition categories and point values used for the overall condition category table. The resulting point values from these three factors were then summed and averaged for each analytical unit to obtain an overall precipitation value for the current conditions table. See Appendix G for climate data, ranking criteria, and condition category by analytical unit.

Large Contiguous Blocks of Native Grasslands Methods – Patch Size and Connectivity

Large contiguous native grassland patches and connectivity among habitat patches are key to healthy regal fritillary populations. To evaluate this factor, we used both patch size and the level of proximity among patches to characterize "connected" areas within each analytical unit. NLCD geospatial data (Dewitz 2019 and Dewitz 2021) again served as a surrogate in the absence of rangewide native grasslands information, though refinement of NLCD data was possible in some states using data from Neimuth et al. (2021); Grassland/Herbaceous, Pasture/Hay, Scrub/Shrub, and Emergent Herbaceous wetland land cover types were assessed, as these areas are presumed to provide habitat.

Condition category criteria for patch size is based on the percentage of land covers that are 1,000 ha (2,471 ac) or more in size. This size threshold is based on literature review (see Table 8 in Chapter 3; , patch sizes of reliable populations noted by Royer and Marrone 1992, p. 25; Swengel and Swengel 2017, p. 4; and J. Shuey 2015 personal communications); while small populations can and do occur in smaller grasslands, long-term persistence of this boom-and-bust species is most likely to occur in the largest, most diverse patches. The Very High condition category criteria require 81% or more of an analytical unit to be composed of areas meeting these type and size thresholds, less than 1 percent is the criteria representing the Extirpated category, and the remaining percentages are distributed evenly among the other condition categories.

We included large patches, as well as those below the 1,000-ha (2,471-ac) threshold, as we developed the condition category criteria for connectivity. We determined that small distances of 3-5 km (1.86-3.11 mi), could potentially be traversed relatively easily and quickly by adult regal fritillaries, perhaps daily, between/among small and large habitat patches. This proximity facilitates repopulation and mixing of individuals to help ensure long-term occupancy of even relatively small habitats. We used geospatial tools to identify the percentage of analytical units composed of the four selected NLCD landcover types in habitat patches meeting our established size and distance thresholds to develop our condition categories for this factor. Patches of 100-500 ha (247-1,236 ac), being relatively smaller, were presumed to require a shorter distance (3 km [1.86 mi]) between them to promote population persistence at these sites. For patches over 500 ha (1,236 ac), that distance was expanded to 5 km (3.11 mi) due to the inherent ability of the larger habitat patches to support long-term populations that wouldn't necessarily require highly frequent dispersals to ensure repopulation of smaller extirpated sites. The acreage of patches meeting these size and distance thresholds were combined and expressed as percentages within each analytical unit. The condition category criteria for this factor aligned with the same scale as that used above for patch size, with 81% or more of an analytical unit composed of areas meeting the thresholds, less than 1% is the criteria representing the Extirpated category, and the remaining percentages are distributed evenly among the other condition categories.

Potential grass areas were identified across the range of the regal fritillary using NLCD 2011 grassland/herbaceous, hay/pasture, shrub/scrub, and emergent herbaceous wetlands data that was refined using additional available grassland data, including Conservation Reserve Program data, for Colorado, Iowa, Kansas, Minnesota, Montana, Nebraska, North Dakota, South Dakota, and Wyoming, per Neimuth et al. (2021). This dataset did not cover the entire range of the regal fritillary, therefore NLCD 2016 (Dewitz 2019) grassland/herbaceous, hay/pasture, shrub/scrub, and emergent herbaceous wetlands data was used to identify potential grass areas in Arkansas, Illinois, Indiana, Missouri, Oklahoma, Pennsylvania, and Wisconsin, though this data was not further refined. Datasets were combined using the "mosaic" function in Arc Pro v2.6.3. Grass patches were established using the "Region Group" function with 8 neighboring cells, reclassified based on designated patch size classes, and buffered to create connectivity zone rasters (Table 1).

Table 1. Patch size classification, description, and buffer distance used to characterize connectivity within each analytical unit.

Patch Size Class	Description	Buffer Distance
< 100 ha (< 247 ac)	Patches less than or equal to 100 ha (247 ac)	NA
100-250 ha (247-618 ac)	Patches greater than 100 ha (247 ac) and less than or equal to 250 ha (618 ac)	3 km (1.9 mi)
250-500 ha (618-1236 ac)	Patches greater than 250 ha (618 ac) and less than or equal to 500 ha (1236 ac)	3 km (1.9 mi)
500-1000 ha (1236-2471 ac)	Patches greater than 500 ha (1236 ac) and less than or equal to 1000 ha (2471 ac)	5 km (3.1 mi)
1000+ ha (2471+ ac)	Patches greater than 1000 ha (2471 ac)	5 km (3.1 mi)

Raster data was coarsened to 60 m by 60 m (197 ft by 197 ft) to facilitate processing. The area of patches within all patch size classes residing within these connectivity zone rasters was calculated by analytical unit. That area was then divided by the total area of an AU to calculate percentage of connected grass area for each AU.

Appendix J – Note About Ongoing Genetics Research

During the summers of 2020 and 2021, the U.S. Fish and Wildlife Service's South Dakota Field Office coordinated with partners to collect regal fritillary genetic samples (a single leg per adult butterfly, standard sampling procedure with no affects to survival or resight (Marschalek et al. 2013, p. 3), approximately 30 samples per site) from areas within the Midwest (Minnesota, Iowa, Illinois, Indiana, and Wisconsin), Northern Great Plains (South Dakota) and Southern Great Plains (Nebraska, Kansas and Missouri). The objectives were to measure changes in genetic diversity for the species as a whole and evaluate differences between the Great Plains populations and Midwest populations, investigating whether populations have lost diversity in the nearly 20 years since (Williams et al. 2003, entire) conducted his analysis. The data, once analyzed, will allow an assessment of overall genetic diversity and connectivity within and between regions. Additional samples may be collected in 2022 from additional portions of the range, particularly North Dakota. Potential morphological differences in regal fritillaries within the Northern Great Plains and Central Great Plains representation units have been observed, prompting other ongoing genetic studies as well (P. Hammond, personal communication, 2021). As of this writing, results are not available; we expect to update future versions of this SSA report with this information when it becomes available.