

Florida Grasshopper Sparrow Disease Risk Analysis

November, 2018



Florida Grasshopper Sparrow Disease Risk Analysis

28 – 29 November, 2018
White Oak Conservation
Yulee, FL

Workshop Report

9 April, 2019

Workshop Organization

White Oak Conservation

Workshop Design and Facilitation

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Florida Grasshopper Sparrow Disease Risk Analysis

Executive Summary

Introduction

The Florida grasshopper sparrow (*Ammodramus savannarum floridanus*, FGSP) is a non-migratory subspecies endemic to dry prairie habitats of south-central Florida. It has been listed as state endangered since 1977 and federally endangered since 1986. Populations have declined at all three major documented breeding sites on public land, including Three Lakes Wildlife Management Area, Kissimmee Prairie Preserve State Park, and Avon Park Air Force Range. The primary causes of this decline have not been fully identified, but known and potential threats to the species include: habitat loss, altered fire regimes, altered hydrology, grazing and land use effects, increased predation pressure, and introduced species. If the Florida grasshopper sparrow continues to decline, it may become extinct in less than a decade.

Disease is perceived to be a potential threat to the continued survival of wild FGSPs, although little is known about the prevalence and intensity of parasites and other pathogens that might affect them. There is some existing evidence regarding the disease threat – parasites that have been found in free-ranging FGSPs include ectoparasites (ticks, feather lice, and feather mites), helminths (*Mediorhynchus papillosum* and an unidentified microfilaria), unidentified fecal coccidia (probably *Isospora* sp.), hemoparasites (*Plasmodium* sp., *Leucocytozoon* sp., and *Trypanosoma* sp.), tick-borne hemobacteria (*Ehrlichia chaffeensis* and *Rickettsia aeschlimannii*, the latter found only from the gut of ticks that fed on FGSP), and mycobacteriosis (*Mycobacterium avium*).

The FGSP captive-breeding program began in 2015 at the Rare Species Conservatory Foundation (RSCF) and in 2016 at White Oak Conservation (WOC) to assist with species recovery. A higher-than-acceptable rate of morbidity and mortality occurred among captive birds during the 2016 and 2017 breeding seasons due to management, nutrition, and disease-related issues. Disease is a common problem in many captive bird populations due to higher densities and stress of birds in captivity. Therefore, steps were immediately taken to identify and manage disease in the captive facilities. Changes in husbandry practices led to a significant reduction in disease in the captive Florida grasshopper sparrow flocks, and no disease-related deaths were reported in 2018. From the management group's perspective, the most pressing infectious disease concerns affecting the captive population (and thus potentially impacting their release) at the present time are extra-intestinal coccidiosis (due to at least four genotypes of *Isospora*), coelomic filariasis (*Aproctella* sp.), and Eastern equine encephalitis (EEE). Based upon current screening and necropsy data, extra-intestinal coccidiosis is the leading infectious cause of morbidity and mortality in both captive flocks. It is currently unknown if the species of coccidia affecting captive FGSP also affect birds in the wild.

In 2018, the U.S. Fish and Wildlife Service and the Florida Fish and Wildlife Commission released a draft 5-year strategic vision that proposed release of captive-reared birds into extant wild populations to supplement wild populations in the face of rapid declines in abundance, while long-term solutions to the threats are developed. The draft strategic vision called for the development of a Disease Risk Analysis (DRA) process to assemble existing information and expert opinion to evaluate the risks associated with releasing captive-reared birds to existing wild FGSP populations in 2019. A workshop was conducted 28-29 November 2018 at White Oak Conservation near Yulee, Florida, facilitated by IUCN's Conservation Planning Specialist Group (CPSG). With over 30 years of experience in designing and facilitating species conservation planning workshops, CPSG has a long history of engaging the wildlife health community.

This includes the development of the joint World Organization for Animal Health (OIE) - IUCN Guidelines for Disease Risk Analysis, which formed the basis for this DRA workshop.

Due to the timing needs of the program, a Rapid Risk Assessment Protocol was followed for this workshop. This method does NOT include the development of a full risk model, but is aimed at combining existing data and expert opinion into a structured decision making process in an attempt to reach consensus around important decision points in support of management and policy decision making. Approximately 20 experts participated in the two-day workshop where consensus was reached in some places, and only convergence on others. This report captures this process and the outputs on the focal points. Assessing and predicting disease risk in wildlife is a dynamic problem with a great deal of inherent uncertainty. While development of a full set of detailed management recommendations to Federal and state agencies was not possible during this workshop, it is our hope that this document helps those decision makers navigate this problem in a world of significant uncertainty for the benefit of the program and the species.

The DRA considered the following fundamental question:

What is the likelihood that an individual Florida Grasshopper Sparrow, held or reared in *ex situ* (captive) conditions, is infected with a particular “high-risk pathogen”, is missed during *ex situ* health screening, and is subsequently released as an infected individual into an existing *in situ* (wild) population?

This question did not include the complete scope of the interest of the management group. In risk analysis language, it covered the risk of “release” of a pathogen via the release of captive birds. It did not fully consider the consequences to the existing *in situ* population(s) since that would entail a detailed understanding of (a) the likelihood of contact with other birds and (b) likelihood of transmission should contact occur. Answering this question in full was deemed a priority of the group through a more formal research project.

In the context of this workshop, a “high risk pathogen” was defined as a pathogen with high severity (resulting in mortality, morbidity), high potential transmissibility, high uncertainty (in consequences, diagnostics, or treatment), or low prevalence/absence in the wild populations.

Information Assembly

Initially participants considered a broad list of potential hazards, but quickly narrowed the focus of the analysis and accompanying discussion to two specific pathogens: filarial nematodes and coccidian protozoa. This was partially due to time limitations, but there was consensus that these were the two most urgent issues as applied to the risk of release in 2019. It was agreed that there was greater consensus around the risk and management of the other pathogens, and that a formal assessment of all pathogens could be facilitated by the working group at a later date. Selected workshop participants gave summary presentations on the nature of these pathogens, and the current state of knowledge regarding their prevalence and impacts in the two captive facilities and in the wild. The presentations and accompanying analyses of available data yielded the summary points identified below. Note that some workshop participants disagreed with the validity of selected assumptions; therefore, the assumptions listed here do not represent full consensus among all those discussing the available information.

Filarial nematodes

Facts

- The parasite has been confirmed to be present at White Oak Conservation (10 of 82 birds tested) and from two wild birds, based on analysis of both blood and tissue samples from birds in the two locations. Preliminary genetic analysis (short-sequence PCR) has classified these organisms as belonging to the genus *Aproctella*. Moreover, short gene region sequences from wild and captive *Aproctella* samples were an exact match, suggesting that the filarid in the captive population may be the same as that identified in the wild.
- While there is no direct treatment for birds infected with *Aproctella*, the prevalence of filarids in captive conditions can be reduced with effective mosquito control.

Assumptions

- Prevalence among wild FGSP is considered to be low due to dispersed distribution of birds in native habitat.
- Severity of filariasis in captive FGSP is low – medium, with impacts to wild birds likely to be less than in captivity.
- Transmission potential among captive birds is higher than in wild populations due to higher *ex situ* densities of both birds and insect vectors, although this potential can be reduced in captivity with targeted mosquito control (primarily through the use of insect netting in outdoor aviaries).

Coccidian protozoa

Facts

- Coccidia have been confirmed to be present in both captivity (White Oak Conservation (17 of 82 birds tested) and Rare Species Conservatory Foundation (sample sizes unknown)) and in the wild, based on analysis of both blood and tissue samples from birds in all locations. Preliminary genetic analysis has classified these organisms as belonging to the genus *Isospora*.
- It appears that there are several strains of *Isospora* in the wild and captive samples. The presence and spatial distribution of specific genotypes in the wild is presently unknown.
- Infection is persistent among captive birds in the absence of effective treatment, leading to high rates of transmission in higher-density captive environments. The specific relationships between bird population density and disease transmission rates and disease severity are unknown.

Assumptions

- Severity (defined in this case as likelihood of spread) of coccidia infection in captive FGSP is high, particularly in younger birds, while presumed to be lower in wild environments because lower population densities may reduce pathogen transmission rates. Severity in the wild is unmeasured and therefore unknown.
- Application of a coccidiostat to captive birds (younger birds under stress and adult birds during the breeding season and, at Rare Species Conservatory Foundation, prophylactically to all year-round) can be effective in suppressing disease expression and reducing the rate of pathogen shedding, thereby significantly reducing the risk of severe disease in *ex situ* populations.

Pathogen Risk Analysis

At the conclusion of these discussions, workshop participants were asked to provide their expert opinion regarding the relative risk that these pathogens posed to the proposed FGSP release program in the context of the primary question outlined above. The following key points/opinions emerged from this discussion:

- Filarial Nematode: Ninety percent (18 of 20) of the participants concluded that the release of captive-reared birds that may be infected with filarial nematodes does not pose a significant threat (low-medium) to existing wild populations, provided there is some effort at mitigating the prevalence of this pathogen in *ex situ* environments; 10 % categorized the risk as high. No confidence estimate surrounding these opinions was captured.
- Coccidiosis: Ninety percent (18 of 20) of the participants categorized the risks associated with releasing a captive bird infected with *Isospora* sp. to the released individual as low or medium. Two of the attendees categorized the risk to the individual as high. Most participants (18 out of 20) categorized the confidence in their assessment of this risk as medium (10) or high (8). Additionally, most participants (18 of 20) categorized the risks associated with releasing a captive bird infected with *Isospora* sp. to the wild FGSP population as low. One of the attendees categorized the risk to the wild population as high. Most participants (17 out of 20) categorized the confidence in their assessment of this risk as medium (12) or high (5).
- Introduced unknown pathogens: There is great concern over the completely unknown risk that captive birds will, in the future, contract a novel pathogen that adversely affects this program. Most workshop participants agreed that current biosecurity protocols at captive facilities make the likelihood of captive birds obtaining a novel pathogen relatively low, although the risk is not zero.
- The risk of doing nothing: Deciding against releases in 2019 likely outweighs the risks associated with the impacts of high-priority pathogens in terms of population/species survival: analysis to date suggests that, if no releases of captive-reared birds are conducted beginning in 2019, there is a high probability of extinction of the Florida Grasshopper Sparrow in the wild within the next decade.
- Despite the fact that most participants characterized the risk of release to the wild population as moderate or low, they still concluded that the precautionary principle should guide release decisions because release of captive-reared birds to the wild with no regard to health status or the risks of pathogen introduction to wild birds is unacceptable on the basis of both individual bird health and overall FGSP population stability.
- Some participants strongly disagreed with the majority views of pathogen risk summarized above, noting in particular that the taxonomic identification of pathogens in the wild is unknown and may be different from those that have been identified in the captive-rearing facilities. Furthermore, the pathogenesis of these organisms is unknown which may enhance the risk to the species.

Preliminary analysis of the management pathways of birds currently housed in the two captive facilities led to identifying the following key points/recommendations related to health risk management.

- Quarantine/Isolation protocols: Quarantine and isolation are specifically defined risk-management strategies aimed at minimizing the likelihood of disease transmission among susceptible individuals. As currently managed, there is no formal quarantine/isolation period for these birds and the group suggests deleting the use of this term in the management strategy. The following “risk management strategies” were recommended:
 - Ex-situ standardized health screening: All birds should undergo standardized health screening (including data standards and management) prior to release, and the FGSP Working Group’s Health Team, in consultation with captive facility staff, will seek to standardize screening protocols to the extent practical.

- The FGSP Working Group's Health Team should determine a threshold microfilarid density that characterizes an individual bird as suitable for release.
- *In situ* pre-release – limited duration: The majority of workshop participants were in favor of recommending relatively short duration of residence (i.e., no more than a week) for birds occupying *in situ* aviaries immediately prior to their release. The preference for shorter duration in *in situ* enclosures was based on 1) reducing perceived risks associated with captivity (e.g., increased exposure to disease, interspecific aggression) and 2) the observation that most workshop participants considered the risk to existing wild populations from known pathogens to be moderate at worst. Therefore, participants viewed time in *in situ* enclosures as acclimation rather than as disease-related pre-release health observation.
- *In situ* pre-release – health screening: Workshop participants identified a need to determine how to handle birds that show obvious signs of illness while in the *in situ* aviaries before release. It may be possible to secure the assistance of a nearby wildlife rehab clinic facility and an associated veterinarian. Representatives from the Federal and State management authorities were tasked with evaluating this possibility and, if appropriate, to add this element to the current release protocol.
- Passive surveillance on avian mortality (pre- and post-release): The FGSP Working Group's Health Team is tasked with developing a carcass disposition protocol. In addition, workshop participants identified the need to bring a project pathologist on to the Health Team to standardize procedures for optimizing data collection from dead birds. This should be implemented in captive facilities as well as into post release monitoring protocols.
- These protocols, once established, will be available upon request to the Health Team chair, Erin Myers (see Appendix I for contact info).

Risk Characterization and Conclusion

The DRA workshop was designed and facilitated so as to provide an objective environment in which experts in Florida Grasshopper Sparrow disease ecology and epidemiology could discuss the relative risks of selecting birds managed in captivity for release to the wild. With this information, State and Federal trust agencies can then make a more informed decision about the feasibility of releases and the contributions they could make to FGSP recovery. These discussions focused on the fundamental question formulated by workshop participants (see page 8), defined in terms of the risk of releasing birds to the wild that may be infected with a high-risk pathogen that escaped detection in that individual and that, subsequently, posed a risk to other birds in the wild recipient population. In this light, the workshop was successful in achieving the goal set forth by those involved in process design and facilitation.

Based on the data and information assembled for this analysis, and the wide-ranging discussions making up the DRA workshop, a majority of participants agreed that the proposed release of Florida Grasshopper Sparrows to an existing wild population (likely at Three Lakes Wildlife Management Area), using birds currently housed at both *ex situ* facilities, should move ahead in 2019. This recommendation emerges from the following conclusions drawn by a significant majority of workshop participants:

- The two priority pathogens of concern – filarial nematodes of the genus *Aproctella* and coccidian protozoa primarily of the genus *Isospora* – are currently found in wild birds;
- In the case of coccidia, functional natural linkages between wild habitats and *ex situ* facilities through mechanisms such as passage of migratory birds significantly reduce the probability of the existence of a novel pathogen in the *ex situ* environment;

Biosecurity protocols currently in place among *ex situ* facilities greatly reduces the risk of introducing and maintaining a novel pathogen in the FGSP population.

The group recognizes that this recommendation is made in the presence of significant data gaps which add to the uncertainty of this assessment. However, while recognizing that pathogen risk to this program is NOT zero, it must be balanced against the estimated high probability of extinction of the remaining wild populations of Florida Grasshopper Sparrows within the next decade, despite ongoing attempts to mitigate the wide range of current threats responsible for the species' rapid decline.

As is evident in this report, the workshop participants were not unanimous in their conclusions and recommendations to the State and Federal management authorities. Some participants expressed significant concerns about the uncertainty around proper identification of pathogen strains/species currently seen in captive birds, the pathogenicity of those strains, and whether those strains/species represent novel threats to potentially naïve wild populations that would receive released birds. As with any risk analysis of this type, particularly those characterized by broad areas of uncertainty, decision-makers must digest the full range of available information, coupled with the weight of FGSP-specific evidence provided by appropriate experts, when deciding on a near-term course of action. Actions taken to improve the status of the species in the wild can be combined with complementary research activities to advance the collective body of knowledge of the role that these pathogens play in the population demography and ecology of the Florida Grasshopper Sparrow as discussed in our 5-Year Strategic Vision (see Appendix VI). Through this adaptive approach to population management, the long-term prospects for the species can be improved by iterative adjustments to evidence-based conservation activities – in captivity and in the field.

Florida Grasshopper Sparrow Disease Risk Analysis: Project History, Iterative Workshop Process, and Expectations

A Short Summary of Programmatic Pathogen Control Efforts

The Florida grasshopper sparrow (*Ammodramus savannarum floridanus*, FGSP) is a non-migratory subspecies endemic to dry prairie habitats of south-central Florida (USFWS 1999). It has been listed as state endangered since 1977 and federally endangered since 1986 (USFWS 1999). Populations have declined at all three major documented breeding sites on public land (Three Lakes Wildlife Management Area [TLWMA; 3,000 ha dry prairie], Kissimmee Prairie Preserve State Park [KPPSP; 19,000 ha dry prairie], and Avon Park Air Force Range [APAFR; 4,200 ha dry prairie]). The primary cause (or causes) of this decline have not been fully identified, but the species' life history likely increases its susceptibility to certain environmental and biological risk factors. Known and potential threats to FGSP include: habitat loss, altered fire regimes, altered hydrology, grazing and land use effects, increased predation pressure, and introduced species. If the Florida grasshopper sparrow continues to decline, it may become extinct in less than a decade.

Little is known about the prevalence and intensity of diseases and parasites affecting Florida grasshopper sparrows (Forrester and Spalding 2003). Diseases and parasites that have been found in free-ranging birds include ectoparasites (ticks, feather lice, and feather mites), helminths (*Mediorhynchus papillosum* and an unidentified microfilaria), unidentified fecal coccidia (possibly *Isospora* sp.), hemoparasites (*Plasmodium* sp., *Leucocytozoon* sp., and *Trypanosoma* sp.), tick-borne hemobacteria (*Ehrlichia chaffeensis* and *Rickettsia aeschlimannii*, the latter found only from the gut of ticks that fed on FGSP), and mycobacteriosis (*Mycobacterium avium*).

The FGSP captive-breeding program began in 2015 at the Rare Species Conservatory Foundation (RSCF) and in 2016 at White Oak Conservation (WOC) to assist with species recovery. A higher-than-acceptable rate of morbidity and mortality occurred in the captive-breeding program during the 2016 and 2017 breeding seasons due to management, nutrition, and disease-related issues. The most prevalent infectious disease concerns affecting the captive population at the present time are extra-intestinal coccidiosis (due to at least 4 genotypes of *Isospora*), coelomic filariasis (*Aproctella* sp.), and Eastern equine encephalitis (EEE). Extra-intestinal coccidiosis is the leading infectious cause of mortality in both captive flocks. It is currently unknown if the species of coccidia affecting captive FGSP also affect birds in the wild. Disease is a common problem in many captive bird populations due to higher densities and stress of birds in captivity. Therefore, steps were immediately taken to identify and manage disease in the captive facilities. Changes in husbandry practices led to a significant reduction in disease in the captive FGSP flocks, and no disease-related deaths were reported in 2018.

In 2018, the U.S. Fish and Wildlife Service and the Florida Fish and Wildlife Commission released a draft 5-year strategic vision that proposed release of captive-reared birds into extant wild populations to supplement declining wild populations while long-term solutions to the threats faced by FGSPs are developed. In an abundance of caution and as part of their due diligence, the trust agencies (U.S. Fish and Wildlife Service and Florida Fish and Wildlife Commission (Service and FWC, respectively)) agreed that a Disease Risk Analysis (DRA), as called for in the draft strategic vision, was needed prior to making a recommendation on whether or not to release sparrows onto the prairie in 2019. A structured, evidence-based DRA process to inform responsible decisions in the face of disease risks was held in November 2018. The DRA was facilitated by experts from the Conservation Planning Specialist Group of the International Union for Conservation of Nature's Species Survival Commission (IUCN – SSC).

The trust agencies had funded several disease studies in the past, and the preliminary results of these studies were expected in November 2018. The DRA was held as soon as possible after the studies

produced initial findings. The intent of the DRA was for the trust agencies to have a clear understanding of the most recent science with regards to the risk from disease:

- To individual birds slated for release;
- To bird populations on the prairie; and
- To capture information regarding the level of (and variance around) uncertainty associated with the risk and how it affected the various options for release.

The information presented at the DRA and summarized in this report was used by staff from the trust agencies to evaluate the pros and cons of various strategies with regards to captive-rearing and release. These strategies were further evaluated at the DRA and by the trust agencies in the context of the risks of not releasing birds, given the precarious state of the wild population and the estimated high probability of species extinction.

The FGSP Recovery Management Group generated a number of outstanding questions that ultimately provided the motivation for designing and implementing the DRA workshop:

- Identify what pathogens/diseases are of most concern to our group for transfer of birds between captive facilities, and what risk level is associated with each of those diseases.
Can we assign a risk level to each of these diseases for:
 - Wild FGSP exposed to released captive FGSP
 - Other wild birds exposed to released captive FGSP
 - Released captive FGSP exposed to wild FGSP
 - Released captive FGSP exposed to other wild bird species
- Is there a difference between the pathogens observed in captivity and the wild? In other words, are we seeing novel pathogens/infectious disease in the captive population?
- Are there standard genetic sequencing methods that may be implemented for our purposes?
- Do we need to know the species of pathogen prior to making a decision to move birds to the wild or between facilities? Or is knowing the genus sufficient? Define the acceptable taxonomic scope.
- If we are seeing novel pathogens, what is the risk to wild individuals and/or the wild population of FGSPs if the pathogen is introduced? What is the risk to other prairie species?
- Are we asking the “right” question to make our decision to release and exchange birds between facilities? Are there other factors that we could control that we should be considering?

At the beginning of the DRA the trust agencies made clear they did not expect a “silver bullet” elicitation or consensus from the workshop. The topic of disease is a difficult one, and the agencies expected variation in the expert opinions regarding the data, data gaps, the risks associated with disease, and how those data might inform a decision regarding potential paths forward. There were no “a priori” decisions made by the trust agencies’ leadership before the DRA. The full suite of potential decisions were on the table—ranging from releasing birds this spring to ending the program if the risk of releasing birds back onto the landscape was too great. The agencies acknowledged that the state of the science is, and would likely continue to be, incomplete. However, from a management perspective, the agencies would be making a decision shortly after the conclusion of the workshop based on the best available science at this time, and the analyses of risk with regards to the various strategies.

The risk from pathogens was just one of the factors the agencies would be considering with regards to moving forward with potential releases. It was a foundational and pivotal factor. However other considerations were evaluated using a weight of evidence approach, including bird behavior and ecology, rapid population declines and the risk of extinction, logistics and money, and other constraints.

Risk Analysis: An Iterative, Adaptive Process

Risk analysis is an inherently iterative process that consists of a structured framework aimed at facilitating the inclusion of available science/evidence into policy and management decision making. Thus, each iteration builds upon the one before it, increasing in sophistication from the rapid assessment through the steps of the analytical phase (Figure 1). Since most of these exercises are driven by decisions made in the “real world,” the first iteration often consists of a rapid assessment conducted by a “working group” of experts. When political will, urgency of action, time and funding allow, teams may progress (or enter upon first iteration in some cases) into the analytical phase where there is usually an expansion of the team of experts and a progression from more qualitative assessments toward the development of long-term standardized health monitoring programs in support of quantitative analytical risk models.

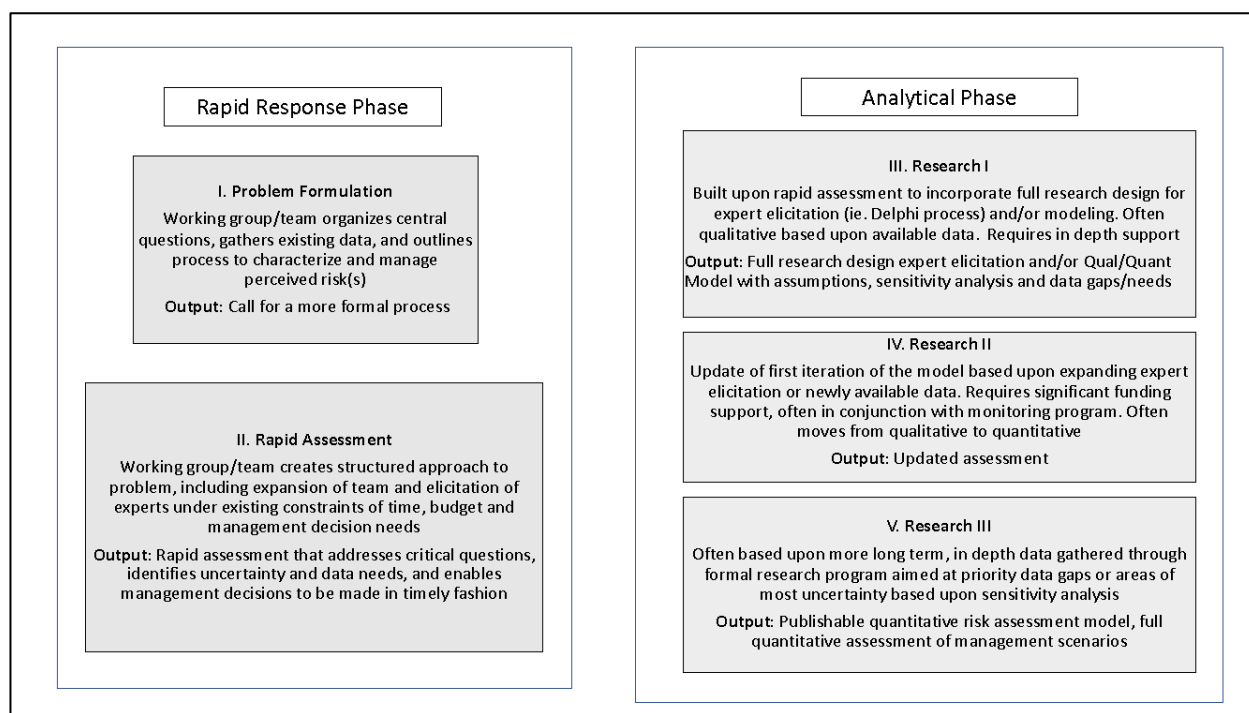


Figure 1. Diagrammatic representation of the progression of steps in a typical disease risk analysis (DRA) process.

From a technical perspective, the existing Florida grasshopper sparrow working group had previously conducted a thorough “problem formulation” exercise and first round rapid assessment that resulted in the identification of several critical points for further discussion and assessment. Thus, the 1.5-day FGSP DRA workshop was designed to: (1) present and discuss the current state of knowledge regarding several points of contention during the rapid assessment process, (2) clearly articulate the risks and associated uncertainty regarding the current state of knowledge with regard to disease, (3) assess/capture the level of consensus among the working group regarding risks associated with these critical points and capture level of risk tolerance around those points when consensus was not reached, (4) outline further data/research needs aimed at major data gaps, and (5) assess the level of consensus and risk tolerance regarding whether birds should be released in 2019 under current protocols.

No risk assessment process is completely clean or satisfying in the real world since at its core it is an attempt to gain consensus at the nexus of science and policy in the face of great uncertainty and the urgent

need for action. Thus, this report represents a hybrid process at the intersection of stages II and III. The editors of this report fully recognize the constraints of this hybrid process given the realities above. Under ideal circumstances, the trust agencies would be encouraged to coordinate a robust expert elicitation in the future to support the development of a full hazard identification and pathway analysis (risk assessment model), from birth to release, including post release monitoring health data. This next step – conducting the first iteration of an analytical risk assessment using the developed risk model – can occur even in the midst of ongoing species management in the wild. With each iteration comes an adaptive management framework based on more data and expert experience, which can only lead to cleaner assumptions and a better description of uncertainty. This, in essence, is the scientific method applied to real world problems.

Key Concepts of Disease Risk Analysis

(Extracted from Jakob-Hoff et al., 2014)

Risk

Risk is simply defined is the chance of encountering some form of harm, loss or damage. It usually has two components:

1. the likelihood, or probability, of something happening and, if it does happen; and
2. the magnitude of consequences of the deleterious activity.

Because of the element of chance, we can never predict exactly what will happen, and thus we start with the premise that there is no such thing as zero risk. Risk can be estimated in many ways, using differing kinds of data (qualitative, quantitative) and/or evidence (i.e., expert opinion), but is usually characterized by a description of the likelihood and magnitude of a prescribed outcome, with an accompanying description of (un)certainly.

Risk Analysis

“Risk analysis is a formal procedure for estimating the likelihood and consequences of adverse effects occurring in a specific population, taking into consideration exposure to potential hazards and the nature of their effects” (Thrusfield, 2007). It is a tool for decision makers to combine science and policy.

Disease

At the most basic level, disease is defined as any impairment of the normal structural or physiological state of an organism. The manifestation of disease is often complex and may include responses to environmental factors such as food availability, exposure to toxins, climate change, infectious agents, inherent or congenital defects, or a combination of these factors (Wobeser, 1997). Infectious microbes are a normal part of the ecosystem and thus disease plays an important role in maintaining the genetic health of populations and in regulating population numbers (Smith et al., 2009). However, in a highly disturbed environment, where significant and relatively permanent changes from earlier ecological states have occurred, disease may threaten the survival of an entire population.

Objectivity

Risk analysis, as for all rational treatments of decision problems, combines subjective and objective elements. On the one hand, the definition of risk is inevitably subjective as risk is always defined relative to the observer (for example, to the stakeholders of a recovery program and their objectives). This subjectivity is natural and, in fact, represents the reason for conducting the DRA. On the other hand, the analysis of risk and the evaluation of the consequences represent the “science” component of a DRA, and should seek to minimize its subjectivity through rigorous estimation. Both the subjective and objective components of the DRA are essential for rational decision-making. The

important aspect being the preservation of their independence ensuring, for example, objectives and estimates of consequences are not confused. For this reason, transparency in declaring all assumptions made is essential (MacDiarmid, 2001).

Acceptable risk

The risk-communication process is essential in helping decision makers to deal with one of the most difficult problems encountered during the risk-analysis process; namely, determining what constitutes an ‘acceptable risk’ (MacDiarmid and Pharo, 2003). Deciding whether or not a particular risk is acceptable depends on the objectives and risk attitudes of the involved stakeholders (MacDiarmid and Pharo, 2003; Thrusfield, 2007), and are often variable within a working group. It is common practice during Risk Analysis stakeholder workshops for participants to be asked to describe their “acceptable level of risk” *a priori* (or before the results), in order to establish independent thresholds for action (or inaction) unbiased by the results. This also further establishes the fact that attaining or requiring “zero risk” is not a viable option within this process.

Assumptions

A risk assessment may sometimes be criticized because some of its inputs are based on assumptions, especially where data are scarce and “expert opinion” is relied upon a great deal. However, all decision-making is based on assumptions, and uncertainty and subjectivity do not mean that valid conclusions cannot be drawn. Even though many of the inputs of a risk assessment are surrounded by uncertainty, one may be able to have confidence that the ‘true risk’ is unlikely to exceed the estimate resulting from a careful and conservative analysis (MacDiarmid, 2001).

Uncertainty

As in all complex situations not all the relevant facts are available when dealing with wildlife disease. As noted above, more often than not, available data are scant. Consequently, qualitative analysis is the most common approach used. A comprehensive literature review, the use of appropriate analytical and decision-making tools and the explicit recording of assumptions and limitations will ensure the best use of available information, identification of significant data gaps for further research and the level of uncertainty decision makers should take into consideration.

Method Used to Conduct this DRA

The process followed for this wildlife DRA generally follows that described by Jakob-Hoff et al. (2014) and Wolf et al. (in press). However, structural adaptations were made to the basic process to accommodate the timeline of the Management Group, and the scope of the uncertainty surrounding these issues. Thus, a rapid assessment process was employed, which relies upon expert opinion to fill significant data gaps when a conclusion must be reached and a decision must be made by managers or policy makers. The broad process is conceptually summarized in Figures 2 and 3, and forms the structure of the analysis that follows.

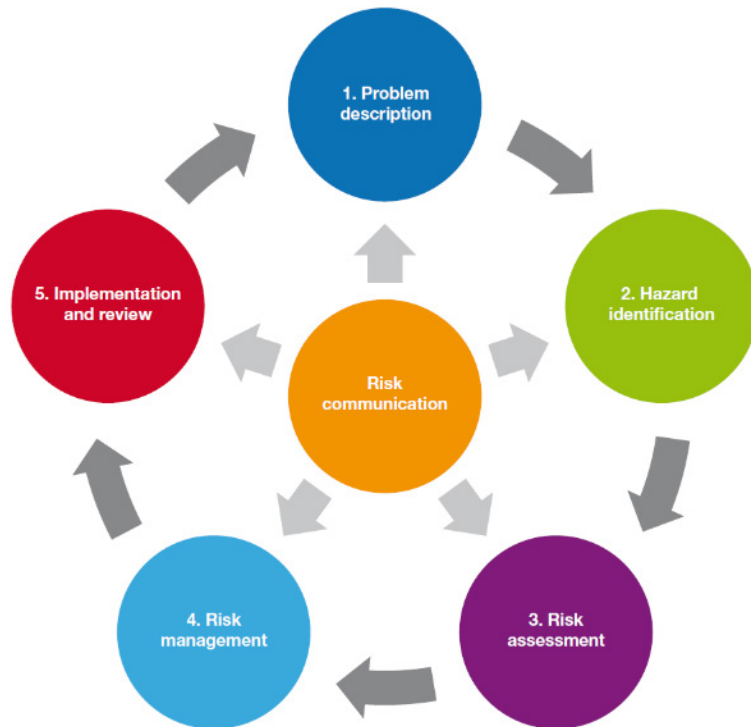


Figure 2. Process steps for disease risk analysis (DRA). From Jakob-Hoff et al. (2014).



Figure 3. Staged consultative process used to conduct the risk analysis for the Florida Grasshopper Sparrow.

The workshop enabled stakeholders to combine existing data with their expertise on critical points of the assessment, including hazard (disease) identification and prioritization, risk assessment and characterization, risk management, data gaps and uncertainty, as well as risk communication (this report).

DRA Context

The context of the disease risk analysis was formulated with stakeholders before the workshop and updated/appended during the process to meet the needs of the working group.

DRA Goal

The goal of this DRA process is to create an appropriate foundation, based on the principles and mechanics of disease risk assessment and management, upon which the trust agencies can make an informed decision on the feasibility of proposed releases of captive Florida Grasshopper Sparrows to the wild. The process is based on a structured, evidence-based analysis of currently available information, utilizing the knowledge and expertise of key specialists. This includes a summary of uncertainty and expert opinion where data are limited and there is great uncertainty. In other words, the goal is to present and discuss the full spectrum of available viewpoints and their implications for program design and implementation.

DRA Objectives

The overall objective of the DRA workshop process is a comprehensive assessment of the ramifications of disease introduction into and across the *in situ* FGSP population(s), as well as potential spillover to other at-risk species (i.e., release, exposure and consequences in risk assessment parlance), via the release of captive-reared birds in this program. Specific objectives on the agenda of this workshop and accompanying report, based upon the time available are:

- The creation of a transparent disease (hazard) prioritization process that may be used to prioritize known and unknown microorganisms with pathogenic potential for the FGSP.
- An assessment of “risk” regarding diseases currently perceived to be of highest concern by the management group. For this purpose, risk is defined as the potential barrier (direct or indirect) that a given disease poses to the successful implementation of the recovery program.
- Development of standard health risk management best practices to support this program.

DRA Scope

The scope, within the constraints of time and other resources, is confined to a qualitative analysis (often relying on expert interpretation of the available data) of relevant published and unpublished information on the susceptibility of FGSP to the two most pressing infectious disease hazards identified by the working group.

DRA Focus

The focus is the identification, assessment and mitigation of health risks associated with the captive-to-wild component of the breed/rear-for-release program for this species. This includes consideration of all birds across all institutions and introduction sites.

Problem Description

An important element in describing the problem for a disease risk analysis is defining the endpoint of the assessment. As a first step, a DRA may be focused only on the risk of pathogen release to the wild once potentially infected birds are moved from a captive environment to the wild. Additional consideration may be given to the risk of pathogen exposure among wild birds that come in contact with the released individuals that may be carrying the pathogen(s) of concern. Finally, the assessment may include consideration of the risk of negative consequences to those wild birds following exposure. Specifying the endpoint of a disease risk analysis is necessary in order to formulate a meaningful problem description that properly frames the subsequent steps.

Workshop participants discussed this issue and agreed that the primary endpoint for this DRA would be the release of potentially harmful pathogens to the wild environment following release of captive-held FGSP to their native habitat. All participants agreed that subsequent exposure of wild birds, with the potential for detrimental impacts following that exposure, was also important in the overall process of disease risk estimates. However, given time constraints for the analysis and key information gaps around high-priority pathogen parameters among wild FGSP (see below), emphasis in this initial analysis was placed on the likelihood of pathogen release.

The fundamental question (problem) for the FGSP disease risk analysis was then defined by workshop participants, as outlined below.

What is the likelihood that an individual Florida Grasshopper Sparrow, held or reared in *ex situ* (captive) conditions, is infected with a particular high-risk pathogen that is missed during *ex situ* health screening and is then released as an infected individual into an existing *in situ* (wild) population?

The group agreed that the analysis would focus on pathogens already known to exist in captive FGSP populations. In other words, the discussions would not broaden out to consideration of a wide array of pathogens not previously found in captive FGSP.

Acceptable Risk

The concept of acceptable risk for release of captive FGSP was discussed in terms of defining one or more unacceptable outcomes following the proposed release. These risks are weighed against the baseline scenario where no releases are implemented, i.e., a “do-nothing” scenario (recognizing, of course, that existing conservation measures designed to mitigate other threats to the species would continue). Given the precipitous rate of observed decline in the species’ abundance across its current range, workshop participants agreed that this baseline scenario features a high probability of species extinction in the wild, probably in the next 5 to 10 years – also an unacceptable outcome in the context of disease-risk management. Consequently, the predicted outcomes of the proposed release efforts, preceded by *ex situ* disease screening as well as post-release monitoring of the wild FGSP population(s), are to be compared to the baseline outcome of likely extinction in the wild within the next decade.

Primary release outcomes deemed unacceptable in the context of the recipient population include:

1. Releasing a novel pathogen to an existing FGSP population that then contributes to an accelerated decline of that population to extinction.
2. Releasing a novel pathogen to an ecosystem that negatively impacts other bird species.
3. No observed net recruitment among FGSP released into the wild by the first spring after release.

4. Releasing a bird from captivity that carries a given pathogen (that may not be identified in the wild) which then dies from associated disease.
5. A given threshold percentage (yet to be determined) of released birds die *in situ* of other diseases.
6. An unidentified pathogen (perhaps with unknown pathogenicity) is knowingly released onto the prairie, regardless of origin or degree of commonality between *in situ* and *ex situ* contexts.

Discussions on the definition of “novel pathogen” continued throughout the workshop. While there may be some precedent for the identification of a novel pathogen based on the extent of DNA sequence divergence between isolated pathogen samples, some workshop participants were not prepared to reach full agreement on a precise definition. Continued discussion of this topic, in conjunction with collection of data on pathogen distributions, both *ex situ* and *in situ*, are required before some resolution of this definition is feasible.

Outcomes #4 and #5 generated significant discussion around the feasibility of unambiguously determining cause of death among wild FGSP and, perhaps more importantly, birds in pre-release enclosures – assuming that dead individuals can be identified and collected early enough for successful post-mortem analysis. Post-mortem and cause-of-death analysis is vital and most tractable for pre-release birds in on-prairie enclosures. The likelihood of recovering telemetered carcasses is higher, offering the opportunity to gather important information on a subset of individuals. Workshop participants appreciated that successful collection and necropsy of pre-release and especially released birds without telemetry would be very difficult, thereby making these outcomes more difficult to monitor. Regardless of the likelihood of detection, there is a clear need for pre and post release standardized health screening and necropsy protocols to support the continued risk management best practices for this program. Likelihood of an event should not drive degree of preparation and planning for disease risk.

Additional outcomes also deemed unacceptable from the *ex situ* management perspective include:

7. The number of birds that can be produced in *ex situ* facilities and considered suitable for release (consistent with program goals) is insufficient through the impact of disease in those facilities.
8. Birds raised in an *ex situ* environment lose their suite of natural behaviors that contribute to *in situ* fitness.

The 5-Year Strategic Vision document (updated 26 November 2018) recommends that a total of 53 females are to be raised in *ex situ* facilities for release each year in order to at least maintain the wild population at its current abundance (estimated at approximately 23 breeding pairs). If *ex situ* disease prevalence and associated impacts prevents that level of production (outcome #7), the proposed release effort is unlikely to prevent extinction of the species in the wild. Additionally, as with outcomes #4 and #5 discussed above, outcome #8 may also be impractical to monitor following release, despite its declared importance among workshop participants.

Hazard Identification

Introduction

This is the phase of the Disease Risk Analysis process where the questions “what can go wrong?”, and “how can it go wrong?” are posed. Usually the discussion is disease-related, but could be any health issue, or even other risks to population survival depending upon the question. This consists of three pieces a) a list of all relevant hazards; b) a list of (prioritized) *ranking criteria* related to the outcome of concern; and c) the development of a tool (e.g., a decision tree) used to assess and rank the list in (a).

List of Relevant Hazards

An initial list of potential health hazards affecting FGSP, both in captivity and in the wild, was generated by workshop participants before the workshop. This list is provided in Table 1.

Table 1. List of potential health hazards affecting Florida Grasshopper Sparrows in both *ex situ* (captive) and *in situ* (wild) environments. The shaded portion of the table highlights the priority hazards identified by workshop participants.

Hazard	Attributed to Mortality?	Number of detections					
		<i>Ex situ</i>		<i>In situ</i>			
		WOC [‡]	RSCF	TLWMA	KPPSP	APAFR	Ranch
Filarial nematode							
<i>Aproctella</i> sp.	Yes	Yes					Yes
Unidentified microfilariae						1*	
Protozoa							
Coccidia (<i>Isospora</i> spp., <i>Atoxoplasma</i> spp., Extra-intestinal unknown)	Yes	Yes	9?	1**	1**		
<i>Leucocytozoon</i> spp.	No?	No					
<i>Plasmodium</i> spp.	No?	No		9	1		0
<i>Trypanosoma</i> spp.	No?	Yes					
Tick-borne bacteria							
<i>Ehrlichia</i> spp.	No?	Yes		0	0		0
<i>Rickettsia</i> spp.	No?	No		0	0		0
Bacteria							
<i>Mycoplasma</i> spp.	Yes	No			1***		
Arbovirus							
Eastern equine encephalitis	Yes	Yes		0	0		0
Other							
Tracheal / bronchial mites	Unknown	Yes					
Chewing lice	No	Yes					
Intestinal nematode (unident.)	Unknown	Yes					
Bile duct trematode (unident.)	No				2 [†]		

[‡] WOC, White Oak Conservation

RSCF, Rare Species Conservancy Foundation

TLWMA, Three Lakes Wildlife Management Area

KPPSP, Kissimmee Prairie Preserve State Park

APAFR, Avon Park Air Force Range

Ranch, Private Ranch

* From Forrester and Spalding (2003). The positive bird was from Polk Co., and therefore presumed from APAFR.

** Two positive birds from Ocala and Okeechobee Cos., and therefore presumed to be from TLWMA and KPPSP.

*** Noss et al. research project at KPPSP (2006 – 2008).

[†] Same bird that died from mycobacterial infection.

After plenary discussion, the workshop participants agreed that, given time constraints for conducting an informative DRA and the emphasis of past discussions on health hazards (pathogens) of concern to FGSP, the remainder of this analysis would be focused on filarial nematodes (primarily *Aproctella* spp.) and coccidian protozoa (primarily *Isospora* spp.) as the primary hazards for consideration in FGSP release scenarios. The group agreed on the value of conducting a more thorough analysis of all the health hazards listed in Table 1, but time constraints dictated that this more detailed analysis would have to be undertaken at a later date.

Hazard Assessment/Ranking Criteria

To establish ranking criteria for the potential hazards listed (Table 1), with an initial focus on filarial nematodes and coccidia, workshop participants were asked to first list criteria, and then rank them in order of importance. The following are presented in rank order, with the number of votes from the body of workshop participants given in parentheses:

- Mortality (17)
- Transmissibility (16)
- Morbidity (9)
- Fecundity (6)
- Emerging / Unknown (5)
- Difficult to diagnose/detect (5)
- No treatment options (3)
- Status in the wild (2)
- Carrier status in sparrows (2)
- Environmental stability [cross species transmission / mutability] (2)

Based upon this, four major categorical criteria were established during discussion:

1. Potential severity of disease (i.e., mortality, morbidity, fecundity, reservoir potential)
2. Transmissibility of the disease (infectiousness, environmental stability, etc.)
3. Level of uncertainty surrounding the disease (e.g., lack of knowledge surrounding consequences, diagnostics, treatment/prevention) (emergence or novel organism)
4. Presence in the wild

There was consensus on criteria #1-#3 above, while a great deal of discussion ensued regarding criterion #4. The discussion converged around the sense that novel diseases with great uncertainty, introduced during a reintroduction program, were higher priority for investigation (not necessarily higher risk) than endemic/normal/expected diseases for which more information is available. Thus, two general pathways emerged from the discussion (endemic and novel diseases) that will be considered in the Risk Assessment.

Hazard Assessment

Tables 2 and 3 on the following pages summarize the information discussed among workshop participants in their plenary analysis of the filarid and coccidian hazards considered high-priority in the context of disease risk for FGSP release to the wild. Information in these tables is presented, where appropriate, for both captive and wild populations.

Table 2. Summary information on the filarial nematode (likely *Aproctella* spp.) hazard for release of captive Florida Grasshopper Sparrows to an existing wild population. Text in red refers to information on the two *ex situ* (captive) facilities (White Oak Conservation and Rare Species Conservatory Foundation), while text in green refers to information on *in situ* (wild) populations (TLWMA = Three Lakes Wildlife Management Area). Asterisks refer to degree of confidence in the stated information among workshop participants: * low; ** medium; *** high.

Mortality	Morbidity	Fecundity	Potential for Carrier	Transmissibility	Uncertainty		Presence in Wild
					Diagnosis	Treatment	
White Oak***: Early stages: some deaths (10%); later, no deaths	White Oak***: Early stages: High Later stages: Very low			Considered to be higher than in the wild due to higher densities of both birds and insect vectors in captive conditions**		Low probability of effective treatment***	
Rare Species***: None	Rare Species***: None						
Low	Medium			Presumably low as wild FGSP don't aggregate in higher densities due in part to habitat characteristics*			Yes – presumed to be at low prevalence across wild sites***
(Based on samples from two birds; presumed to be low at the population level but can likely be mitigated*) Difficult to estimate in wild populations				Full suite of insect vectors is currently unknown			
				Transmission is expected to be higher in wet seasons**			

Table 3. Summary information on the coccidian protozoan (likely *Isospora* spp.) hazard for release of captive Florida Grasshopper Sparrows to an existing wild population. Text in red refers to information on the two *ex situ* (captive) facilities (White Oak Conservation and Rare Species Conservancy Foundation), while text in green refers to information on *in situ* (wild) populations. Asterisks refer to degree of confidence in the stated information among workshop participants: * low; ** medium; *** high.

Mortality	Morbidity	Fecundity	Potential for Carrier	Transmissibility	Uncertainty		Presence in Wild
					Diagnosis D _x	Treatment T _x	
Can be high without mitigation***	Can be significant, with poor condition commonly observed***		Persistent infection not uncommon**	Can be high, but impacts of infection are now reduced with mitigation**		Coccidiostat reduces shedding rate, can be a significant effect***	
	Potentially age-dependent?*		Coccidiostat reduces shedding rate, can be a significant effect***				
	Now reduced with proper mitigation***						
Probably low*	Probably low to medium*		Persistent infection likely not uncommon*	Presumably lower than in <i>ex situ</i> conditions as wild FGSP typically don't repeatedly aggregate at a single site in densities comparable to captive birds **		Primary management approach is dilution**	Yes*** – but specific genotype presence / distribution is unknown
Likely to be dose-dependent, and a function of habitat conditions, etc.*	Likely to be dose-dependent, and a function of habitat conditions, etc.*						

Hazard Assessment Summary and Conclusions

We can summarize the information presented in Tables 2 and 3 for the two primary pathogen hazards identified in this hazard analysis.

Filarial nematodes

Facts

- The parasite has been confirmed to be present at White Oak Conservation (10 of 82 birds tested) and from two wild birds, based on analysis of both blood and tissue samples from birds in the two locations. Preliminary genetic analysis (short-sequence PCR) has classified these organisms as belonging to the genus *Aproctella*. Moreover, short gene region sequences from wild and captive *Aproctella* samples were an exact match, suggesting that there the filarid in the captive population may be the same as that identified in the wild.
- While there is no direct treatment for birds infected with *Aproctella*, the prevalence of filarids in captive conditions can be reduced with effective mosquito control.

Assumptions

- Prevalence among wild FGSP is considered to be low due to dispersed distribution of birds in native habitat.
- Severity of filariasis in captive FGSP is low – medium, with impacts to wild birds likely to be less than in captivity.
- Transmission potential among captive birds is higher than in wild populations due to higher *ex situ* densities of both birds and insect vectors, although this potential can be reduced in captivity with targeted mosquito control (primarily through the use of insect netting in outdoor aviaries).

Coccidian protozoa

Facts

- Coccidia have been confirmed to be present in both captivity (White Oak Conservation (17 of 82 birds tested) and Rare Species Conservatory Foundation (sample sizes unknown)) and in the wild, based on analysis of both blood and tissue samples from birds in all locations. Preliminary genetic analysis has classified these organisms as belonging to the genus *Isospora*.
- It appears that there are several strains of *Isospora* in the wild and captive samples. The presence and spatial distribution of specific genotypes in the wild is presently unknown.
- Infection is persistent among captive birds in the absence of effective treatment, leading to high rates of transmission in higher-density captive environments. The specific relationships between bird population density and disease transmission rates and disease severity are unknown.

Assumptions

- Severity (defined in this case as likelihood of spread) of coccidia infection in captive FGSP is high, particularly in younger birds, while presumed to be lower in wild environments because lower population densities may reduce pathogen transmission rates. Severity in the wild is unmeasured and therefore unknown.
- Application of a coccidiostat to captive birds (younger birds under stress and adult birds during the breeding season and, at Rare Species Conservatory Foundation, prophylactically to all year-round) can be effective in suppressing disease expression and reducing the rate of pathogen shedding, thereby significantly reducing the risk of severe disease in *ex situ* populations.

Risk Assessment for Priority Hazards

After extensive discussion of this information, workshop participant experts were asked to assign a level of overall risk for each of these two hazards, in the specific context of risk to wild FGSP populations following release of captive-reared birds (e.g., with respect to the identified risk assessment question). For both hazards, participants noted that biosecurity protocols at both facilities combined with the known histories of birds brought in from the wild made it unlikely that diseases novel to the prairie were introduced to the captive flock.

Risk Assessment: Filarial Nematodes

For filarial nematodes, the results of this individual ranking exercise are found in Table 4.

Table 4. Individual scores among workshop participants relating to perceived risk to wild Florida Grasshopper Sparrow populations following release of captive-reared birds that may be infected with filarial nematodes (*Aproctella* spp.). See accompanying text for more information.

Degree of Risk	Number of Instances
Low	12
Medium	6
High	2

Based on this information, **a large majority (90%) of workshop participants concluded that the release of captive-reared birds that may be infected with filarial nematodes poses a Low to Medium threat to existing wild populations, provided there is some effort at mitigating the prevalence of filarids in *ex situ* environments. Notably, two participants identify this risk as High based on uncertainty in the group's understanding of the taxonomic identity and potential severity of this type of pathogen.**

Risk Assessment: Coccidian Protozoa

In the case of coccidian protozoa, a more detailed analysis of the participants' perceived risk was conducted in which risk was assessed separately for (a) an individual bird being released and (b) the recipient population as a whole. Moreover, workshop participants were asked to provide their level of confidence in their assignment of risk. [Unfortunately, this confidence assessment was not conducted for the filarial nematode analysis.] The results of this assessment are presented in Table 5.

Table 5. Individual scores among workshop participants relating to perceived risk to wild Florida Grasshopper Sparrow populations following release of captive-reared birds that may be infected with coccidian protozoa (*Isospora* spp.). Risks are assessed at two levels: the fate of individual captive-reared birds following release (columns 2-4), and the overall impact to the recipient wild population (columns 5-7). The degree of confidence in assessing these risks among workshop participants is included in the table. See accompanying text for more information.

Risk	Confidence in Assessment					
	Individual Bird Fate			Recipient Population Fate		
	Low	Medium	High	Low	Medium	High
Low	0	4	5	2	11	5
Medium	2	5	2	0	0	0
High	0	1	1	0	1	1

Overall, interpretation of the results in Table 5 indicate that **a large majority (90%) of workshop participants assess the risks – to the individual being released – associated with releasing a captive-reared bird that may be infected with coccidian protozoa as low or medium. Additionally, most participants assessed the risks – to the recipient population as a whole – associated with releasing a captive-reared bird that may be infected with coccidian protozoa as low.** Note that two out of the 20 participants identified both of these risks as high, with medium to high confidence in their assessment. This assessment is particularly noteworthy in the case of the risk to the recipient wild population, where the remainder of the participants assigned a low risk for the release of a potentially infected bird.

When considering infection with coccidian protozoa, these results indicate that a large majority of the workshop participants identify the short-term health and long-term success (recruitment) of released birds as the primary concern in the design, implementation and monitoring of a program featuring release of captive-reared Florida Grasshopper Sparrows to existing populations occupying suitable habitat. The data summarized in Table 5 suggests that there is comparatively less concern about the risk of population-level impacts of such a release program, although these risks are certainly not to be discounted. This result appears to come from two important conclusions that emerged from plenary discussions on the general characteristics of coccidia. Firstly, while at least some coccidia infecting captive FGSP have not yet been found on the prairie, likely due to difficulties in finding carcasses suitable for post-mortem analysis, this pathogen appears to be a rather common component of passerine populations in the area of interest. Secondly, experts in the *ex situ* management of species like FGSP commonly characterize coccidia infections as a “disease of captivity” owing to the consequences of artificially high bird population densities in *ex situ* environments. Thus, one would expect to find exposed birds as a rule, and achieving “freedom from coccidian parasites” may not be realistic. Risk is then based upon transmission more than prevalence of the parasite; risk management is then focused less on proof of parasite absence and more on husbandry, stress and treatment protocols.

One area of major uncertainty surrounds the diagnosis and characterization of this pathogen. As part of the overall DRA, this uncertainty manifests itself in the discussion of the novel pathogen pathway as a number of experts were concerned that this opens up the possibility for a greater degree of risk that novel diseases amplified in the *ex situ* environment could be released and negatively affect the recipient population and similar species. Alternatively, if genotypes are species-specific, this could limit the spread of infection among species – there are several potential pathways to consider and manage depending upon the taxonomic level one defines as a “positive case”. The workshop participants recognized the taxonomic uncertainty around the identification of specific coccidian pathogens known to exist in captivity. Some participants strongly argued that the specific genotypes of potentially fatal *Isospora* and other genera identified in captive-reared birds have not been detected in the wild and may not occur there. Other participants noted that a comprehensive effort to characterize all coccidia found on the prairie to ensure that no captive strains are novel would be time consuming and logistically challenging.

Risk Assessment: Transfer Pathway Analysis

When a thorough disease risk analysis is undertaken, we can utilize a graphical depiction of the pathway through which a bird will move, from the time it is hatched to the date on which it is released to the wild. This visual pathway model allows users to specify individual stages of residence along the pathway, the length of time that individuals are expected to reside in that stage, and potential points where pathogens are likely to be introduced to or transmitted among individuals of concern. These points are often referred to as critical control points (Jakob-Hoff et al., 2014) and represent places where disease mitigation efforts (and data collection) can or should be targeted to reduce risk of pathogen introduction, exposure, or negative consequences to exposed individuals.

Before the workshop, a preliminary transfer pathway diagram was constructed to serve as an initial tool for discussion among participants at the workshop itself (Figure 3). This simple pathway was based on the proposed release effort described in the Florida Grasshopper Sparrow 5-Year Strategic Vision Document (updated 10 September 2018). Important elements of this diagram include the timing of proposed health screenings, and the differential timing of transfers of fledglings (hatch-year or HY birds) and older adults (second-year or SY birds) to field aviaries prior to release to the wild. The separate release pathways comprise an adaptive management experiment to determine the relative success of releasing younger vs. older birds in terms of the capacity of *ex situ* facilities to produce individuals suitable for release, and their ability to survive and reproduce following release. In addition, the pathway diagrams explicitly identify the periods of time during which birds are actively held in outdoor aviaries in the *ex situ* facilities for pathogen screening (originally referred to as “quarantine”, although this was subsequently redefined in the workshop discussions), and for acclimation to the wild environment after transfer to *in situ* field aviaries.

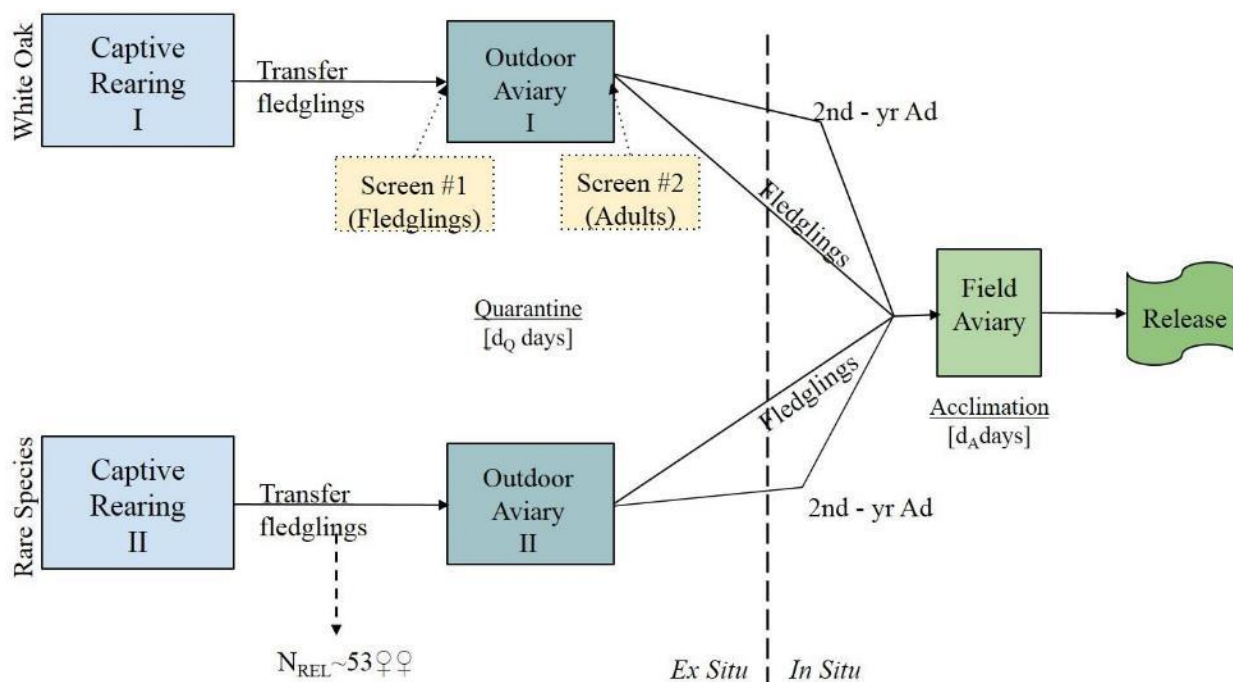


Figure 3. Proposed transfer pathway diagram for Florida Grasshopper Sparrows. Diagram based on proposed release effort described in the USFWS / FWC 5-Year Strategic Vision document (updated 10 September 2018).

Using this simple diagram as a starting point, each of the two institutions currently housing breeding FGSP populations – White Oak Conservation and Rare Species Conservatory Foundation – were asked to develop their own transfer pathway diagrams to provide specific details on their proposed methods for housing, screening and transferring birds over time. The two separate diagrams were combined into a single diagram to perhaps more easily compare and contrast the proposed approaches (Figure 4). Note that workshop participants agreed that birds slated for release would be reared at both captive institutions. This recommendation provides justification for developing some standardization of *ex situ* management and health screening protocols so that decisions regarding release candidates can be made under more effective coordination across institutions.

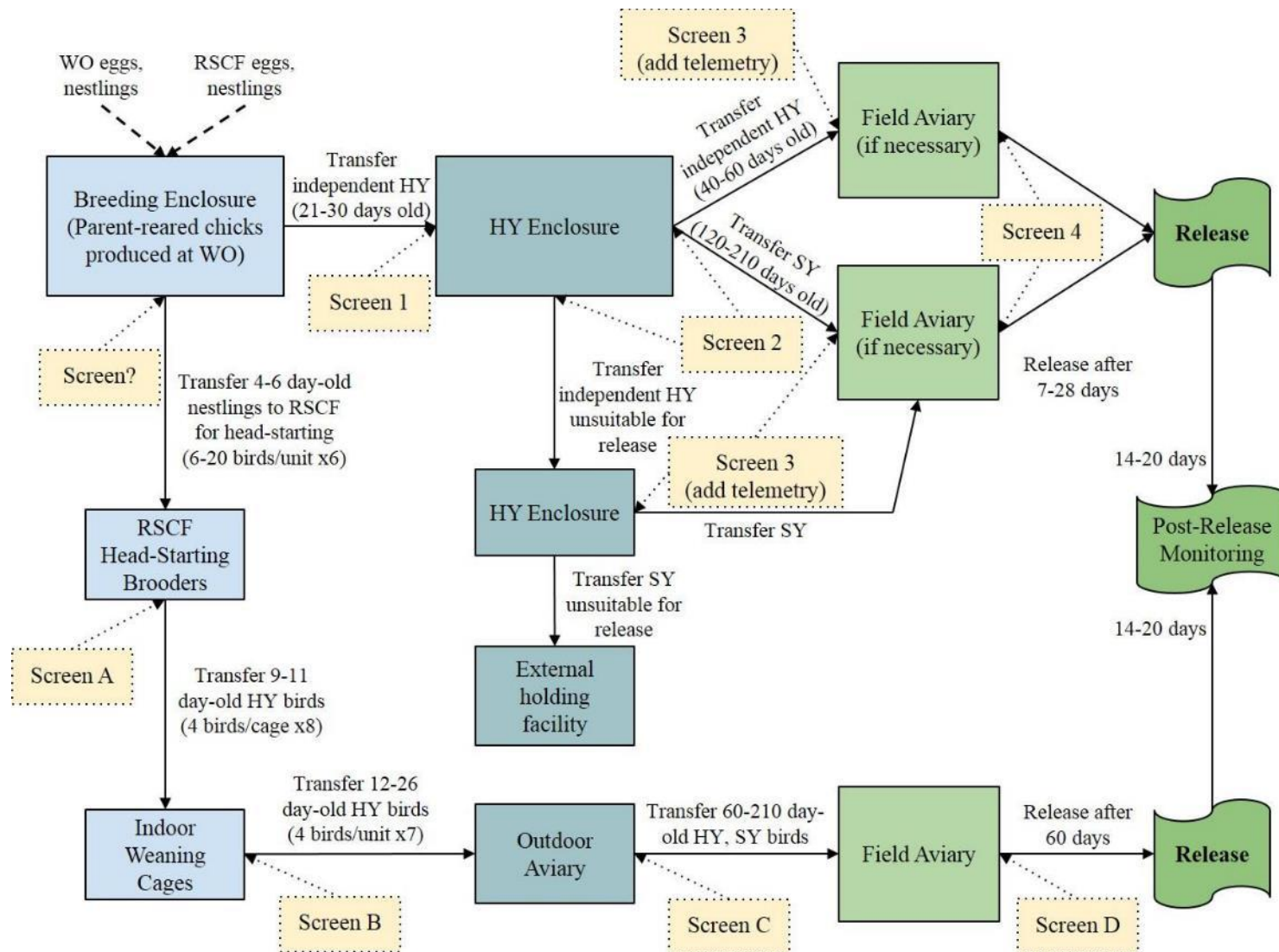


Figure 4. Proposed transfer pathway diagram for Florida Grasshopper Sparrows. Diagram is a composite graphic using separate diagrams created at the FGSP DRA workshop by staff from White Oak Conservation (WO – center and upper right portions of diagram) and Rare Species Conservatory Foundation (RSCF – left and lower portions of diagram).

Discussion of management alternatives

The workshop participants outlined FGSP management alternatives that define the spectrum of disease risk tolerance – from zero-risk tolerance, effectively defining a do-nothing management scenario; to full-risk tolerance, where birds would be released with little to no consideration of risk of pathogen/disease introduction and/or transmission among released birds and their wild counterparts.

1. Zero-risk tolerance (do nothing): No birds are released in 2019, or beyond, because of significant concern of releasing novel pathogens into the environment. Although the group recognized that the concept of “zero-risk” effectively does not exist in the practical world, there may be great hesitance to release birds to the wild, even if the risk of adverse consequences is low. Under this scenario, analysis to date suggests a high probability of extinction of the Florida Grasshopper Sparrow in the wild within the next decade. Although data indicate that recruitment is comparable to similar passerines, researchers have observed low nest survival and high rates of adult mortality.

If extinction of the species in the wild were to occur, the existing *ex situ* population would then serve as the lone insurance population against full extinction of the species. This would therefore require careful genetic and demographic population management, in accord with general principles of captive population management of endangered species – most notably those laid out in the IUCN’s *Guidelines on the Use of Ex Situ Management for Species Conservation* (IUCN 2014). Support for such an effort – logistical, financial, and otherwise – would likely need to come from the *ex situ* community as Federal support would instead be focused on *in situ* habitat protection or other programs.

A poorly-planned or poorly-executed release attempt in the face of scientific uncertainty could jeopardize continued persistence of the species in the wild. Therefore, as an alternative management option, a release effort could be delayed for at least a year, perhaps pending the results of additional data collection and analysis. There is some concern among both Federal and State management authorities that a failure to demonstrate significant progress on improving the species’ status in the wild may lead to reduced support for continued conservation efforts. This concern must be balanced against the urgency of the release effort that comes from the observed rapid population declines and the very low current abundance of the species in the wild.

2. Full-risk tolerance (releasing without regard for health status): The design and implementation of an aggressive release strategy that is designed to maximize the production of birds from both *ex situ* facilities, with release protocols that do not take into account the health status of birds for release. This alternative was also quickly discounted, as the probability of realizing one or more of the primary unacceptable outcomes is too high.

In light of the above discussion, workshop participants clearly recognized that a release program must be designed that strives to improve the status of the species in the wild, while actively minimizing the risks of one or more unacceptable outcomes in the context of disease management.

Filarial nematode treatment

There are two risks associated with microfilarids – survival of the infected individual and pathogen transmission through the population. There is currently no effective treatment protocol that is realistic, so prevention is more important in this case than control. Once infected, morbidity and mortality are correlated with intensity of infection, while spread is the product of the likelihood that a mosquito feeds on an infected bird, becomes a carrier, and subsequently infects another bird. Risk is likely correlated with intensity of infection either way. Presence and intensity of infection is determined through blood smears, which are imperfect surveillance tools. Each of the captive facilities currently make their own

judgments about the suitability for release of individual birds that are infected with microfilarids. However, there is currently no standardized threshold nematode density that would prevent a bird from being released. Some workshop participants suggested that they would be comfortable releasing a bird with a relatively large number of microfilarids on a standard slide (e.g., <50), while others stated a much lower threshold for characterizing release suitability (e.g., <5). There was a general recognition of the practical difficulty in accurately determining microfilarid loads in a given bird and associated outcomes, particularly in light of the disagreement among workshop participants about the ability to identify filarid taxa. Overall, the group recommends that the FGSP Working Group's Health Team attempt to determine a threshold microfilarid density that characterizes an individual bird as suitable for release.

The primary method of microfilarid control appears to be the implementation of broad mosquito control measures in order to reduce the risk of initial infection. Mosquito netting is currently in place at White Oak Conservation in light of the presence of microfilaria in captive birds at this facility.

Coccidian protozoa treatment

The body of workshop participants generally agreed that a high proportion of wild passerines are typically infected with one or more forms of coccidia. Consequently, the main goal of *ex situ* treatment is to control the levels of infection in individual birds instead of eliminating all pathogenic and/or non-pathogenic coccidia. As discussed in a previous section of this report, *ex situ* treatment of infected birds with coccidiostats can be an effective means of reducing the severity and impact of infection with coccidians such as *Isospora*. Currently, White Oak Conservation staff give coccidiostats by dusting food items such as crickets, while Rare Species Conservatory Foundation staff give them in the water that the birds drink. Furthermore, White Oak Conservation staff removes coccidiostat treatment of adults after the breeding season, and have observed no negative effects of this mode of treatment. At this point, there is no consensus favoring one method of delivery over another, although there are management logistical differences such as the fact that birds will not drink water from artificial sources in one institution, instead preferring to get their water from dew that accumulates on grass in their surrounding natural habitat. The choice of *ex situ* treatment protocol may be made on the basis of husbandry mechanics.

Coccidiostat treatment would likely target younger birds, as they are at the highest risk of mortality from infection with this pathogen. Release candidates may be positive for coccidia but still remain in the pool of available birds if they appear healthy in the presence of infection. This is consistent with the interest in maximizing the probability of survival of released birds as a primary goal of FGSP disease risk management. All of this of course is based upon the acceptance of the following assumptions: (a) there is a willingness to release birds that are infected (zero risk does not exist) and may continue shedding after release, and (b) there also is a willingness to accept the uncertainty surrounding taxonomic classification of the organisms, creating a possibility that novel organisms are released, and (c) the goal of management is risk reduction, not elimination. If so, risk-management research can be conducted to optimize screening and treatment protocols. Models exist in other captive avian spp. to address this and this would be identified as a priority for further research. Risk characterization can be updated as new information is discovered.

Duration of "quarantine and isolation"

Current release protocols describe a period of quarantine, in either *ex situ* or *in situ* aviaries, which is designed to identify birds that would be unsuitable for release based on health status. Discussion at this workshop, however, revealed uncertainty regarding whether the current protocol actually constituted a quarantine in the traditional sense – holding birds and screening them for pathogens to ensure that they are in good health before they are released.

The Dictionary of Epidemiology provides the following definitions for “quarantine” and “isolation”:

Quarantine

Restriction of the activities of well animals who have been exposed to a case of communicable disease during its period of communicability (i.e., contacts) to prevent disease transmission during the incubation period if infection should occur.

- Absolute or complete quarantine: The limitation of freedom of movement of those exposed to a communicable disease for a period of time not longer than the longest usual incubation period of that disease in such manner as to prevent effective contact with those not so exposed.
- Modified quarantine: A selective, partial limitation of freedom of movement of contacts, commonly on the basis of known or presumed differences in susceptibility and related to the danger of disease transmission.

Isolation

Separation, for the period of communicability, of infected persons or animals from others under such conditions as to prevent or limit the transmission of the infectious agent from those infected to those who are susceptible or who may spread the agent to others. There are further definitions available through AZA AHC

Currently, there is no proper system for a strict quarantine in either *ex-situ* or *in-situ* aviaries. After some discussion, the group generally agreed that what is currently called a “quarantine” period is actually more of a “holding” period for proper health screening, monitoring or observation. Rather, it consists of a number of recommended risk mitigation procedures. Therefore, the recommendation is to remove any mention of “quarantine” and replace with risk management or risk mitigation, habituation or monitoring period, and thereby moving discussion to the efficacy of these procedures.

Health Screening: as shown in Figure 4, health screening activities are undertaken as birds enter and exit *ex situ* aviaries and field (*in situ*) aviaries. When conducted in *ex situ* aviaries, health screening can be used to filter out birds considered unsuitable for release on the basis of standardized testing protocols for both microfilaria and for coccidian protozoa, as well as for general health concerns unrelated to either of these pathogens. The *in situ* aviary holding period would also include time for acclimation of the birds to the wild environment, i.e., to reduce stress levels after movement to the field, etc. The frequency of health screening (as laid out in Figure 4) must be balanced with the potential harm that can result from repeated handling of the birds and the subsequent stress.

A related issue – and one that was discussed at length in this workshop – is the length of time during which the birds are held in a proposed *in situ* aviary. Current thoughts on this issue range from less than one week to as long as 60 days. A longer holding time is based on the desire to include a prepatency period for coccidia infection, i.e., the length of time it would take between infection with the parasite and the ability to detect that infection with proper testing of blood or fecal material. [Estimated prepatency period is approximately 2-3 weeks.] This would be especially important if treatment with a coccidiostat is terminated before moving birds to the *in situ* aviary to test their response to the possibility of re-infection. Additionally, the longer time period would potentially facilitate the retrieval of carcasses to determine cause of death from any agent or circumstance prior to release – adding another diagnostic data collection node in the health monitoring protocol. On the other hand, it may be important to reduce the length of time in the *in situ* aviaries in order to reduce individual stress levels that can ultimately lead to a higher burden of coccidia infection. Following this logic, releasing those individuals to the wild – birds that have already been screened multiple times before entering the *in situ* aviaries – may possibly allow them to successfully adapt to their wild environment as rapidly as possible.

Ultimately, the workshop participants were asked to provide their expert opinion on the appropriate length of time that birds should be held in *in situ* aviaries before release to the wild. The results of this elicitation are summarized in Table 6.

Table 6. Elicitation of preferred duration of holding period for Florida Grasshopper Sparrows occupying *in situ* aviaries before release to the wild. See Figure 4 and accompanying text for more information.

0 – 3 days	≤ 7 days	2 – 4 weeks	4 – 8 weeks	≥ 8 weeks
4	7	4		3

Slightly more than 50% of workshop participants (11 of 18) viewed one week or less as a reasonable holding period for birds occupying *in situ* aviaries, while the remainder believe this duration should be at least 2-4 weeks and perhaps as long as about 60 days. While workshop participants did not reach consensus on this issue, the discussion brought forth valuable information for the Federal and State management agencies to consider when making decisions on design and implementation of potential release strategies. This node of great uncertainty presents another opportunity for continued research that could provide data for updated assessment.

During this discussion, workshop participants identified a need to determine how to handle birds that show obvious signs of illness while in the *in situ* aviaries before release – where to hold these individuals, for how long, etc. It may be possible to secure the assistance of a nearby wildlife rehabilitation clinic facility and an associated veterinarian. Representatives from the Federal and State management authorities were tasked with evaluating this possibility and, if appropriate, to add this element to the current release protocol.

Post-release monitoring

The primary purposes of a dedicated post-release monitoring phase include: tracking survival of released birds; retrieving carcasses to help determine the cause of death; tracking movements of released individuals; and tracking elements of individual behavior such as song composition, mating tendency, and recruitment. In order to get truly useful information, a monitoring program needs to have (a) a significant sample size, and (b) radios on birds for as long as is practical. The current plan is to place radio transmitters on approximately 25 birds just prior to release.

An important element of the monitoring plan is the proper handling of carcasses when they are available. It is crucial to establish a standard protocol for handling and sampling carcasses that are obtained from the field; moreover, this protocol should be aligned with the necropsy protocol currently used by the *ex situ* management facilities. The FGSP Working Group's Health Team will be tasked with developing this carcass handling protocol. In addition, workshop participants identified the need to bring a project pathologist on to the Health Team. Working Group participants from the *ex situ* community can assist with the identification of qualified candidates for this position.

A Preliminary Analysis of Further Research Needs

This disease risk analysis, and the substantial work by the various experts that preceded this workshop, has identified many areas of uncertainty in the taxonomy of the high-priority pathogens and their optimal management. In order to address these important information gaps, the workshop participants identified a series of potential research efforts that could ultimately improve the quality of the current disease risk analysis. Future research opportunities include:

- More detailed understanding of the life cycles of both *Aproctella* sp. and *Isospora* sp. pathogens as it relates to FGSP infection.
- Identification of coccidian genotypes in wild birds.
- Better understanding of the genetic characteristics that define species differences in protozoa such as coccidia.
- More definitive information on the value of resolving coccidia genotypes to the species level. Is this level of resolution necessary to determine potential for causing disease?
- Develop better clarity on how pathogens may differentially lead to disease in Florida Grasshopper Sparrows and Eastern Grasshopper Sparrows.
- Better determination of a threshold pathogen load that precludes birds from being released.

Preliminary Conclusion from This Analysis

The DRA workshop was designed and facilitated so as to provide an objective environment in which experts in FGSP disease ecology and epidemiology could discuss the relative risks of selecting birds managed in captivity for release to the wild. With this information, the trust agencies can then make a more informed decision about the feasibility of releases and the contributions they could make to FGSP recovery. These discussions focused on the fundamental question formulated by workshop participants (see page 8), defined in terms of the risk of releasing birds to the wild that may be infected with a high-risk pathogen that escaped detection in that individual and that, subsequently, posed a risk to other birds in the wild recipient population. In this light, the workshop was successful in achieving the goal set forth by those involved in process design and facilitation.

Based on the data and information assembled for this analysis, and the wide-ranging discussions making up the DRA workshop, a majority of participants agreed that the proposed release of FGSPs to an existing wild population (likely at Three Lakes Wildlife Management Area), using birds currently housed at both *ex situ* facilities, should move ahead in 2019. This recommendation emerges from the following conclusions drawn by a significant majority of workshop participants:

- The two priority pathogens of concern – filarial nematodes of the genus *Aproctella* and coccidian protozoa primarily of the genus *Isospora* – are currently found in wild birds;
- In the case of coccidia, functional natural linkages between wild habitats and *ex situ* facilities through mechanisms such as passage of migratory birds significantly reduce the probability of the existence of a novel pathogen in the *ex situ* environment;
- Biosecurity protocols currently in place among *ex situ* facilities greatly reduces the risk of introducing and maintaining a novel pathogen in the FGSP population.

Taking this information together, some workshop participants concluded that the risk of introducing a novel strain of either pathogen to the wild, which may lead to significant negative demographic impacts to the recipient wild population, is acceptably low. Others strongly disagreed with this conclusion and recommended a much more conservative, careful approach to pilot releases and the need for additional pathogen research and assessment. While workshop participants recognized that the risk of novel

pathogen introduction through release of captive-reared birds may not be zero, that risk needs to be considered in light of the high probability of extinction of the remaining populations of Florida Grasshopper Sparrows in the wild within the next decade, despite ongoing attempts to mitigate the wide range of current threats responsible for the species' rapid decline. Despite the fact that most participants characterized the risk of novel pathogen release to the wild population as moderate or low, they still concluded that the precautionary principle using available reliable information should guide release decisions. Release of captive-reared birds to the wild with no regard to health status or the risks of pathogen introduction to wild birds is unacceptable on the basis of both individual bird health and overall FGSP population stability.

As is evident in this report, the workshop participants were not unanimous in their conclusions and recommendations to the State and Federal management authorities. Some participants expressed significant concerns about the uncertainty around proper identification of pathogen strains/species currently seen in captive birds, the pathogenicity of those strains, and whether those strains/species represent novel threats to potentially naïve wild populations that would receive released birds. As with any risk analysis of this type, particularly those characterized by broad areas of uncertainty, decision-makers must digest the full range of available information, coupled with the weight of FGSP-specific evidence provided by appropriate experts, when deciding on a near-term course of action. Actions taken to improve the status of the species in the wild can be combined with complementary research activities to advance the collective body of knowledge of the role that these pathogens play in the population demography and ecology of the Florida Grasshopper Sparrow as discussed in our 5-Year Strategic Vision (see Appendix VI). Through this adaptive approach to population management, the long-term prospects for the species can be improved by iterative adjustments to evidence-based conservation activities – in captivity and in the field.

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Appendices

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Appendix II

Comments from Disease Experts Regarding Pre-Release Health Screening and Quarantine for Florida Grasshopper Sparrows

Compiled by Florida Fish and Wildlife Conservation Commission staff in preparation for the November 2018 Disease Risk Analysis Workshop

Last edited 11/26/2018

(Expert names have been redacted in the text, but a full list of experts contacted is at the end of the document.)

Questions

How important is it to test the resistance of the birds to the coccidia prior to release by stopping the medication and observing the birds? Are you aware of other programs that used an in situ quarantine site to evaluate the effects of stopping the anti-coccidian medication? If warranted, could this type of exploratory monitoring period be accomplished ex situ?

Respondent A: See responses below (next question).

Respondent B: I think first you would have to determine how to test the potential resistance to the coccidia, you can do this by doing McMasters counts of oocysts per gram of feces over 3-4 weeks during treatment and after discontinuing treatment, in our experience we get multiple phenotypes of *Isospora* in the feces which makes difficult to establish if this will translate into a decrease of the systemic infection. Obtaining blood samples and doing a percentage of infected leucocytes will give a more accurate view of the severity of the infection. Our population of captive population of BCLT has not suffered any new casualties due to systemic *Isospora* for the last 4 years, we still have relatively large numbers of coccidia in feces but no infected leucocytes in blood smears. My advice would be to discontinue the meds, it is possible that the treatment is not as effective as you think and your birds have developed some resistance. I am not aware of any other programs facing your issue. I do think that keeping the birds ex-situ will make monitoring the status more challenging specially if you want to obtain samples.

Respondent C: Well, theoretically, releasing the resistant birds would be probably worst outcome from the epidemiological point of view as they will carry the infections they survived but others might not. Just theoretically. No idea about any other programs, usually it's cheaper to kill the birds instead of releasing them. However, I have never used the first option. One of the reasons is that coccidiosis seems not to be the serious health problem (at least in the wild) for the greenfinches I used to work with.

Respondent D [phone call notes]: Both approaches [in situ and ex situ quarantine] sound reasonable. Ex situ would allow better control over safety and care. In situ, you would have to be really careful about predators. You might consider putting the wire for the enclosure in a trench and angling it outward 3-4 feet to prevent digging. Also consider double-wired enclosures to limit raccoon and snake predation. It is nice having them in the field but could be challenging logistically.

Respondent E: I feel both pre-release programs are reasonable and perhaps testing both would be ideal to see which might work better. Yes, I think monitoring period could be done ex situ. I think this is the most progressive program in regards to disseminating coccidia management that I am aware of. I do feel having the aviaries set up prior to release e.g. soft release would be beneficial.

Respondent F [phone call notes]: It is important to be sure the birds have had some exposure to coccidia to build immunity. I would not advise taking birds off anti-coccidia medication because this could make it harder for them to survive once released [increased stress in combination with high parasite loads].

Respondent G: See phone call notes below.

Respondent H [phone call notes]: Any type of movement or containment will cause stress for the birds, and coccidiosis is stress-related. Therefore it is important to continue Toltrazuril (anti-coccidian medication) during these stressful times. For the Cirl bunting project, it might have been advantageous to continue treating the supplemental food they received post-release. Assuming the parasite is native (and it likely is), you want the birds to still be infected when they are released, but not overwhelmed with the parasite load. More handling means more stress, which means more opportunities for mortality from coccidiosis.

Is it appropriate to continue or discontinue anti-coccidian medications once birds are moved to field aviaries for acclimation?

Respondent A: For questions 1 and 2, I'm not aware of any programs using that approach. Another species having *Isospora* problems in conservation breeding programs is the Blue-crowned Laughingthrush from China. In that program (and other species I've worked with), the anticoccidial drugs are primarily used before the breeding season to reduce shedding by the adults so fledglings have lower exposure. Most of the evidence suggests that fatal *Isospora* infections are primarily a problem in younger birds, but in all ages, it is likely dose-dependent, so if your release candidates are in large field aviaries where soil oocyst burdens are likely to be low, I think you could end the treatment after the initial stress of movement and acclimation.

Respondent B: I would discontinue the medication before you transfer your birds into the field aviaries and monitor them for a while before moving them.

Respondent C: I would recommend stopping medication if you believe it's dangerous to release the carriers.

Respondent D [phone call notes]: It would be good to test the birds without their meds. Meds won't eliminate coccidia; will just limit numbers. If good husbandry, burden should be reasonable. It would be interesting to see what burden they might have when removed from meds. This seems like a reasonable approach.

Respondent E: I would test both if possible. I do not think we know. The key would be to keep the levels as low as possible so that when they are released they will not be as likely to transmit the organism. I guess my preference would be to leave them on the drug until release.

Respondent F [phone call notes]: You want to release them in the best condition possible, therefore keep them on the medication. If you believe this strain of coccidia is not found in the wild population, then you should not release them at all. Look at the path from wild to captivity to wild, and the conditions of the breeding facilities (are they isolated from other avian species? What are the chances they have picked something up from the facility) to determine that likelihood.

Respondent G: See phone call notes below.

Respondent H [phone call notes]: Keep them on the anti-coccidian medications in the field aviaries. Assuming it is native (and it likely is), you want to retain the coccidian. Suggest fecal float to make sure they have some coccidia present before release.

Does 8 weeks seem like an appropriate length of time to assess whether coccidiosis re-emerges and causes mortality after stopping anti-coccidian medications?

Respondent A: We don't actually know much about the lifecycle of *Isospora* so it's difficult to say what kind of time frame would be required, but 8 weeks would be more than enough in my view if you wanted to pursue that option. I would probably recommend a shorter interval of 4 weeks or even less. I get concerned when birds are held for too long in quarantine or isolation settings (though acclimation to a field aviary followed by a soft release with supplemental feeding works really well with most species).

Respondent B: Two points here, we saw recurrence of clinical signs with very severe infection 4-6 weeks after discontinuing the treatment in the BCLTs, but we have noted also a strong seasonality in BCLTs deaths due to systemic *Isospora*, especially around winter months, possibly linked with molt? so you may need to consider this.

Respondent C: Sounds reasonable to me. Depends on how pathogenic your parasite is. My experience with greenfinches tells me that coccidiosis is not the problem in the wild, usually we don't see any oocysts

Respondent D [phone call notes]: Would have to double-check the life cycle, but 8 weeks sounds long. Thinks 4 weeks would be fine.

Respondent E: Yes I think so. Obviously the longer the better if you are truly trying to assess this. I am not sure you can truly rid them of the coccidia but if you can reduce it enough to stop shedding prior to release so that there is less environmental contamination, that would be best.

Respondent F and Respondent I [phone call notes] : Depends on the individual immune system of the birds, the conditions within the aviary, the behavior of the birds [congregating by the food dishes], and how stressed they are from the aviary. The medication does not eliminate coccidia but keeps it at low loads. Why do you want to answer this question? How will this help answer if a bird can be released or not? In the case of the Cirl bunting, we found it was important to maintain host/parasite connection and not completely remove it from the captive flock (need exposure to combat it in the wild).

Respondent G: See phone call notes and follow-up e-mails below.

Respondent H [phone call notes]: Does not see the reason for the 8 weeks for coccidia, assuming the coccidian is a native species. The situation will not change from the start of the 8-week period to the end. The key question: What is the purpose of the quarantine? You cannot base the purpose solely off of one hazard; you need a full DRA to look at what is preventing transfer of infectious agents to the sparrows (e.g., from other passerines at the facility, native birds with access to the cages, personnel, or equipment brought into contact with the birds). The DRA will help determine the appropriate length of quarantine to address all hazards.

We already know that unmedicated FGSP struggle with coccidia in captivity. We also know that high densities of birds can increase the concentration of coccidia in the environment. How large would the field aviary need to be to prevent increased coccidia loads as a result of artificially high densities in captivity? We want to ensure that mortalities in the field aviary are not the result of increased exposure to coccidia.

Respondent A: I'm not sure that anyone has concrete data on enclosure size or density required to keep exposure at low enough levels, but if your field aviaries are going to be 20x40 ft, that should be more than enough space provided you aren't putting huge numbers of birds in them. Zoos have been very successful in managing Isospora problems in much smaller aviaries.

Respondent B: Not sure about specific dimensions of the aviary, apart from direct re-infection from infected food/water, the parasite also replicates asexually in the animal's body which can amplify the infection regardless of the levels in the environment, I suppose that you could reduce the levels of re-infection by increasing hygiene, cleaning daily food and water containers. Species behavior may play a role, large number of birds may lead to increased stress aggravating the infections?

Respondent C: No idea, I have never used any field aviaries, just kept the birds inside in individual cages.

Respondent D [phone call notes]: 20' x 40' aviary seems reasonably large for 16 birds. May wish to consider a visual barrier of some sort to keep birds from flying into the sides of the enclosure and to make them less visible to predators, though you do want the birds to see their environment. Consider sanitation for the food and water stations – that's where the birds are likely to ingest oocysts and become "superinfected." Make sure you can access feeders from the outside, and limit exposure of the birds to people.

Respondent E: I am not sure I can answer this but the larger the better while still being small enough that the birds can be managed for what you need to do. Being able to clean the enclosure post release would also be ideal. If they are coming into the enclosure without shedding I would think you would be OK.

Respondent F [phone call notes]: Advise to keep them on anti-coccidia medication.

Respondent G: See phone call notes below.

Respondent H [phone call notes]: Keep them on anti-coccidian meds. Aviary size is the wrong question. A better question is what is the best aviary design and husbandry for the lowest stress level? Whatever

size the cage is, the birds will get re-infected with coccidia. More handling means more stress, which means more opportunities for mortality from coccidiosis. For Cirl buntings, release aviaries were hard-sided, 2x2x1 meters, whole nest kept together, so 2-4 chicks in cage. Birds did fly into the mesh sometimes when predators were present.

Can coccidia in the gut become extra-intestinal when concentrations become high enough? Can the stress and high densities in captivity lead to this occurring?

Respondent A: There are at least two views about this. One is that established intestinal infections can become extra-intestinal in times of stress. The other is that extra-intestinal infections only occur after ingestion of infective oocysts - the larger the dose the more severe the extra-intestinal manifestations are. I lean towards the latter hypothesis, but no one really knows. We are hoping to answer this question in the study we are doing now.

Respondent B: I think there are species of Isospora that can complete the life cycle exclusively in the intestine while others have a systemic phase, the higher concentrations should not affect the life cycle just the severity of the infection.

Respondent C: Absolutely.

Respondent D [phone call notes]: Of the two species in his crane study, one looked like it left the GI tract and became extra-intestinal, but the other stayed in the GI tract. This could be affected by stress. It's hard to predict which species will leave the GI tract and which won't.

Respondent E: Yes but this has not been well studied and probably depends on a lot of factors. Stress and high densities in captivity can lead to this occurring.

Respondent I [phone call notes]: Yes, depending on the strand of Isospora, this is possible, but it is not a necessary phase of the parasite.

Respondent G: See notes below.

Respondent H [phone call notes]: Depends on the species of parasite and the host species, but, yes, possible.

Based purely on disease risk, is ex situ quarantine sufficient to ensure birds are healthy enough to release, or is an in-situ quarantine warranted to see how birds react to pathogens to which they may not have exposure in captivity?

Respondent A: From the quarantine and disease risk standpoint, I think ex-situ is fine, particularly if the ex-situ location is in the same general region as the release site.

Respondent B: I would prefer to have an in-situ quarantine as new environment, meteorological conditions, novel pathogens could cause some undesirable effects or aggravate infections that may be already present.

Respondent C: As I said before, releasing seemingly healthy birds might mean that you just release carriers of the disease.

Respondent D [phone call notes]: Birds in enclosures may not be exposed to many of the disease factors in the wild, even in the in situ enclosures, so there's not a strong argument here. Being in an enclosure can limit disease exposure, so there may not be enough interaction with disease organisms until the birds are released and interacting with other birds. [CF: what about mosquito-borne illnesses?] Can't do much about vector control, especially if you're releasing them in a few weeks anyway.

Respondent E: I think these would be questions that you would need to address by trying different scenarios.

Respondent F and Respondent I [phone call notes]: Ex-situ quarantine and health monitoring (i.e., monitoring the weight) is sufficient-- given the risk of them having a novel disease picked up in captivity is acceptably low. The Disease Risk Analysis will help you answer this question.

Respondent G: See notes below.

Respondent H [phone call notes]: Doesn't see a reason to distinguish between in situ and ex situ quarantine for coccidia. For other hazards, the length of quarantine, the location, etc. will be answered at the DRA. In the, DRA you will determine the best duration and location of quarantine for each hazard (among other questions), then you'll look at the whole picture of all hazards and develop a disease risk management and release protocol to encapsulate all hazards and management options to address them.

Do you have any recommendations from your projects that might help us navigate the risks associated with releasing captive-reared birds known to carry coccidia that may or may not be native to the prairie of origin?

Respondent A: What we are finding so far in our study is that many (maybe most) passerine species on nearly all continents have a host-adapted *Isospora* species or strain that they handle quite well, provided exposure levels are kept low, as they would be in an intact ecosystem setting. Without more information, I can't comment on the probability that your GRSPs have a native or exotic *Isospora* (or co-infections with multiple *Isospora* species), but it is certainly reasonable to argue that that they would have their own host-adapted species or strain, and that it would cause significant problems in captive settings, as we have seen in other programs.

Respondent B: We have been reintroducing red-billed choughs, a corvid species that had gone extinct in Jersey, we planned a very prolonged soft release with supplemental feeding in a field aviary that would allow us to check the birds regularly, despite not having any particular issues with parasites in the captive population, as soon as we released the birds they acquired high levels of coccidia, without any obvious ill effects, while they acquire some nematodes, specifically *Syngamus trachea* and *Syngamus bronchialis* that led to some deaths in hatchlings. So, my main advice would be to monitor the birds as close as possible following transfer in release site aviaries or monitor.

Respondent C: No. I worked with greenfinches which are partial migrants, so never worried about this.

Respondent E: I think what you have laid out sounds amazing and very thorough. If you can experimentally evaluate these difference scenarios, I think that would be ideal.

Respondent F and Respondent I [phone call notes]: What we have seen in most cases, and with the Cirl bunting, is that coccidia is found in the wild population and is host-specific. Stress induces the disease, and therefore it is more likely to see the effects manifested in captivity and not in the wild.

Respondent G: See phone call notes below.

Respondent H [phone call notes]: Suggests conducting a DRA.

Additional Comments

Respondent G (phone call notes)

Summary

1. Respondent G thinks either ex situ or in situ quarantine both would work fine.
2. He suggested pulsed treatments with anti-coccidian medication to allow birds to be exposed to the pathogens and develop immunity. Early exposure is key. He suggests looking at fecal floats while the birds are in quarantine to see if they are shedding oocysts.
3. He thinks 8 weeks is plenty – the Isospora life cycle in birds is typically 5-7 days (but takes 24-36 hours to become infective, so it's a total 10-day cycle).
4. The CO3 region does a good job differentiating coccidia species using SNPs. He offered his lab's help if needed, and he has primers he can share for CO3. He doesn't think sequencing the whole genome is necessary.
5. Stress reduction is very important if EIC is the problem. If intestinal coccidia are the issue, density of birds in enclosures is important.

Full notes

- Immunity is very species-specific (i.e., immunity to one coccidian doesn't copy over to other parasites).
- Immunity could be developed ex situ.
- Toltrazuril doesn't allow much immunity to develop, especially if birds are in really clean spaces. Some other drugs allow "leakage" so birds are exposed to the pathogen, but the pathogen doesn't replicate. Suppressing the cycle completely would mean birds would be bad for the birds. He recommends pulsed treatment (see below).
- Distributed feeding rather than concentrated feeding at bird feeders is one way to help reduce exposure, but at the densities we're discussing, he thinks all birds will get exposed in the aviaries. A larger pen distributes transmission pressure.
- If taking them off their meds, you could observe for sick animals then treat them and release them back into the pens to knock down the infection.
- He suggests collecting material in the areas where the birds will be released to see if oocysts of the coccidia species are already present.
- He said that it is hard to test resistance experimentally – it's a tough call, because you could end up hurting the birds. He suggests screening the birds as you go and medicating when necessary. E.g., you could put Kraft paper drop cloths under roosts or feeding stations.

- Isospora life cycle in birds typically 5-7 days (but takes 24-36 hours to become infective, so it's a total 10 day cycle).
- If birds are shedding oocysts, the birds certainly have exposure and should do fine when released. If no oocysts are present and you take them off the drugs, one animal could get sick, and, although that animal might be fine, it will shed lots of replicated oocysts that will get other birds sick and will amplify among the population in the aviary.
- Treating parents limits the transmission to the young birds.
- Some isospora can be transmitted via nest mites.
- Some isospora can develop resistance to drugs.
- The birds need exposure when young. After that, they can withstand heavy challenges when older. Initial exposure is key.
- He leans toward a longer quarantine time, but he thinks it could be either in situ or ex situ.
- He suggests doing a pulse of drugs rather than consistent drug treatment. E.g., no drug for 3-5 days, then on for a week, off a week, then on again. Let the parasite cycle through so "leakage" occurs but they don't replicate enough to kill the birds.
- Respondent G offered to do the genetics for us if Dr. Ritchie or Dr. Wellehan get backed up.
- He thinks the CO3 gene is really good for differentiating coccidia species – 1.5x better than the CO1. He doesn't know of any valid species that didn't get at least 0.9% variation at CO3. Most species diverge much more than that. "Wobbles" are almost always the third position in the codon and don't matter.
- Oocysts are viable in the environment for a year, maybe 1.5 in Florida.
- Killing the oocysts is very difficult. He suggests one of two methods:
 - Paraformaldehyde boiled off in the room (paraformaldehyde "bomb").
 - Or home brew surface spray made of 1 gallon windshield fluid (lowest temp available, like -40 C) mixed with 200 ml neat Ammonium Hydroxide (37%). Spray, leave on for 2-3 minutes, wipe or dry off. Wear a carbon filter mask!
 - The oocysts are not killed by bleach – they simply float.
- If the intestinal phase is the problem, the density of the birds is important. If EIC is the problem, stress is very important. If EIC is the problem, pulsed treatment is the way to go. Allows exposure but knocks it down. You can expand the period of exposure as you get nearer to release.
- One method to reduce transmission: could lift the feeding tray or bottom of enclosure so vegetation grows up through it, but feces fall down below the bottom, so birds aren't exposed to as many oocysts.
- Isospora can move from intestinal to visceral. Atox is usually just the EIC form of intestinal Isospora.
- If birds are indoors: One way to reduce stress might be to drop the light level or to use something like brown plastic "mops" that could be autoclaved. (this is because the birds like to be in the grass in lower light environment).
- If the birds are kept in an open-topped enclosure where other birds can perch (e.g., not double-layered), he wouldn't worry about the "unknowns." The birds are probably exposed to everything. Insects (dung beetles, flies, etc.) can carry coccidia in or out of pens.
- He suggests talking to Adrianna Pastor, a vet at Calgary who was involved in black-footed ferret and used pulsed treatment for coccidia.
- One take home: reduce stress!
- For pulsed treatment, use baby-steps – build tolerance to challenge over time.
- Quarantine could be in or ex situ.

- Newly hatched birds are not great hosts for coccidia. Usually it is after the bird's gut develops that they get sick – just like we observed with FGSP.
- There is often a narrow age window when the birds are highly susceptible. Take the age at which they are dying, back up maybe a week from there, allow them some exposure, then medicate them.
- Whole genome not necessary. Can do mitochondrial SNPs – works well if you get a couple. He highly recommends CO3.

Respondent G (follow-up e-mail correspondence)

The scenario: a group of independent hatch-year or second-year passerine birds that have been exposed to an extra-intestinal Isospora sp. in captivity are placed in a large, outdoor aviary (maybe 20' by 40'), taken off their anti-coccidian medication, and then observed over a period of time.

Even if the birds have been reared in captivity, it is possible that they've been exposed and, with a couple exposures, they might already be immune or partially so.

FWC: Do I remember correctly from our conversation that it typically would take 5-7 days before the birds would start shedding oocysts again, and that it would take an additional 1-1.5 days for the shed oocysts to become infective?

Most coccidia would start shedding at ~4-5 days (but up to 7 d) after ingesting oocysts and sporulation to infectivity would take about 24 to 36h.

FWC: If so, that would mean that other birds in the aviary could start picking up shed, infective oocysts at around 7-9 days (+ or -). If the birds had not developed sufficient immunity to the Isospora sp. while in captivity and picked up shed oocysts, how long would it typically take for a passerine challenged with an extra-intestinal coccidian to start showing visible signs of illness?

I would suspect that infective oocysts could be available at 6 days but might take as long as 9 days - if colder, it will take longer. If you have an immunologically naive host, the time it takes for development of clinical illness depends on how many oocysts are ingested. If the outside challenge is really high (10's of thousands of oocysts) then I would expect extra-intestinal development to be accelerated with clinical disease evident in perhaps as little as 4 or 5 days (i.e. at or before patency). At lower levels of initial infection, I would suspect clinical disease to appear later, perhaps weeks (?? - realize that this is a SWAG - I have no data to support this). Until there is immunological control, the parasites are likely to double in the tissues every 8 - 12 hours; if the clinical signs are largely because of inflammatory responses then you'd expect around 2 weeks, maybe a bit later with lighter challenges. Your previous experiences with nestlings and fledglings are likely to be more informative than my guesses, I'm afraid.

Respondent J (e-mail correspondence)

What I know from the European house sparrow is, that under outdoor aviary condition almost all birds became infested with coccidians but without any apparent serious illness. The only important issue was the fresh water: birds started to die immediately we slowed down with changing their water every day. However, I am not sure that coccidians had any effect here. Perhaps you should treat the birds against coccidians but keeping a control, untreated group for comparison, if you haven't done yet.

Respondent A (e-mail correspondence)

Just a couple of final comments. I don't know much about the recent history with the Florida Grasshopper Sparrow, so my previous comments should be taken as fairly generic. I might modify my responses in light of the additional information that would be coming from your risk analysis workshop.

I assume that the IUCN CPSG will be facilitating the workshop. If that is the case, it will be tremendously helpful for your program. They do an outstanding job with these things. A couple of concepts can get lost in the course of an intensive workshop, though. The first is that it's impossible to really evaluate the risks of animal movements if you don't have comparable information about all the populations involved (e.g., captive-reared reintroduction cohort, the wild recipient populations, non-target species in the ecosystem, etc.), and you almost never have comparable information. There are ways of dealing with that, but it's a bit too complicated to get into in an email.

The second is that there are risks associated with not taking certain conservation actions that are often not given adequate consideration. People naturally tend to focus on the risks of taking an action rather than the risks of alternate actions, or no action, which can be even riskier for a species. That's one of the reasons the Po'ouli is now extinct. The CPSG workshop facilitators are generally really good at making sure all the possible actions or inactions are considered.

The third thing to be aware of is that the average person, including the average biologist or veterinarian, is not very good at intuitively evaluating the risks associated with multi-step processes. For example, if there is a concern about introducing a foreign pathogen into a wild population, there are multiple independent steps involved, each of which entails its own risk (e.g., the released animal has to be carrying the pathogen, it must be shed in a transmissible form, susceptible wild individuals must be exposed, infection and replication must occur, the cycle must repeat itself, there must be negative population consequences as a result, etc.). The probability of a negative population outcome is determined by multiplying, not adding, the probabilities at each step. So if there are many independent steps required, the cumulative probability of a negative population outcome is often much lower than most people intuitively think. And then you have to consider the probability that the foreign pathogen will be introduced into the wild population by some other means, completely independent of any conservation actions.

Respondent B (e-mail correspondence)

Hi Erin, thank you for your email, I would not consider by any means an expert in systemic coccidiosis in passerines, but I can share our experience and findings with this pathology in Blue crowned laughing thrush kept in captivity. See below some of my comments, I am away until next week, but if you wish to have a chat on the phone happy to do so when I come back to Jersey.

From the point of view of the disease risk analysis, I would wait until you can confirm that the coccidia in your ex situ population is the same as the wild population, even if your captive birds have become resistant the risk of introducing a novel pathogen into the potentially naive wild population seems quite severe.

Regarding treatment with toltrazuril, in our experience the treatment was effective to stop the shedding of oocysts in feces was administered orally by gavage tube, but the birds had to be treated daily for several weeks in order to reduce the percentage of infected lymphocytes and as soon as the treatment was discontinued the severity of the infection went back to pre-treatment status. Our treatment in the drinking water was not effective to reduce the coccidia fecal shedding.

Respondent K (e-mail correspondence):

Who is looking at the pathology of these cases? Do you know which genus of coccidian you have in these sparrows? Species of *Isospora* are most likely involved if you have development outside the gut from my experience. We used to call these *Atoxoplasma*, but now *they are considered Isospora*. I am still mulling over what you sent yesterday. What is the estimate on the size of the wild population of the FGS?

FWC: Coccidia found in tissues from birds that died in captivity at White Oak Conservation Holdings have been submitted and sequenced (using SNPs) by Dr. Jim Wellehan at UF (Dr. Scott Citino's group at WOCH performed the necropsies). Dr. Wellehan has also searched for coccidia DNA in frozen fecal samples from wild birds, but none was positive. Dr. Jim Austin and his master's student Jen Eells have run PCR on blood samples from wild and captive Florida grasshopper sparrows but have not been able to detect coccidia DNA in the blood.

*Infected carcasses from the second captive population (Rare Species Conservatory Foundation, Loxahatchee) as well as a few infected wild fecal samples have been sent to Dr. Branson Ritchie at UGA. We are still waiting for his formal report, but he has successfully amplified the entire mitochondrial genome from a captive sample and is in the process of comparing that to the wild sample. It doesn't appear to match any coccidia in the existing library, but the closest match was an *Isospora*.*

We estimate there were ~151 individuals in the wild at the end of the 2018 breeding season (including adults and banded nestlings). There are another 81 individuals held in captivity (~40 of these may be eligible for release if we can sort out the release protocols).

So no one has looked at the feces to look at the morphology of the sporulated oocysts? This has to be done as all zoological species names are based on morphology, not PCR data. The latter can place the taxon in the correct genus, but without sporulated oocysts, you will never have a name unless someone has run the genetics on a known species of coccidian. Am I to presume that no hematozoan sequences came up in the blood study, as these could be contributors to mortality. If you have blood smears, someone needs to look at them for blood parasites as you may have more than one agent in action here.

FWC: One of the captive facilities has done fecal float tests, but I do not believe they have used the oocysts for identification based on morphology. I was under the impression that they were difficult to identify to species correctly, but if that's not the case, then we should probably look into this like you have suggested.

Several of the wild and captive blood samples from living birds have tested positive for Plasmodium/Haemoproteus sp. and Leucocytozoon sp. during PCR screening. The other major pathogen discovered was an Aprocetella sp. filariid. This has been detected in both wild and captive blood samples.

OK so I still have not addressed your efforts to look at timing as I am still trying to determine what parasites you are dealing with. I think Terry would be a good contact for this information. Although now he is primarily working on sea turtles.

If you are doing fecal flotations, then you could be detecting the oocysts that go with your coccidian parasite. The oocyst morphology will tell you which genus of coccidian you have in your birds. The oocysts of most genera pass unsporulated and you then have to let the oocysts develop or sporulate (a form of asexual reproduction). I know that Heather has her hands full of projects and I am not saying that her lab would determine the genus as that will be up to her as I am copying her on this message. More importantly at this point is the genus as that will give you useful information as to the life cycle. Species identification can come later as that may require making a series of measurements of the oocysts to describe the species as it may be undescribed or match it to a species name already.

As to the blood parasites (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) blood smears with a decent stain (Giemsa) and someone with training can look at the stages on the smear and determine which species may be present. I may be able to help with this as I have experience with these parasites. I also have my own microscope at home that I can use to do this. What I would need are some of the smears (if you have made them) from birds that PCR says are positive. If you are not making smears, please start doing that. Use only a tiny drop of blood to make the smear. You will not get a species ID from PCR, with very few exceptions.

As long as you are doing wildlife disease work, I would encourage you (or your team) to attend the ad hoc meetings that Mark organizes. It is a nice informal manner to communicate with others that might be able to help your progress. These are usually every other month and take up about 2 hours in an afternoon.

Respondent D (phone call notes)

- You can't get rid of coccidia, can only reduce numbers. Most birds can handle low numbers, and exposure in the wild is less. Problems usually occur in captivity. You want the feces to drop somewhere where they won't accumulate too much.
- The coccidian is probably host-specific. Nonetheless, it would be good to establish that it is present in the wild population. With the cranes, he and his colleagues didn't feel comfortable releasing the birds until they had found the parasite in the wild.
- James knows both Branson Ritchie and Jim Wellehan. They are good people to have.
- Parent-reared birds will likely have better chances than hand-reared birds. You might be able to increase production if pulling eggs or nestlings in for hand-rearing, but they would likely be less quality individuals.
- Recommends minimizing exposure to humans.
- One concern is that the birds may be naïve to predators, but not sure what we could do (maybe fly raptors over?).

Respondent F and Respondent I (phone call notes)

Summary

- Coccidia is usually host-specific, and effects of it manifest in captivity, but it is usually also present in the wild
- Respondent I noted that nematode effects manifest under stress and may be expressed in captivity but not the wild
- Need to decide on a whole how much of a risk there is that the birds in captive facilities picked up a novel pathogen (the DRA will help with this). You cannot screen for everything.
- Recommends keeping time in captivity to a minimum
- May need to offer hand-reared birds more support, but this could be in the form of post-release support, not just a long in-situ acclimation
- When releasing birds, you want to release birds with as minimal stress as possible (stress caused from a cage, stress caused from high coccidia levels...) to give them the best chance

Full Notes

- Time in captivity in general should be kept to a minimum (keep sparrows wild and not habituated/acclimated to humans and captivity)
- Respondent F: What are the risks with the release strategy and what are the key objectives
 - If the key objective is the health of the sparrow, do not want to release them with a compromised immune system (i.e., no exposure to coccidia, or high loads of coccidia)
 - Different objective is the risk of co-introducing a disease, manage them differently. If you are truly concerned the birds have a novel parasite, then should not release any of them
- Normally coccidia shows up in captivity when they are stressed and usually is in the wild
- On the 8 weeks in-situ quarantine to assess whether coccidiosis re-emerges and causes mortality after stopping anti-coccidian medications:
 - All it will show is whether you will get an accumulation of load again
 - Unlikely that you have completely removed coccidia from the birds, treating is just a decrease of infection intensity

- If you find something in the environment affecting the birds, and the outcome is probably death, that is not different than letting them go sooner and tracking them to that end (except may be less stressful out of the cage)
- Keeping them in an aviary for 8 weeks prolongs the stress, especially off the anti-coccidia medication
- Release will be stressful for them as well, so you don't want them to have high loads of the coccidia when they are released
- Never release birds completely naïve to coccidia (asked about pulse treatment, agreed that would work if worried they have not been infected)
- Learned from the Cirl bunting that it is better to maintain host/parasite connection by not trying to completely remove the parasite in captivity
- Is eight weeks an appropriate amount of time to assess whether coccidiosis re-emerges and causes mortality after stopping meds?
 - Depends on a lot of factors, such as the immune system of the birds, the loads of coccidia [how water and food is kept in cage, if the birds congregate around bowls of food or water, how the birds interact with each other in the cage, and the stress of the birds in response to the cage]
 - Should think about why you need to know this, what you will learn from keeping them for 8 weeks and if that is necessary to release the birds
- Field aviary size needed to prevent increased coccidia loads
 - Recommend keeping them on coccidia medication
 - Coccidia is a disease of captivity
 - Depends on feeding areas, how likely they are to congregate around feeding area, density of birds, individual immune systems
 - Also depends on how stressed birds are
- Some strands of Isopora can go extra cellular [extra-intestinal] in a stage of a life cycle, but it is not necessary/common for the Isopora to do so.
- Respondent F: Why do you need to know if the bird is infected with coccidia before letting it go? Why do you want to see if they show signs of infection?
 - The choice is to let the bird go or not. Will you only let the bird go if it doesn't show signs of coccidia? What if one bird shows signs and the others do not?
 - Birds should have some coccidia anyway. Generally, not a problem in the wild.
 - What is the desired outcome of keeping them for 8 weeks?
- Hihi work-Monitored weight change, selected birds that were physically healthy and had not lost weight. Dosed them with anti-coccidia medicine to mitigate for possible stress induced load, and then let them go
- More important than the aviary may be the support hand-reared birds will need post-release [supplemental food, protection when they nest]. Holding them longer might not be what they need. They probably won't do well [because hand-reared], but if they reproduce, their offspring will need less and contribute more.
 - Pit tags might help with monitoring if they are coming back to a feeding area
- Figure out what the explicit role of the aviaries is
 - Is it coccidia? Disease?
 - Or support / acclimation?
 - How long does a captive bird need to learn? Is the time in the aviary doing good?
- Population problem (I expressed the reason the group is heavily focused on disease is because the FGSP population did a nose dive that looks like disease could have been culprit) —

Respondent F would be surprised if sharp decline in wild was actually due to coccidia. Important not to get blinded by the disease seen in captivity.

- It is useful to critically look at the translocation pathway from wild to captivity to wild
 - Context of where the facilities are in the natural range of the wild bird
 - And Biosecurity of the facility
 - Together can determine the likelihood of a novel disease in the birds
- Respondent I: Filarids are a similar hazard to coccidia, a carrier hazard, commensal to the birds and normally don't get sick from it but can under stress
 - Need to see if they could have picked it up from another species in the context of the facility
- Need to clearly articulate three different factors: Vitality of the introduced population as a whole [survival and recruitment], welfare of the individual birds, and the risks to the wider ecosystem
- For welfare of the individual birds
 - How to measure this, acceptable risks the group is willing to take

On coccidia and filarids: Respondent F and Respondent I said it was important to look at the actual risk the birds had at picking up something novel in captivity based on the biosecurity of the facility and the amount of contact with other birds. This way you are covering all possibilities of pathogens, not just the ones manifested in captivity.

Consulted Experts

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Appendix III

A Literature Review of pathogens that may cause morbidity or mortality in the critically endangered Florida Grasshopper Sparrow

Prepared by: Jennifer Eells

May 22, 2018

For: Florida Grasshopper Sparrow Disease Risk Assessment Team

General Introduction

Florida Grasshopper Sparrows (*Ammodramus savannarum floridanus*, FGSP) are critically endangered and have been declining steadily since 2009. In an effort to prevent extinction, a captive breeding population was started from captured wild individuals. The detection of coccidians and microfilarial pathogens in FGSP and GRSP (Eastern Grasshopper Sparrows) at the White Oak facility raised a number of difficult questions pertaining to the recovery options available for managers. For example, are these same pathogens present in wild populations or were they acquired in captivity? As a means to help evaluate the risks associated with releasing captive reared or head-started birds from facilities like White Oak, I conducted a literature review. The purpose of this literature review was to address a release scenario exercise and potentially answer questions that have not been satisfactorily answered through traditional laboratory and field methods.

Two separate pathogens of high concern were addressed according to the release scenario: coccidian sp. (Protozoans) and microfilarial nematodes (parasitic worms). Specifically, the review was carried out with the following questions in mind:

1. How frequently is the pathogen diagnosed in wild passerine birds, and grassland birds in particular?
2. What is the likelihood that the pathogen has population level impacts to wild passerine birds?
3. What is the mode of transmission for the pathogen?
4. What is the likelihood of transmission to wild FGSP?
5. Is this pathogen species-specific, or is transmission to other dry prairie birds possible?
6. What are the mitigation options that can be performed by the captive facilities to reduce possible spread of infection?
7. How reliable are screening methods for this pathogen?
8. What are the health consequences of infection by the pathogen (can birds survive and reproduce while infected)?

Methods

This literature survey utilized Google Scholar, J-Stor, and PubMed. Because of the limited research specific to FGSP I included all relevant literature on passerines with an emphasis, if possible, on grassland birds and sparrows. I searched for keywords using the following combinations: “Disease AND wild birds”, “Disease AND Grassland birds”, “Disease AND Florida Grasshopper Sparrows”, “Coccidia AND wild birds”, “Coccidia AND Passerines”, “Microfilaria AND wild birds”, and “Microfilaria AND Passerines”. Where possible, an effort was made to limit data information to the years 2014-2018 to ensure collection of the most up to date studies. The field of disease ecology is a fast paced field with new data rapidly replacing the old. Search terms used were a combination of key terms from the above questions as well as pathogen name. Where it was not possible to find information from the last five years, papers that were highly quoted were used instead.

Below I summarize the information found in the literature that most accurately reflects the answer to the questions posed by the health team. Entries are divided first into pathogen of interest and then question and answer sections. In the end, I summarize general findings in the research and potential future steps needed for more information.

Coccidians (*Isospora* sp. and *Eimeria* sp.)

Coccidians are intracellular parasitic protozoans that inhabit domestic and wild birds and are capable of causing health concerns (Dolnik et al., 2010; Schrenzel et al., 2003). Coccidians invade the intestinal epithelium cells and reticuloendothelial system of the host species where they can undergo both asexual and sexual reproduction and produce oocysts that are shed in host feces (Adkesson et al., 2005).

I) How frequent is the pathogen diagnosed in wild passerine birds, and grassland birds in particular?

Coccidians are considered the most common endoparasite in captive and wild birds and are the cause of Coccidiosis (Globokar et al., 2017; Dolnik & Hoi, 2010). Globokar et al. (2017), recorded a ~22% infection rate out of 398 samples from wild Passeriformes. In their study of Estonian Greenfinches, Horak et al. (2004) found an 89% *Isospora* infection rate in birds pre-study. Coccidians have been found to be host specific (Hafeez & Barta, 2017; Lopez et al., 2007). Dolnik & Loonen (2) state that “*Isospora* species are believed to be narrow host-specific on the level of genus... Therefore, each *Isospora* species found for the first time in a bird genus can be considered a new species.”

II) What is the likelihood that the pathogen has population level impacts to wild passerine birds?

Dolnik & Hoi (2010), found that male house sparrows infected with *Isospora* suffered weight loss resulting in less energy to defend dominant positions in the social hierarchy. This further caused increasingly stressful and aggressive encounters with subordinate males and loss in social status. In 2012, Sainsbury and Vaughan-Higgins, wrote a disease-risk assessment plan for the reintroduction of Eurasian Cranes to England. In the article, they listed coccidia as a high risk hazard with population and immunodeficiency effects in captive birds. They predicted that upon release, these effects would be negated with the cessation of capture stress and crowded conditions.

Xie and Nevis (2012), studied the effects of an anti-coccidial drug Ponazuril on orphaned American Robins in captivity. Over a three year period, despite the treatment, they saw their mortality rate increase from 1.6% to 13.3%. They attribute most of this increase to the presence of coccidians in the birds and discovered that orphans without immunity under stressful conditions were the most likely to be stricken and suffer ill effects. Consequently, efforts to restock wild birds from captive birds could be curtailed if this holds true for FGSPs as well.

III) What is the mode of transmission for the pathogen?

Transmission is fecal-oral by ingestion of the oocysts shed by infected birds which can occur via contaminated water, nest debris and uncleaned floors (Dolnik & Hoi, 2010; Krautwald-Junghanns et al., 2009) “Coccidiosis becomes important as a disease when animals live under crowded or dense populations that allow buildup of infective oocysts in the environment and ingestion by susceptible hosts” (Botzler & Brown, 2014). In captive birds, immunosuppression related to poor diet, stress, loss of genetic diversity and drug therapy are factors of severity for infection and spread (Allen & Fetterer, 2002; Xie & Nevis, 2012).

IV) What is the likelihood of transmission to wild FGSP?

Our study has found *Isospora* in wild and captive FGSPs showing this parasite already exists in populations. Dolnik et al. (2010) found a correlation between prevalence and intensity of infection and host foraging and feeding habits. Ground dwelling birds had a higher rate of exposure to contaminated feces and thus a higher probability of infection rates. Repeated exposure likely also cause a greater intensity of risk due to reinfection. However, she also stated that this could cause a greater tolerance to

infection and found that insectivores had the lowest intensity of infection. This could be one reason that levels of detection in wild FGSPs are low.

V) Is this pathogen species-specific, or is transmission to other dry prairie birds possible?

Coccidians are generally host specific but horizontal transmission probably occurs between hosts (Norton et al., 2007). *Eimeria sp.*, is often associated with domestic fowl, but has been found in wild birds such as Passeriformes, Anseriformes and Galliformes (Yabsley, 2007). In geese, *E. truncata* has been known to cause renal and intestinal coccidiosis and in cranes, *E. gruis* and *E. reichenowi* cause Disseminated Visceral Coccidiosis which can lead to death (Spaulding et al., 2007). In 2011, Berto was the first researcher to find two new species of *Eimeria* in songbirds belonging to the *Tyrannidae* family in South America. He reported no ill effects.

Isospora sp. is considered the most common endo-parasite in captive and free ranging Passeriformes and occurs on several continents (Dolnik et al., 2010; Globokar et al., 2004; Yang et al., 2016; Yang et al., 2018). Outside of the FGSP disease project, there was only one other record of coccidians in the FGSP. In 1997, Foster et al. found two birds that tested positive for *Isospora sp.* in Okeechobee and Osceola. As this was based on unpublished data quoted in Parasites and Diseases of Wild Birds in Florida (Forester and Spaulding, 2003), there was no more specific information.

VI) What are the mitigation options that can be performed by the captive facilities to reduce possible spread of infection?

Mitigation in captive facilities can be achieved by cleaning cages frequently, quarantining new birds, separating wild and domestic birds, reducing fecal contamination of food and water and hand rearing chicks to prevent infection from adults (Krautwald-Junghanns et al., 2009; Norton et al., 2007; Sainsbury & Vaughan-Higgins, 2011). Several drugs, such as Tetracyclines and Sulfachlorpyrazine can be used to reduce shedding of oocysts but won't eliminate tissue infection completely (Adkesson et al., 2005; Arabkhazaeli & Madani, 2014; Horak et al., 2004; Sood et al., 2018).

Vaccinations for *Eimeria sp.* in chickens have been developed (Allen & Fetterer, 2002) but this treatment is not feasible for wild birds (Yabsley, 2008). In fact, it has been found that low levels of the organism may be needed to stimulate the immune system and no exposure (due to antibiotic or vaccine application) may prevent this from happening (Norton et al., 2007; Xie & Nevis, 2012; Yabsley, 2008). For example, birds without this immunity and exposed to the parasites causing Atoxoplasmosis, often result in high mortality events (Norton et al., 2007).

VII) How reliable are screening methods for this pathogen?

The use of PCR and sequencing via blood samples is the most reliable method of screening according to all the papers reviewed. While fecal float tests can be used, they are often not accurate due to low level, intermittent shedding of oocysts during the day (Adkesson et al., 2005; Dolnik et al., 2010; Lopez et al., 2007; Norton, 2007). Swabs adjusted to be taken in the late afternoon or evening and from juvenile birds provide better representatives of infection (Globokar et al., 2017).

Adkesson et al. (2005) also state that other methods of detection can be used such as histopathological exam of tissue samples, buffy coat smears, organ impression smears, transmission electron microscopy examination and liver biopsies. He warns, however, that organ samples and buffy coat smears may not be reliable as organisms can be in low enough concentrations that they are missed or not present. Additionally, it can be difficult to identify coccidians based on visual inspection (Hafeez & Barta, 2017).

The use of PCR and amplicon comparisons in BLAST or GenBank are more reliable (Bertram et al., 2014; Dolnik et al., 2009).

VIII) What are the health consequences of infection by the pathogen (can birds survive and reproduce while infected)?

While most birds remain asymptomatic, Coccidiosis can cause diarrhea, decreased activity, anorexia, weight loss, ruffled feathers, potentially decreased reproductive performance and even death (Adkesson et al., 2005; Dolnik, 2010; Lopez et al., 2007; Xie & Nevins, 2012). Dolnik et al., (2010) listed three factors that are important in increasing the intensity of coccidian infection in wild birds. They include the frequency of re-infection that occurs, the number of infections with a high level of oocysts, and the presence of other infections.

Coccidian parasites can also alter the physiology of birds, by destroying the cells lining the intestines and inhibiting the uptake of essential dietary elements (Brawner et al., 2000; Horak et al., 2004; McGraw & Hill, 2000). Furthermore, they can effecting body mass and size of secondary sex characteristics and reduce fertility (Buchholz, 1995; Horak et al., 2004; Dolnik & Hoi, 2010). Wild birds with infection could also “face unpredictable food availability, direct stress from risk of predation, and a more diverse array of parasites and diseases, all of which could make it more difficult to invest resources in overcoming an acute infection by coccidians” (Horak et al., 2004).

Microfilarial nematodes (*Aproctella* sp.)

Microfilaria are the immature stages of nematode worms. They are often found in the blood or under the skin depending on the species (Bartlett, 2009; Huang et al., 2016). Adults inhabit the cardiovascular, pulmonary or lymphatic systems (Anderson, 2000).

I) How frequent is the pathogen diagnosed in wild passerine birds, and grassland birds in particular`?

Microfilaria are quite common with over 16 genera found in avian hosts (Huang et al., 2016). Silveira et al., (2010) found a 6.6% infection rate in passerine species in Brazilian grasslands and Hamer et al., (2013) showed an infection rate of 11.1% in American Robins. Other studies have shown rates as high as 20% and as low as 1% (Dusek and Forrester, 2002; Benedikt et al., 2009; Londono et al., 2007).

II) What is the likelihood that the pathogen has population level impacts to wild passerine birds?

Species infected with multiple parasites can interact and have negative health consequences (Clark et al., 2016). The researchers in that study found microfilaria infections are positively correlated with malarial infections in birds. He found that nematodes caused raised levels of heterophils and decreased levels of lymphocytes in host blood. This affected the host’s ability to use antigens to respond to other infections and thus increased host susceptibility. Another study found that an unidentified filarial parasite combined with Haemosporida was shown to cause a 90% mortality rate in wild purple martins, with young birds more likely to be stricken (Davidar and Morton, 2006).

III) What is the mode of transmission for the pathogen?

Several biting arthropods can be vectors of microfilaria, including sandflies, black flies, lice, biting midges, and *Culex* spp. mosquitoes (Bartlett, 2007). These arthropods act as both vectors and intermediate hosts; microfilaria undergo development in the vector before being transferred to a new avian host where they mature into adults (Anderson, 2000; Huang et al., 2017).

IV) What is the likelihood of transmission to wild FGSP?

Thus far, microfilaria have been confirmed in two cases for wild FGSP. Currently, one of the species remains undescribed. BLAST (Basic Local Alignment Search Tool) results indicate it is most closely related to *Dirofilaria repens* and *Loa Loa*. The other wild sample has been identified as *Aproctella sp.*, the same species found in captive birds at White Oak. Originally, the microfilaria at White Oak was identified as *Dirofilaria repens* but upon further characterization were amended to *Aproctella sp.* (pers. comm. April Childress, 2018). Further comparison is needed to see if this second wild sample is also *Aproctella sp.* All literature mention the difficulty in identifying microfilaria without the presence of adult worms.

V) Is this pathogen species-specific, or is transmission to other dry prairie birds possible?

This pathogen appears to be host specific. Sehgal et al., (2005) found evidence of microfilaria host specificity in African birds between ecosystems and countries separated by large swaths of savannah. Benedikt et al., (2009) found 6 specialist nematode taxa related to families and genera of their tropical bird hosts in Costa Rica. The authors theorize the possibility of vectors spreading microfilaria amongst differing avian hosts but conclude their findings show that to be untrue.

VI) What are the mitigation options that can be performed by the captive facilities to reduce possible spread of infection?

Mitigation for microfilaria in captive birds is similar to that recommended for coccidians. Quarantine, regular cleaning of cages and control of vectors are urged (Lloyd, 2003). Ivermectin, Levamisole, Fenbendazole and Mebendazole have all been used with varying success for treatment (Bartlett, 2007). However, dead adult worms left in situ after treatment can cause inflammatory responses and lesions leading to clinical disease (Bartlett, 2007). Lloyd (2003) also warns against the effects of toxic doses, methods of administration and reactions in captive birds. Thus, any treatment undertaken should be highly regulated if it occurs at all.

VII) How reliable are screening methods for this pathogen?

Both Hamer et al. (2013) and Clark et al. (2016) recommend a multi approach use for the identification of microfilaria. They recommend the use of molecular methods or PCR as the first step in detection. Clark et al. (2016) also uses the counting of blood cell types from blood films while Hamer et al. (2013) prefers antibody testing for further diagnosis. There are several issues reported with the use of blood film analysis, however. Bartlett, (2007) and Hamer et al., (2013) state that blood slides collected from the brachial vein face limitations as microfilaria normally reside in the main body of the host. Another limitation mentioned by Bartlett, (2007) is that while blood slides can be used to confirm diagnosis of microfilaria, they can't be used to identify species.

VIII) What are the health consequences of infection by the pathogen (can birds survive and reproduce while infected)?

Adult filarial nematodes are not generally considered pathogenic in birds when found in the lumen of blood vessels (Larrat et al., 2012). Issues tend to arise when the worms invade major organs such as those Bartlett (2009) found in American Crow hearts. In contrast, Larrat et al. (2012) found that microfilaria in the lung blood vessels of boreal owls, already stressed by environmental conditions, contained such heavy loads of microfilaria that the circulation system was effected and probably contributed to the death of several birds. Mechanical irritation, dyspnea, anorexia, pneumonia and lethargy have also been documented in wild birds (Santiago-Alarcon and Merkel, 2018).

An important interaction that can occur between arboviruses and microfilaria in vector species (Vaughan & Turell, 1996) is the enhancement of arboviruses. This process happens when a vector ingests an arbovirus, such as Eastern Equine Encephalitis (EEE), and a nematode parasite at the same time. Consequently, the incubation time for the virus in mosquitoes is lowered, the infectious dose is lowered and incompetent vectors can be transformed into competent ones (Vaughan & Turell, 1996). While the rate of occurrence in the wild is unknown, it is suspected that this process plays an important role in arbovirus transmission cycles especially in the tropics (Vaughan & Turell, 1996). For the FGSP, this process could cause increased risk of exposure to a fatal arbovirus such as EEE.

Gaps in knowledge

The gaps in our knowledge of the prevalence of pathogens in FGSPs are related to the fact that this is the first formal effort to address disease concerns in the critically endangered FGSPs. The subsequent listed items are other identified gaps that could provide crucial future information for management of the FGSP.

1. The incidence of pathogen prevalence and infection in wild FGSP.
2. The species identification of documented parasites in the FGSP.
3. The role of immunosuppression in disease and pathogen virulence carried by the FGSP.
4. Stress levels and their contribution to morbidity and mortality experienced in the wild by the FGSP.

Closing and summary remarks

The following points have been suggested by the above review of literature:

1. The pathogens in question are mostly spread through close contact and crowding whether in captivity or in the wild. As a critically endangered and mostly solitary species, the FGSP might not be at high risk in the wild. Contact between mates and between parents and nestlings, may provide an exception, although this has not been conclusively determined. Captive birds face different challenges due to enforced intimacy in pens. These often crowded conditions are ideal for transmission amongst species (Adkesson, 2005; Norton et al., 2007; Yang et al., 2016).
2. Stress and subsequent immunosuppression can render pathogens more pathogenic particularly in captivity. Wild birds can also experience stress related to the breeding season, migration and habitat fragmentation (Allen & Fetterer, 2002; Dolnik & Hoi, 2010; Globakar et al., 2007; Horak et al., 2004; Norton et al., 2007; Xie & Nevins, 2012). Until more is known about the stress levels of the FGSP in the wild, it will be hard to predict the role immunosuppression plays in infection rates.
3. Double or multiple infection consisting of more than one type of parasite or of a parasite and a virus can also cause these pathogens to become more pathogenic. Some parasites have an additive effect by suppressing the immune system or bypassing barriers that allow other species to invade. Others may cause the evolution of more virulent strains of the particular organism (Adkesson et al., 2005; Clark et al., 2016; Dolnik & Hoi, 2009; Dolnik et al., 2010; Globaker et al., 2007; Hamer et al., 2013).
4. Medicine and treatment may not always be the best course of action for the presence of parasites. Negative effects that can occur include loss of immunity, reduced microbiome from antibiotics, overdoses and clinical disease (Bartlett, 2007; Gerhold et al., 2011; Horak et al., 2004; Lloyd, 2003). Drugs may also only temporarily suppress infections (Norton et al., 2007; Xie & Nevins, 2012; Yabsley, 2008) and treatments in both wild and captive populations could be an incredibly expensive exercise in terms of resources, time and effort.

5. The use of molecular techniques such as PCR and DNA or RNA sequencing is the most common, fastest and accurate way to test for and identify pathogens. The quick turnaround time of these methods allows for time sensitive management decisions to be made much faster than previously allowed. Unfortunately, false negatives can also occur if viremia levels are below those of detection. In an effort to help prevent this from occurring, where possible multiple methods of detection should be used such as blood film analysis, PCR and ELISA tests (Adkesson et al., 2005; Bertram et al., 2014; Clark et al., 2016; Dolnik et al., 2009; Dusek & Forrester, 2002; Hafeez & Barta, 2017; Hamer et al., 2013; Huang et al., 2017; Norton et al., 2007; Schnitzler et al., 1998; Schrenzel et al., 2003; Yang et al., 2016; Yang et al., 2018).

Annotated Bibliography

Adkesson, M.J., Zdziarski, J.M., and Little, S.E., 2005. Atoxoplasmosis in Tanagers. *Journal of Zoo and Wildlife Medicine*, 36(2), pp 265-272.

This article described the use of PCR on various tissues to determine the presence of Atoxoplasmosis. It also discussed treatment options, signs of infection and general information about coccidians.

Allen, P.C. & Fetterer. 2002. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clinical Microbiology Reviews*, 15(1), pp. 58-65.

This paper was about the study and use of vaccines in domestic poultry i.e. chickens. It also discussed immunology and had good information on immunosuppression in sick animals.

Anderson RC (2000) Nematode Parasites of Vertebrates. CABI Publishing, New York.

This book gave information on the characteristics and ecology of nematode worms.

Arabkhazaeli, F., & Madani, S.A. 2014. Visceral Coccidiosis in a Common Mynah (*Acridotheres tristis*) due to *Isospora* sp. infection. *Iranian Journal of Veterinary Medicine*, 8(1), pp. 59-62.

This paper was about the presence of coccidians in a dead common mynah, what the necropsy found and what treatments were tried to save the bird.

Bartlett, C.M. (2007). Filarioid Nematodes. In Thomas, N.J., Hunter, D.B., Atkinson, C.T. (Eds.), *Parasitic Diseases of Wild Birds* (pp. 439-462). Ames, IA: Blackwell Publishing.

This was a good informational chapter on Filarioid nematodes with plenty of examples.

Benedikt V., Barus V., Capek M., Havlicek M., Literak I. 2009. Blood parasites (*Haemoproteus* and microfilariae) in birds from the Caribbean slope of Costa Rica. *Acta Parasitology*, 54:197–204.

This paper was cited a lot and had good information on frequency of microfilaria in tropical birds as well as host specificity.

Bertram, M.R., Hamer, G.L., Snowden, K.F. Hartup, B.K. & Hamer, S.A. 2014. Coccidian Parasites and Conservation Implications for the Endangered Whooping Crane (*Grus americana*).

In their study, they discuss in depth the molecular methods used in diagnosis. They also extensively used BLAST to map out the phylogenetics of the *Eimeria* species they found in Whooping Cranes. The impact of this species on endangered birds were also informative.

Berto, B.P., Flausino, W., McIntosh, D., Teixeira-Filho, W.L. & Lopes, C.W. G Coccidia of New World passerine birds (Aves: Passeriformes): a review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) *Systems in Parasitology*, (80):159–204.

This paper is an overview of coccidia that are found in New World birds. It also discusses the two new species that Berto et al. found in New World passerines.

Botzler, R.G. & Brown, R.N. 2014. *Foundations of Wildlife Diseases*. Oakland, CA: University of California Press.

This text book contained relevant information on the diseases that can be found in wildlife. It gave good general information on coccidians especially *Eimeria* sp.

Brawner, W. R. I., Hill, G. E. & Sundermann, C. A. 2000 Effects of coccidial and mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. *Auk* 117, 952-963.

This paper describes the effect of coccidians on plumage as well as dietary uptake restrictions.

Buchholz, R. (1995) Female choice, parasite load and male ornamentation in wild turkeys. *Animal Behaviour*, 50: 929-943.

This article discussed the effect that coccidians have on sexual characteristics in male wild turkeys and how these effected female choice as an indicator of make energy reserves.

Clark, N.J., Wells, K. Dimitrov, D., & Clegg, S.M. 2016. Co-infections and environmental conditions drive the distributions of blood parasites in wild birds. *Journal of Animal Ecology*, 85, pp. 1461–1470.

This was a well conducted study on the ways that double infections from microfilaria and Malaria can drive infection. It also gave alternative methods for detection such as the counting of blood cell types.

Davidar P. & Morton ES. 2006. Are multiple infections more severe for purple martins (*Progne subis*) than single infections? *Auk* 123(1):141–147.

This article is about the study of microfilaria on purple house martins. It's full of surprising information about the effects of double infection on wild, young birds.

Dolnik, O.V., Dolnik, V.R., & Bairlein, F. 2010. The effect of host foraging ecology on the prevalence and intensity of coccidian infection in wild passerine birds. *Ardea*, 98(1), pp 97-103.

This paper discussed the role that host foraging, feeding habitats, and social behavior played on transmission of coccidian infection. The authors found that large flocks of ground dwelling, fruit eating birds were the most likely to be infected.

Dolnik, O.V. & Hoi, H. 2010. Honest Signalling, dominance hierarchies and body condition in House Sparrows *Passer domesticus* (Aves: Passeriformes) during acute coccidiosis. *Biological Journal of the Linnean Society*, 99: 718-726.

This paper was very informative about how sexual characteristics and body condition are changed by the presence of coccidian infection in birds with dominance hierarchies.

Dolnik, O.V. & Loonen, M.J.J.E. 2007. *Isospora plectrophenax* n. sp (Apicomplexa: Eimeriidae), a new coccidian parasite found in Snow Bunting (*Plectrophenax nivalis*) nestlings on Spitsbergen. *Parasitology Research*, 101:1617–1619.

This article discusses the discovery of an *Isospora* sp. and its host specificity.

Dolnik, O.V., Palinauskas, V. & Bensch, S. 2009. Individual oocysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian feces: from photo to sequence. *Journal of Parasitologists*, 95(1), pp. 169-174.

This article had great information about the laboratory work involved in identifying coccidians.

Dusek R.J., Forrester D.J. 2002. Blood parasites of American crows (*Corvus brachyrhynchos*) and fish crows (*Corvus ossifragus*) in Florida, USA. *Comparative Parasitology*, 69:92–96.

This paper had useful information about the presence of microfilaria in crow species in Florida as well as their effects on the birds. It also mentions that blood film analysis is not the best diagnostic tool.

Foster et al. 1997. University of Florida: Unpublished data.

The entry for the unpublished data on FGSP coccidians. Found in: Forrester, A.J. and Spalding, M.G. 2003. Parasites and diseases of wild birds in Florida. Gainesville, FL: University Press of Florida.

An informative book with lots of local information. It needs to be updated.

Friend, M. and Franson, J.C. 2001. Field Manual of Wildlife Disease: General Field Procedures and Diseases of Birds. A U.S. Geological Survey Publication, https://www.nwhc.usgs.gov/publications/field_manual/field_manual_of_wildlife_diseases.pdf

This publication provided background information and descriptions of coccidians.

Gerhold, R. W., A. L. Fuller, L. Lollis, C. Parr, and L. R. McDougald. 2011. The efficacy of anti-coccidial products against *Eimeria* spp. in Northern bobwhites. *Avian Diseases*, 55(1): 59–64.

This paper discusses a study of controlling coccidiosis with treatments used on chickens. It also gives information about the effects of the disease and a list of anti-coccidial medicines tested in the study.

Globokar, M., Fischer, D. and Pantchev, N. 2017. Occurrence of Endoparasites in captive birds between 2005 to 2011 as determined by faecal floatation and review of literature. *Berlin and Munich Veterinary Weekly*. Published online: <https://vetline.de/open-access/158/3126/>.

This article was about a study performed in Germany, whereby scientists evaluated large amounts of fecal samples taken from wild birds over six years. It contained relevant information on coccidians in wild birds, how to test for them, and what species were found.

Hafeez, M.A. & Barta, J.R. 2017. The complete mitochondrial genome sequence of two *Isospora* species (Eimeriidae, Eucoccidiorida, Coccidiasina, Apicomplexa) causing coccidiosis in superb glossy starlings, *Lamprotornis superbus* (Aves: Sturnidae). *Mitochondrial DNA Part B: Resources*. 2(2): 895-896.

This paper provides information on the discovery of new species of *Isospora* in Passerines. This paper is also one of the papers recommended from the BLAST search for the *Isospora* found in the wild FGSP.

Hamer, G.L., Anderson, T.K., Berry, G.E., Makohon-Moore, A.P., Crafton, J.C., Brawn, J.D....et al. 2013. Prevalence of filarioid nematodes and trypanosomes in American robins and house sparrows, Chicago, USA. *International Journal for Parasitology: Parasites and Wildlife*, 2, pp. 42-49.

This is a great paper talking about a microfilaria study performed on songbirds that are gregarious and thus at higher risks of transmission. The authors discuss enhancement of arboviruses, molecular techniques for diagnosis and identification, and interactions of parasites.

Heatley, J. J., Payne, S. and Tizard, I. 2017. Avian Vaccines: Current Options and Strategies. *Veterinary Clinics of North America: Exotic Animal Practice, Therapeutics*, 21(2), pp. 379-397.

This was a report about the issues surrounding avian vaccinations. It mostly discussed that not enough reliable vaccines are commercially available vs how many have been tested and that that needs to change despite difficulties.

Horak, P., Saks, L., Karu, U., Ots, I., Surai, P.F., & McGraw, K.J. 2004. How coccidian parasites affect health and appearance of greenfinches. *Journal of Animal Ecology*, 73, pp. 935-947.

This paper analyzed the effect of coccidians on the health and expression of traits in greenfinches. It also described treatment effects and different ways to test for coccidians.

Huang, Y.L., Tsai, S.S., Thongchan, D., Khatri-Chhetri, R. & Wu, H.Y. 2017. Filarial nematode infection in eclectus parrots (*Eclectus roratus*) in Taiwan. *Avian Pathology*, 46(2), pp. 188-194.

The article describes the methods used to find filarial nematodes in wild parrots. Also, has lots of good background information on coccidian locations in the bird body.

Krautwald-Junghanns, M.E., Zebisch, & R. Schmidt, V. 2009. Relevance and Treatment of Coccidiosis in Domestic Pigeons (*Columba livia formadomestica*) with Particular Emphasis on Toltrazuril. *Journal of Avian Medicine and Surgery*, 23(1): 1-5.

This article had a lot of great information about how to prevent and treat coccidiosis in domestic pigeons.

Larrat, S., Dallaire, A.D. & Lair, S. 2012 Emaciation and larval filarioid nematode infection in boreal owls (*Aegolius funereus*). *Avian Pathology*, (41):4, pp. 345-349.

This study had great general information about nematodes as well as a very interesting study about the effects of nematodes in potentially causing the deaths of Boreal Owls.

Londono, A. P., Pulgarin, R. C. & BLAIR, S. 2007. Blood parasites from the lowlands of northern Colombia. *Caribbean Journal of Science*, 53: 87-93.

This paper gave examples of microfilaria presence in South America.

Lopez, G., J. Figuerola, and R. Soriguer. 2007. Time of day, age and feeding habits influence coccidian oocyst shedding in wild passerines. *International Journal for Parasitology*, 37(2007): 559-564.

This article discusses the effects of coccidiosis as well as shedding of oocysts in birds.

Lloyd, C. 2003. Control of nematode infections in captive birds. *In Practice*, 25, pp. 198-206.

An article in a veterinarian magazine, this contained lots of concise helpful information about treatment, diagnoses and examples of nematodes.

McGraw, K.J. & Hill, G.E. (2000) Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. *Proceedings of the Royal Society of London B: Biological Sciences*, 267, 1525-1531.

This paper also gave information about the effects of *Isospora* on colorization in American Goldfinches.

- Norton, T.M., Neiffer, D.L., Seibels, B., Benson, K., McAloose, D. Travis, D. Gentz, N. (March 27th, 2003; updated 2007). *Atoxoplasma* medical protocols recommended by the Passerine *Atoxoplasma* working group. Retrieved from: <https://www.aazv.org/general/custom.asp?page=545>
This article was a summary of studies that had been done on Atoxoplasmosis in zoological birds. It contained information on treatment, studies, and diagnosis of the organism.
- Sainsbury A.W., & Vaughan-Higgins R.J. 2012. Analyzing disease risks associated with translocations. *Conservation Biology*, 26(3):442-52.
There was a lot of great information in this paper about how and why to conduct a disease risk assessment when enacting a translocation. This paper mentions coccidiosis in an endangered bird.
- Santiago-Alarcon, D. & Merkel, J. 2018. Chapter 7: New Host-Parasite Relationships by Host-Switching. P.G. Parker (ed.), *Disease Ecology, Social and Ecological Interactions in the Galapagos Islands*, Springer, Cham.
This chapter talks about the possibility of host switching in microfilaria, symptoms, techniques and the effects of more than one parasite on birds.
- Sehgal R.N.M., Jones H.I., Smith T.B. 2005. Molecular evidence for host specificity of parasitic nematode microfilariae in some African rainforest birds. *Molecular Ecology*. 14:3977–3988.
This paper talked about host specificity in microfilaria in African birds.
- Schnitzler, B.E., Thebo, P.L., Mattsson, J.G., Tomley, F.M., & Shirley, M.W. 1998. Development of a diagnostic PCR assay for the detection and discrimination of four pathogenic *Eimeria* species of the chicken. *Avian Pathology*, 27: 490-497.
This paper gave great detail on how to run and analyze PCR results on *Eimeria*.
- Schrenzel, M.D., Maalouf, G.A., Gaffney, P.M., Tokarz, D., Keener, L.L., McClure, D., Griffey, S., McAloose, D. and Rideout, B.A. 2003. Molecular characterization of Isosporoid coccidia (*Isospora* and *Atoxoplasma* spp.) in passerine birds. *Journal of Parasitology*, 91(3): 635-647.
This paper had good details about what coccidians are and how to detect then using PCR and phylogenetic analysis.
- Silveira, P., Nayara, N.O., Rodello, D., Pinheiro, R.T., and Braga, E.M. 2010. Microfilariae Infection in Wild Birds from the Brazilian Cerrado. *Journal of Wildlife Diseases*, 46(4):1305-1309.
This paper had information about the frequency of microfilaria in grassland birds.
- Spalding, M.G., Carpenter, J.W., & Novilla, M.N. (2007). Disseminated Visceral Coccidiosis in Cranes. In Thomas, N.J., Hunter, D.B., Atkinson, C.T. (Eds.), *Parasitic Diseases of Wild Birds* (pp. 181-194). Ames, IA: Blackwell Publishing.
This was a special chapter devoted entirely to Disseminated Visceral Coccidiosis in Cranes and gave great descriptions of the disease.
- Vaughan, J.A., & Turell, M.J. 1996. Dual Host Infections: Enhanced Infectivity of Eastern Equine Encephalitis Virus to *Aedes* mosquitoes mediated by *Brugia* Microfilariae. *The American Journal of Tropical Medicine and Hygiene*, 54(1), pp. 105-109.
A carefully led laboratory study on the enhancement of arboviruses caused by microfilaria in mosquitoes.

Woodford, M.H. and Rossiter, P.B. 1993. Disease risks associated with wildlife translocation projects. *Scientific and Technical Review of the Office International des Epizooties*, 12(1), pp. 115-135.

This paper was good background reading as well as talked about the use of the vaccine for Whooping cranes.

Xie, S. & Nevis, J, 2012. Coccidiosis as a Cause of Death in Orphaned American Robins: A Pilot Study in Assessing the Effectiveness of Ponazuril as a Means of Reducing Mortality Rate. *Journal of Wildlife Rehabilitation*, 32(3): 7-10.

This study was very informative about the effect of coccidia infection on young American Robins in captivity and the use of medication in treatment.

Yabsley, M.J. (2007). Eimeria. In Thomas, N.J., Hunter, D.B., Atkinson, C.T. (Eds.), *Parasitic Diseases of Wild Birds* (pp. 162-180). Ames, IA: Blackwell Publishing.

This was an informative chapter in this book specifically about Eimeria in wild birds. It was very helpful.

Yang, R., Brice, B. & Ryan, U. 2018. Morphological and molecular characteristics of *Isospora butcheriae* n. sp. in a silvereye (*Zosterops lateralis*) (Latham, 1801). *Parasitology Research*, 117: 1381-1388.

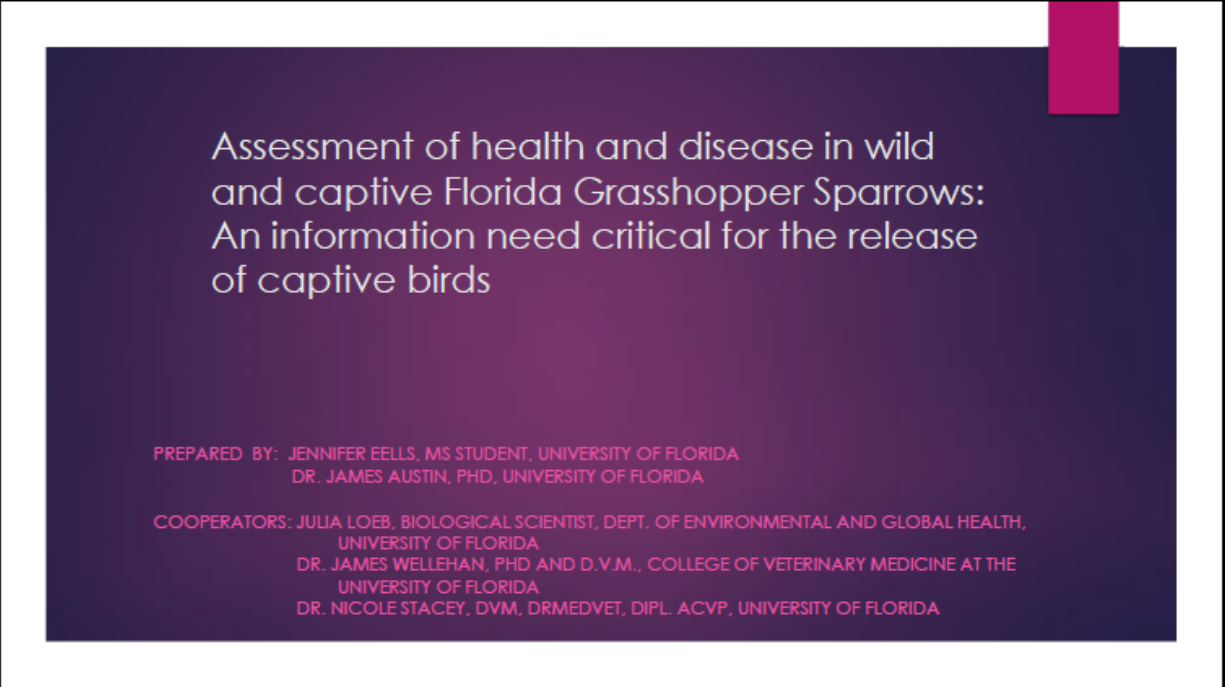
This paper was recommended during the BLAST search for the *Isospora* sp. found in the wild FGSP. It also gave helpful information about PCR analysis

Yang, R., Brice, B. & Ryan, U. 2016. Morphological and molecular characteristics of *Isospora neochmiae* n. sp. in a captive-bred red-browed finch (*Neochmia temporalis*) (Latham, 1802). *Experimental Parasitology*, 166: 181-188.

This paper was recommended during the BLAST search for the *Isospora* sp. found in the wild FGSP. It also gave helpful information about PCR analysis.

Appendix IV

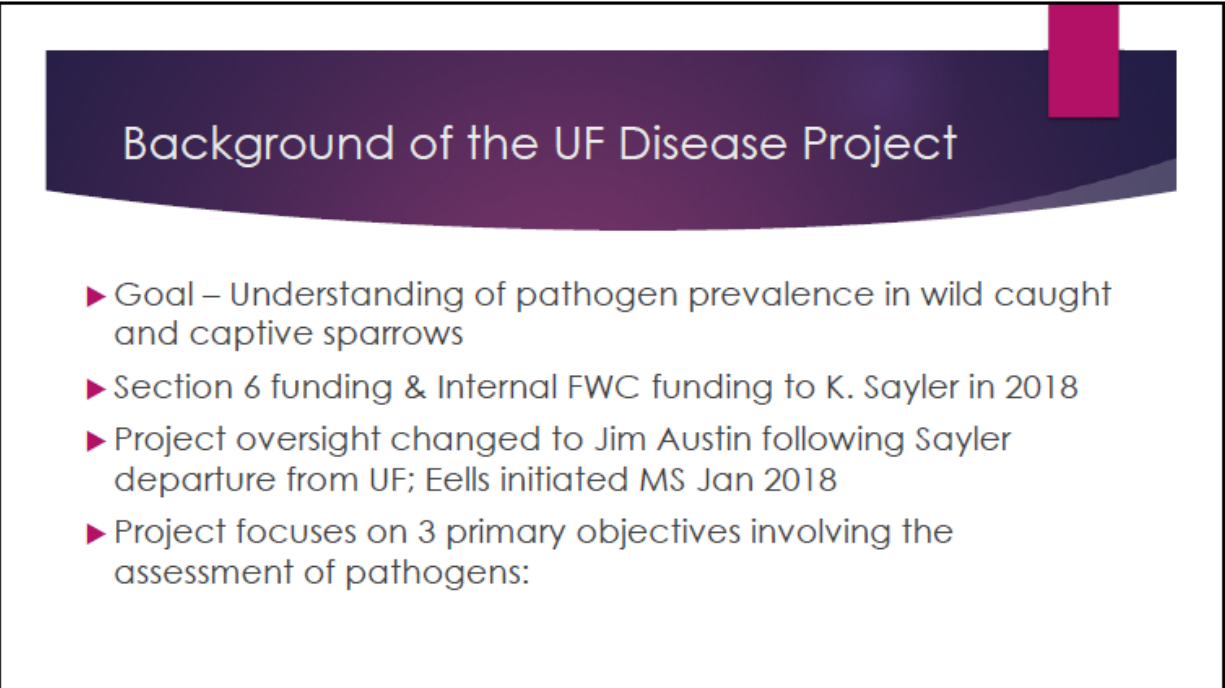
Data Summary Presentations from Workshop



Assessment of health and disease in wild and captive Florida Grasshopper Sparrows: An information need critical for the release of captive birds

PREPARED BY: JENNIFER EELLS, MS STUDENT, UNIVERSITY OF FLORIDA
DR. JAMES AUSTIN, PHD, UNIVERSITY OF FLORIDA

COOPERATORS: JULIA LOEB, BIOLOGICAL SCIENTIST, DEPT. OF ENVIRONMENTAL AND GLOBAL HEALTH, UNIVERSITY OF FLORIDA
DR. JAMES WELLEHAN, PHD AND D.V.M., COLLEGE OF VETERINARY MEDICINE AT THE UNIVERSITY OF FLORIDA
DR. NICOLE STACEY, DVM, DRMEDVET, DIPL. ACVP, UNIVERSITY OF FLORIDA



Background of the UF Disease Project

- ▶ Goal – Understanding of pathogen prevalence in wild caught and captive sparrows
- ▶ Section 6 funding & Internal FWC funding to K. Sayler in 2018
- ▶ Project oversight changed to Jim Austin following Sayler departure from UF; Eells initiated MS Jan 2018
- ▶ Project focuses on 3 primary objectives involving the assessment of pathogens:

Project objectives

- ▶ **Objective 1:** Assess the prevalence of pathogens with vector borne transmission in wild and captive populations
- ▶ **Objective 2:** Assess the prevalence of potential pathogens with direct transmission either fecal or oral in wild and captive populations
- ▶ **Objective 3:** Characterize the virome diversity in adult and nestling sparrows from wild and captive populations

2017/2018 Samples from Field Sites

Site	Whole Blood	Blood Film Slides	Fecal samples
Three Lakes WMA	80	31	~112
Avon Park	1	1	0
Kissimmee Prairie State Park	4	0	4
The Ranch	14	8	15
White Oak (WO)	58	34	0

West Nile Virus (Objective 1)

- ▶ Birds tested:
 - ▶ Wild birds → 43 (0 pos)
 - ▶ WO birds → 30 (0 pos)
- ▶ Methods:
 - ▶ Q-PCR on RNA



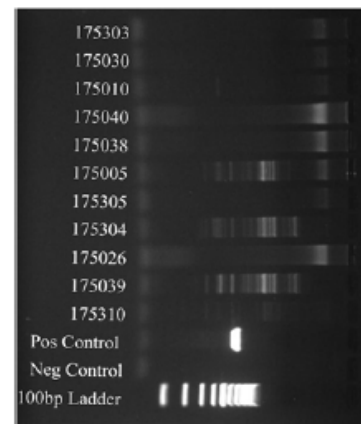
Eastern Equine Encephalitis (1)

- ▶ Birds tested:
 - ▶ Wild birds → 53 (0 pos)
 - ▶ WO birds → 30 (1 pos)
- ▶ Methods:
 - ▶ Q-PCR on RNA



Rickettsia sp. (1)

- ▶ Birds tested:
 - ▶ Wild birds → 64 (0 pos)
 - ▶ WO birds → 11 (0 pos)
- ▶ Methods:
 - ▶ PCR: 631 bp region of OmpA (rOmpA) gene



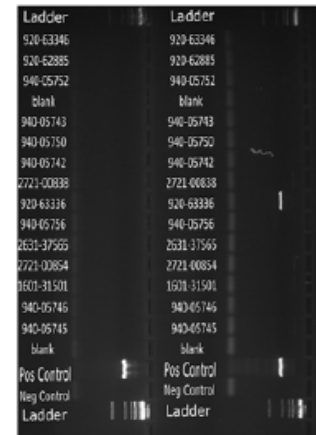
Ehrlichia sp. (1)

- ▶ Birds tested:
 - ▶ Wild birds → 64 (0 pos)
 - ▶ WO birds → 11 (0 pos)
- ▶ Methods:
 - ▶ PCR: groESL heat shock operon



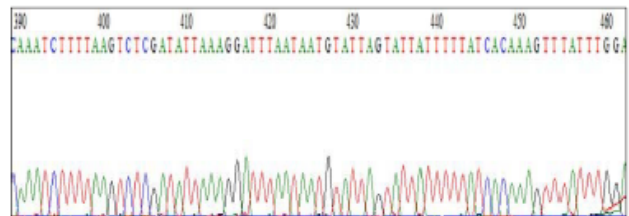
Haemosporidians (1)

- ▶ Birds tested:
 - ▶ Wild birds → 63 (2 pos)
 - ▶ WO birds → 11 (2 pos)
- ▶ Methods:
 - ▶ PCR: 479 bp region of mtDNA (cytochrome b gene)
 - ▶ Sanger Sequencing



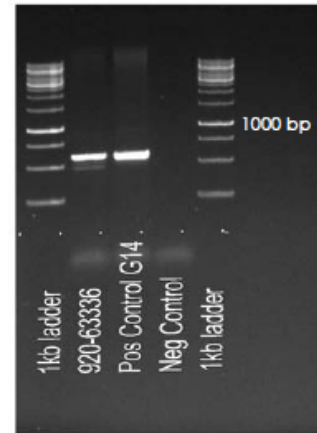
Haemosporidians (1)

- ▶ Species:
 - ▶ Plasmodium P04-B
 - ▶ 99% match
 - ▶ Cosmopolitan
 - ▶ Wild & WO birds



Microfilaria (1)

- ▶ Birds tested:
 - ▶ Wild birds → 71 (2 pos)
 - ▶ WO birds → 21 (8 pos)
- ▶ Methods:
 - ▶ PCR of 18s rRNA nuclear gene & CO1 mtDNA
 - ▶ Sanger sequencing
 - ▶ Visual blood film analysis



Microfilaria sequence comparisons

Species	18S (nuclear)		CO1 (mitochondrial)	
	% identical	Accession #	% identical	Accession #
<i>Aproctella</i> sp.	98%	AB973229	95-96%	FR823334
<i>Loa loa</i> (African eyeworm)	98%	XR_002251421	89%	AJ544875
<i>Dirofilaria repens</i>	98%	AB973229	88%	MH780816

Ranch/Rare Species bird 940-05707:

- ▶ Positive in 2017 (Wild)
 - ▶ Confirmed UF PCR
 - ▶ Confirmed Blood slide
- ▶ Negative in 2018 (Captive)
 - ▶ Confirmed UF PCR
 - ▶ Confirmed UGA PCR

WHY????

Periodicity of microfilaria in blood?
False negative?
Drug Treatment?

Coccidians (Objective 2)

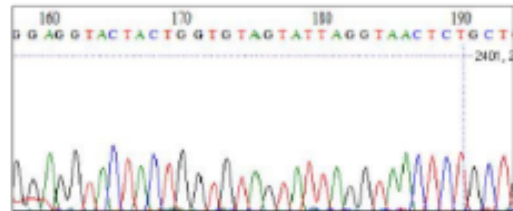
- ▶ Birds tested:
 - ▶ Wild birds → 54 (1 pos)
 - ▶ WO birds → 22 (13 pos)
- ▶ Methods:
 - ▶ PCR: Subunit I of the cytochrome c oxidase gene (C01)
 - ▶ Fecal Samples: 6 sent to Wellehan Lab
 - ▶ All negative



Coccidians (2)

► Species:

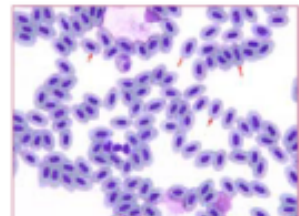
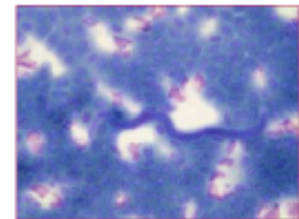
- *Isospora* sp. RY2015c (wild)
 - 97% match
- *Isospora greineri* (captive)
 - 99% match
 - Cosmopolitan
- Wellehan Lab has 4 unique *Isospora* genotypes identified



Blood Film Slides (2)

► Samples tested:

- ~51 wild slides → 1 pos for microfilaria
 - 940-05707, The Ranch
- WO birds → 4 pos for microfilaria
- 9 wild slides → small round distinct basophilic inclusions or signet ring structures of unknown origin



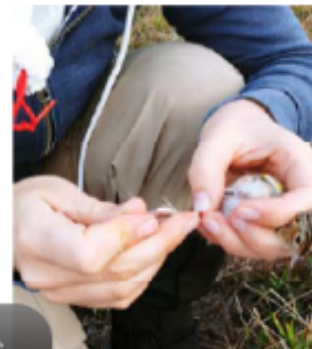
Viral Fecal Samples (Objective 3)

- ▶ Samples tested:
 - ▶ Wild birds → ~76 samples (0 pos)
- ▶ Methods:
 - ▶ Vero E6 (ATCC CRL-1586) cell culture
 - ▶ Chicken embryo fibroblast cells
 - ▶ Blind passaged at later date



2017/2018 Coinfections

- ▶ Coccidia and Microfilaria
 - ▶ WO birds → 3
- ▶ Coccidia and Plasmodium
 - ▶ WO birds → 2
- ▶ Plasmodium and Microfilaria
 - ▶ Wild birds → 1
 - ▶ 920-63336, TLWMA



Summary

- ▶ Haemosporidian infection rate
 - ▶ Wild → 3%
 - ▶ WO → 18%
- ▶ Coccidian infection rate
 - ▶ Wild → 2%
 - ▶ WO → 60%
- ▶ Microfilaria infection rate
 - ▶ Wild → 3%
 - ▶ WO → 38%



Photo by Neil Pearson/FWC Fish and Wildlife Research Institute (Creative Commons)

Questions?

- ▶ Jennifer Eells
 - ▶ jeells0@ufl.edu
- ▶ James Austin
 - ▶ austinj@ufl.edu



White Oak Conservation

Ammodramus savannarum

floridanus

Updates

○○○

11/26/2018

2018 Disease Risk Assessment Meeting

Basics:

82 FGSP (*A. s. floridanus*) over time at WO

44 animals have died, 35 necropsies performed

17 had evidence of coccidian parasitism (2 had inflammation only, no organisms)

10 had evidence of microfilaria, 3 with evidence of filarial nematodes in air sacs

34 fecal samples performed (some pooled), 7 positive for coccidians (cytology)

29 blood smears from 27 individuals, 6 positive for coccidians (cytology), 1 positive for microfilaria (cytology)

Adult air sac filarioidal nematodes and air sacculitis cases

	Case #1 - 0.1	Case #2 - 1.0	Case #3 - 1.0
DOB	8/10/16	Unk	6/3/17
Arrival at WO	8/14/16	8/16/16	6/3/17
Bloodwork results	NEG 9/26/16 POS 11/16/16	N/A	N/A
Date of Death	12/10/16 (124 days)	12/9/17*	8/18/17 (76 days)
Comorbidities	Coelomitis, dermatitis, epicarditis - unknown if all changes related to filarioid	Acute pulmonary hemorrhage	Intestinal Isospora, Squamous metaplasia, ureteral stones and rupture
Sequencing performed	No	<i>Aproctella</i> (Jim Austin's lab?)	No

*Last microfilaria seen on 12/9/17 via cytology or histopathology in FGHS

Microfilaremia in FGSP at WO (all born at WO)

	DOB	Age at death (days)	EIC?	Vit A def?	Other
4	5/24/17	37	X	X	
5	5/24/17	44	X	X	
6	5/24/17	75	X	X	
7	6/3/17	30	X		
8	6/4/17	53	X	X	
9	6/8/17	30	X		Only 1 microfilaria seen
10	6/8/17	54	X	X	

Microfilaria additional information

Hematology information:

27 individuals, 29 samples, 22 still alive

1 positive for microfilaria

Age of venipuncture - Mean 48.6d, Range 10-231d

Data skewed towards fledgling/independent hatch year birds

Coccidian additional information

Cases confirmed on necropsy:

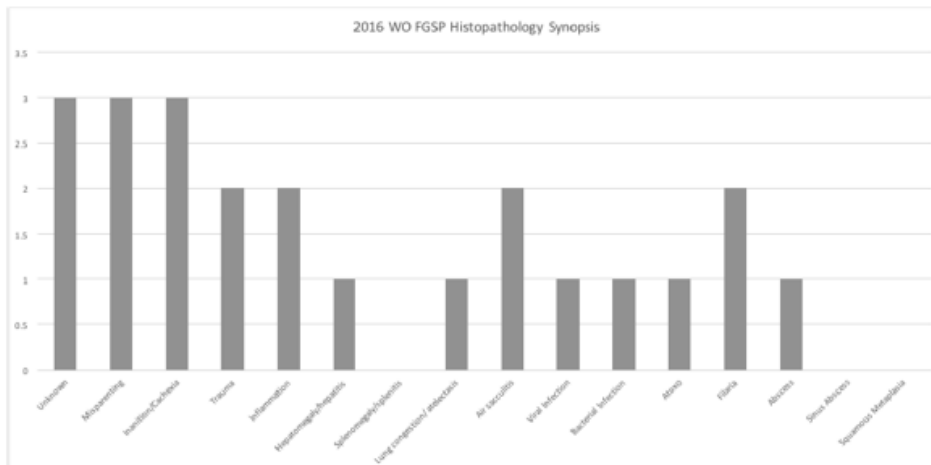
Mean age 37 days, range 10-76 days

8/17 cases also had microfilaria present

2 had venipuncture performed antemortem with positive ID cytologically

4 genotypes identified via molecular techniques

2016 Necropsy Data (N = 4)



Prominent Findings:

Trauma

Misparenting

Air sacculitis

2017 Necropsy Data (N = 24)

Prominent findings:

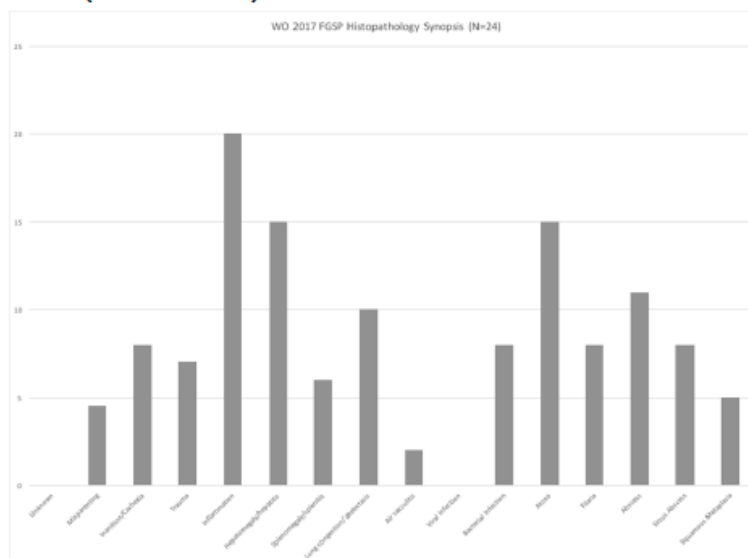
Inflammation

Abscess and squamous metaplasia

Coccidian disease

Hepatomegaly/hepatitis

Filarial disease



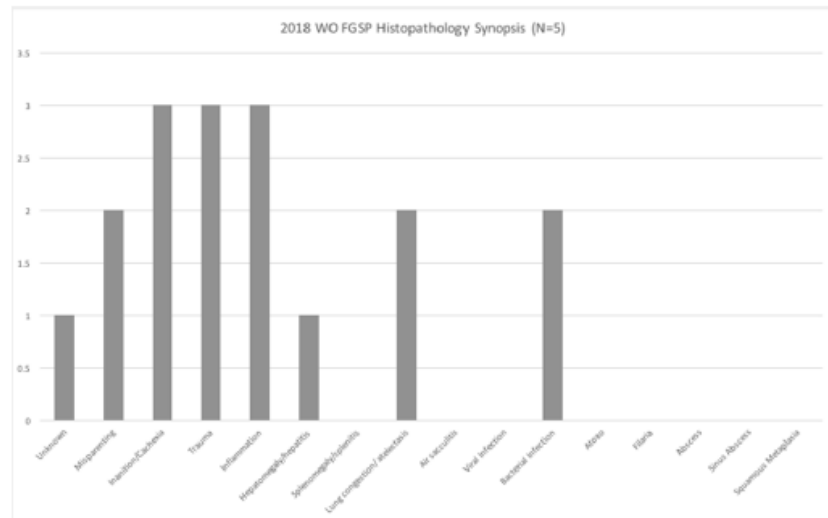
2018 Necropsy Data (N = 5)

Prominent Findings:

Trauma, inflammation,
bacterial infection

NO Coccidial disease

NO Filarial disease



Appendix V

Documents and literature provided to participants in advance of the Disease Risk Assessment Workshop

Protocols, meeting notes, progress reports, and presentations

- Flip chart and collection plan from the Florida grasshopper sparrow planning meeting, March 2018.
- Reports from captive-breeding facilities
 - 2016 Rare Species Conservatory Foundation Annual Report
 - 2016 White Oak Annual Report
 - 2016-2017 Rare Species Conservatory Foundation PowerPoint
 - 2017 Rare Species Conservatory Foundation interim report
 - 2017 White Oak PowerPoint (October)
- Health and disease documents
 - White Oak 2017-18 Pathology presentation “From the Path Lab to the Flock”
 - White Oak FGSP Isospora genotype spreadsheet
 - White Oak FGSP blood smear analysis
 - Rare Species presentation for 2016-2017 WO Health Meeting
 - FGSP WO Health Meeting minutes, February 2017
 - Morris Animal Foundation FGSP Disease Abstract/Proposal, Katherine Saylor
 - Ticks and the Endangered Grasshopper sparrow presentation, Katherine Saylor
 - Dr. Branson Ritchie’s response to risk questions regarding Filarids and EIC
 - Dr. Scott Citino’s response to risk questions regarding Filarids and EIC
 - 1st Interim Report for Jim Austin’s UF team FGSP pathogen analysis
 - FGSP health summary factsheet, May 2018
 - FGSP health team directory, February 2018
 - Captive health threats, April 2018
 - Various necropsy and bacteriology reports
- Husbandry documents
 - Rare Species hand-rearing protocol
 - Rare Species general protocols
 - White Oak captive breeding husbandry improvement plans
 - White Oak handling and diet protocols sparrows

Reports and peer-reviewed literature

- Bartlett, C.M. 2008. Filarioid Nematodes. Pages 439–461 in *Parasitic Diseases of Wild Birds* (C.T. Atkinson, N.J. Thomas, and D.B. Hunter, eds.). Wiley and Sons Press, New York, New York, USA.
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- Carleton R.E. 2007. Disease ecology of a population of Eastern bluebirds (*Sialia sialis*) in Georgia: A starting point. Dissertation thesis. University of Georgia, Athens, Georgia, USA.
- Dolnik O.V., V.R. Dolnik, and F. Bairlein. 2010. The effect of host foraging ecology on the prevalence and intensity of coccidian infection in wild passerine birds. *Ardea* 98:97–103.
- Eells, J. 2018. A literature review of pathogens that may cause morbidity or mortality in the critically endangered Florida grasshopper sparrow. Internal report. University of Florida, Gainesville, Florida, USA.
- Forrester D.J. and M.G. Spalding. 2003. *Parasites and Diseases of Wild Birds in Florida*. University Press of Florida, Gainesville, Florida, USA.
- Hamer G.L., T.K. Anderson, G.E. Berry, A.P. Makohon-Moore, J.C. Crafton, J.D. Brawn, A.C. Dolinski, B.L. Krebs, M.O. Ruiz, P.M. Muzzall, T.L. Goldberg, and E.D. Walker. 2013. Prevalence of filarioid nematodes and trypanosomes in American robins and house sparrows, Chicago USA. *International Journal for Parasitology: Parasites and Wildlife* 2:42–49.
- Hõrak P., L. Saks, U. Karu, I. Ots, P.F. Surai, and K.J. McGraw. 2004. How coccidian parasites affect health and appearance of greenfinches. *Journal of Animal Ecology* 73:935–947.
- Knight A., J.G. Ewen, P. Brekke, and A.W. Santure. 2018. The evolutionary biology, ecology, and epidemiology of coccidia of passerines birds. *Advances in Parasitology* 99:35–60.
- Larrat S., A.D. Dallaire, and S. Lair. 2012. Emaciation and larval filarioid nematode infection in boreal owls (*Aegolius funereus*). *Avian Pathology* 41:345–349.
- Mcgill I., Y. Feltrer, C. Jeffs, G Sayers, R.N. Marshall, M.A. Peirce, M.F. Stidworthy, A. Pocknell, and A.W. Sainsbury. Isosporoid coccidiosis in translocated ciril buntings (*Emberiza cirilus*). *The Veterinary Record* 167:656–660.
- Norton T.M., D.L. Neiffer, B. Seibels, K. Benson, D. McAloose, D. Travis, E. Greiner, K. Latimer, S.E. Little, J.M. Zdiarski, M. Schrenzel, B. Rideout, M. Vince, and N. Gentz. *Atoxoplasma* medical protocols recommended by the passerine *Atoxoplasma* working group. American Association of Zoo Veterinarians.
- Pranty B. and J.W. Tucker Jr. 2006. Ecology and management of the Florida grasshopper sparrow. Pages 188–200 in *Land of Fire and Water: The Florida Dry Prairie Ecosystem* (R. Noss, ed.). Proceedings of the Florida Dry Prairie Conference.
- Ragheb E.L.H., and K.E. Miller. 2016. Demographic and disease assessment of the declining Florida grasshopper sparrow at Three Lakes Wildlife Management Area. Section 6 Final Report. Florida Fish and Wildlife Conservation Commission, Tallahassee, Florida, USA.
- Ragheb E.L.H., and K.E. Miller. 2018. Population monitoring and predator exclusion for Florida grasshopper sparrows at Three Lakes Wildlife Management Area. Section 6 Final Report. Florida Fish and Wildlife Conservation Commission, Tallahassee, Florida, USA.
- Ragheb E.L.H., and K.E. Miller. 2018. Identification and exclusion of nest predators of Florida dry prairie birds using video surveillance techniques and predator deflection fencing. Section 6 Final Report. Florida Fish and Wildlife Conservation Commission, Tallahassee, Florida, USA.
- Staley M. and C. Bonneaud. 2015. Immune responses of wild birds to emerging infectious diseases. *Parasite immunology* 37:242–254.
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Appendix VI

Florida Grasshopper Sparrow 5-Year Strategic Vision

Second Draft

Last updated 26 November 2018

EXECUTIVE SUMMARY

Florida grasshopper sparrow (FGSP; *Ammodramus savannarum floridanus*) populations have been in sharp decline despite intensive management and research efforts. Habitat loss, alteration of hydrology and fire regimes, and possibly disease, have played a role in the decline. As breeding pairs reached critically low numbers in 2015, the U.S. Fish and Wildlife Service (FWS) began a captive-breeding program to augment the wild population via captive-reared birds. The FWS and Florida Fish and Wildlife Conservation Commission (FWC), with input and feedback of the FGSP Working Group, present this FGSP 5-year Strategic Vision to guide management actions for the species. The goal of the vision is to stabilize and grow the wild population over the next five years through habitat management, wild population management, and captive rearing and release, while identifying management actions that can reverse the population decline and reduce and eventually eliminate the need for future captive rearing.

The FGSP 5-year Strategic Vision outlines necessary objectives and actions to achieve the goal stated above. The appendices delve into specific details of the captive-rearing program, release strategies, and health screening protocol.

The **Introduction** reviews the history of FGSP conservation and the necessity of a time bound vision moving forward. The **Conservation goal** clearly states the desired outcome of the vision, to stabilize and grow the wild population over the next five years.

Six measurable **Objectives**, to be implemented at all sites with extant FGSP populations, outline how the conservation goal will be achieved:

- Habitat Management (Objective 1)
- Nest Protection (Objective 2)
- Monitoring and Database Management (Objective 3)
- Captive Management and Release (Objective 4)
- Research (Objective 5)
- Outreach (Objective 6)

The **Methods** list clear, attainable actions for each objective.

Stakeholder Engagement and Decision-Making Framework summarizes the development of the vision and the process of future decision making. As the trust managers, FWS and FWC will make final decisions if group consensus within the FGSP Working Group cannot be reached.

Check points and criteria for success are quantifiable criteria to guide decisions and evaluate the program. The checkpoints at years two and five serve as an assessment of success for the captive-rearing program and a decision point to continue agency support.

The annual **Budget** outlines estimated costs, sources for funding, and anticipated shortfalls and how they will be addressed. The timeline details funding for each action that will facilitate long-range planning.

Appendix A describes the rationale for the details in the captive management and release strategies. Decisions on the size of the release cohort, captive-rearing methods, location of the captive program, magnitude of the captive effort, age at release, quarantine, acclimation, and release location are presented with rationales based on scientific literature and the best available data on FGSP biology.

Appendix B provides an adaptive management framework for testing aspects of the release plan. Appendix B specifies the composition of captive release cohorts as well as methods related to quarantine and acclimation, post-release monitoring via radio transmitters, and systematic point-counts. Criteria for success and evaluation of release methods provide check-ins and procedures if release methods require modification.

Appendix C specifies pre-transfer screening protocol to ensure continuity of treatment and health information gathered from captive FGSPs.

Appendix D defines protocols of captive-rearing facilities, both for present and possible future partners. These best practices have consistently shown high survivorship and productivity, and their application at current and future partner facilities will optimize the success of the captive-breeding program.

Appendix E contains literature cited for the document, including appendices.

Recommendations from a Disease Risk Analysis (DRA) in late November 2018 will inform and possibly alter proposed methods for quarantine, health-screening protocols, and release decisions. Contingency plans are outlined in the vision for alternative DRA outcomes.

ACKNOWLEDGEMENTS

This vision would not have been possible without input from the FGSP Working Group. Many contributed to the vision with helpful feedback and suggestions. These include Rob Aldredge (FWS), Emily Angel (Archbold Biological Station [ABS]), Jim Austin (University of Florida [UF]), Mark Cunningham (FWC), Kelly Currier (ABS), Jennifer Benson-Hughes (Kissimmee Prairie Preserve State Park [KPPSP]), Robin Boughton (FWC), Reed Bowman (ABS), Scott Citino (White Oak Conservation Holdings [WOCH]), Jim Cox (Tall Timbers Research Station [TTRS]), Jennifer Eells (UF), Jessica Emerson (WOCH), Steve Glass (FWC), Paul Gray (Audubon), Shayna Jacques (FWC), Troy Hershberger (Avon Park Air Force Range [APAFR]), Archer Larned (University of Maryland – Baltimore), Alan Lieberman (San Diego Zoo [retired]), Erin Meyers (FWS), Kris Pitcher (FWS), Paul Reillo (Rare Species Conservatory Foundation [RSCF]), Andrew Schumann (WOCH), Rebecca Schneider (FWC), Lisa Shender (FWC), Greg Thompson (ABS), Jim Wellehan (UF), Rebecca Windsor (ABS), and Tatiana Villante (RSCF).

The FGSP 5-year vision is a collaboration effort between FWS and the FWC. Primary authors are Ashleigh Blackford (FWS), Andrew Cox (FWC), Adrienne Doyle (FWC), Craig Faulhaber (FWC), Karl Miller (FWC), Mary Peterson (FWS), and Erin Ragheb (FWC).

INTRODUCTION

Despite ongoing management efforts, sharp declines in Florida grasshopper sparrow (FGSP; *Ammodramus savannarum floridanus*) populations have been observed in recent years, and the subspecies is now nearing extinction. As of the 2018 breeding season, there were an estimated 23 breeding pairs left in the wild. Habitat loss, altered fire regimes and hydrology, and land-use change likely have been responsible for population declines, and disease has been hypothesized as a potential contributor. Recent research confirms that adult survival and productivity rates are too low to support a stable population. Data indicate that survival rates of unprotected nests in most years is so low that adult survival rates would have to be greater than almost any value reported for another temperate passerine species to overcome the observed poor productivity (FWC unpub. data, Pizarro Muñoz et al. 2018).

To date, conservation partners have employed a multi-pronged approach to address the declines. Partners have conducted land management to restore and maintain suitable habitat. Research projects have provided guidance on habitat management and have identified limiting life history stages. Based on the results of this research, partners have instituted emergency actions (e.g., predator fencing, nest lifting, red-imported fire ant [*Solenopsis invicta*] treatments) that have substantially improved nest success, though they do not provide long-term solutions. In 2015, the U.S. Fish and Wildlife Service (FWS) initiated a captive-breeding program to augment the wild population via release of captive-reared birds. FGSP breeding pairs are currently held at Rare Species Conservatory Foundation (RSCF) in Loxahatchee and White Oak Conservation Holdings (WOCH) in Yulee, with additional unpaired adult males held at Santa Fe College Teaching Zoo (SF) in Gainesville. This effort is likely to produce substantial conservation benefits through release of captive-reared birds beginning as early as 2019, depending on the outcome of a Disease Risk Analysis (DRA) that will assess the risk to wild populations of releasing captive-reared birds. Looking into the future, reversing FGSP declines will require continued investment in land management and nest protection, captive rearing to augment the wild population, and research to identify solutions in the wild that eliminate the need for continued captive rearing.

The FWS and the Florida Fish and Wildlife Conservation Commission (FWC), with input from the FGSP Working Group, present this 5-year Strategic Vision for the management of the FGSP. The vision provides a clear goal, objectives, and success criteria, along with an integrated set of actions designed to achieve them. Estimates of the resources required for full implementation are included. The strategic vision is time-bound, and success criteria are intended to provide agencies and partners the information they need to assess the degree to which continued investment is warranted in each facet of current FGSP conservation efforts.

CONSERVATION GOAL

Stabilize and grow the wild population over the next five years through habitat management, wild population management, and captive rearing and release, while identifying management actions that can reverse the population decline and reduce and eventually eliminate the need for future captive rearing.

OBJECTIVES

(To be implemented at all sites with extant FGSP populations)

1. Restore and maintain optimal conditions in occupied and potential FGSP habitat through continued use of land management practices, such as prescribed fire, mechanical treatments, and, where appropriate on private lands, prescribed grazing.
2. Improve reproductive success by locating and protecting FGSP nests with fences, by lifting nests at risk of flooding, and by conducting treatments to control fire ants when appropriate.
3. Conduct demographic monitoring of wild FGSP on public and private lands (with landowner permission) wherever FGSP occur.
4. Augment the wild FGSP population in a manner that stabilizes and grows the wild population (current stabilizing target is 23 breeding pairs, based on the estimated size of the 2018 wild population) through captive rearing, genetic management of captive and wild populations, and the release of captive-reared individuals in an adaptive management context.
5. Conduct research that will assess the likelihood of meeting overall population goals and will identify management actions that can stop the decline, grow the population, and eliminate the continued need of a captive support population.
6. Increase public knowledge of the status and trends of the FGSP, its recovery needs, and opportunities for the public to participate in the FGSP's recovery.

STAKEHOLDER ENGAGEMENT AND DECISION-MAKING FRAMEWORK

This vision was developed with input from the FGSP Working Group (working group), a public-private partnership that has worked collaboratively on FGSP conservation since 2002. The FWS and the FWC distributed a draft of the document to the working group on September 11, 2018 and conducted a facilitated working group meeting on September 17, 2018, in an effort to reach consensus among working group members for the elements of this plan. Consensus is defined to mean that all members actively support the plan or at least can accept it. When the working group was unable to reach consensus on an issue, the FWS and FWC, who serve as trust managers for the people of the United States and Florida, respectively, made the final decision. The FWS and FWC considered written and verbal comments provided by working group members when developing the second draft of this plan.

METHODS

HABITAT MANAGEMENT (Objective 1)

Continued habitat management is fundamental to the persistence of wild FGSP. FGSP require open, frequently-burned dry prairie habitat. FGSP habitat will be maintained in an early successional stage through frequent prescribed fire (every 1 to 2 years, recommended in Feb-Mar [prairie occupied by FGSP] or Apr-May [prairie not occupied by FGSP]). Prescribed grazing may be an option for maintaining habitat in some cases, with the prescription decided on a case-by-case basis in coordination with FWS and FWC staff. Roller-chopping and tree removal help to expand and improve FGSP habitat. Priority will be given to the maintenance of dry prairie habitat in locations known to currently support FGSP populations followed by the restoration and maintenance of potential habitat in unoccupied areas.

Action 1: Conduct prescribed fire, tree removal, roller-chopping, and exotic grass control at all field sites (Three Lakes Wildlife Management Area [TLWMA], Avon Park Air Force Range [APAFR], Kissimmee Prairie Preserve State Park [KPPSP], and a private ranch [Ranch]) to maintain dry prairie habitat in suitable condition for FGSP.

Action 2: Create an appendix that contains guidance on habitat management (e.g., prescribed fire, mechanical treatments, prescribed grazing) for FGSP.

NEST PROTECTION (Objective 2)

Poor nest success has been identified as one of the major proximate causes of population decline for FGSP. Working group partners have demonstrated dramatic improvements in nest success by installing predator deflection fences around known nests, lifting nests that are at risk of flooding, and treating red imported fire ants near known FGSP nests. A nest fenced at the start of incubation has a 53% chance of fledging young, compared to 4% for an unfenced nest (2017 TLWMA estimates; FWC unpub. data). Continuing these management actions is critical until larger-scale management solutions can be identified and implemented.

Action 3: Continue to locate wild FGSP nests and install predator deflection fences at those nests at all field sites.

Action 4: Continue to lift FGSP nests at risk of flooding at all field sites using established working group protocols.

Action 5: Treat red imported fire ant mounds found near FGSP nests using established hot water techniques (King and Tschinkel 2006; G. Thompson, pers. comm.) or via manual removal (with shovel).

MONITORING AND DATABASE MANAGEMENT (Objective 3)

Understanding the current wild population size, population trends, and reproductive effort is necessary for making informed conservation decisions. Demographic monitoring has been critical to identifying limiting life history stages and crafting management strategies and research to address threats.

Action 6: Continue to survey all known wild populations and recently unoccupied habitat annually using standardized point count and band-resighting methods.

Action 7: Continue coordinating with private landowners to identify and monitor other FGSP populations on private lands should they exist.

Action 8: Continue demographic monitoring of wild FGSPs and monitor captive-reared FGSP released into the wild. This includes capturing and affixing color bands on all newly discovered adults and nestlings and locating and monitoring nests to obtain estimates of reproductive success.

Action 9: Standardize demographic, point count, and habitat data collection across sites and merge into a master archival database. Demographic data will then be summarized annually to generate vital rate estimates and pedigree analysis tables for each subpopulation.

CAPTIVE MANAGEMENT AND RELEASE (Objective 4)

The objective of the FGSP captive program (2019-2024) is to produce and release enough FGSPs to maintain a stable or increasing wild population. This means that, at a minimum, enough birds would be released to maintain at least 23 wild breeding pairs, based on the size of the 2018 wild population. Ideally, enough releases would occur to grow the wild population to a size that is more resilient to demographic and environmental stochasticity. However, we recognize that captive-rearing efforts are expensive and logistically challenging, and, ultimately, management actions will need to produce a substantial improvement in wild vital rates to grow the population in a manner that is fiscally feasible and sustainable. Without these improvements to wild population vital rates, the scale of the captive effort would need to grow annually for an undetermined amount of time in order to result in a growing wild population. Although we hope to exceed the minimum presented here, the captive program must at least produce enough recruits into the wild population to keep the population stable while ways to improve wild vital rates are sought (Objective 5).

Captive management and release portions of the vision will be executed within an adaptive framework. Recommendations from the DRA will inform quarantine and release decisions. The captive program will be a cooperative effort, as reflected by rapid data sharing among partners and joint authorship of peer-reviewed scientific products that arise from program activities. The strategy below, for which a thorough

rationale is provided in Appendix A, is based on what we consider to be the most likely outcome of the DRA, but we also present contingency plans for other potential DRA outcomes.

1. *Determination of minimum annual cohort size.* We estimate that at least 8 captive-reared females need to be recruited into the breeding population each year to stabilize the wild population at 23 breeding pairs. Assuming a minimum post-release survival and recruitment threshold of 15%, we estimate that at least 53 captive-reared females need to be released annually to meet our target of 8 recruited females (Appendix A). Our target is specific to females because they are the limiting sex, but any males not needed to support the captive population will also be released. Assuming a balanced sex-ratio of captive-reared birds, the number of released males will approximate that for females. The 15% target was chosen as a realistic minimum starting point to establish initial estimates for the minimum cohort size. We will refine the recruitment rate (and hence the minimum cohort size) once we have data from releases.
2. *Captive-rearing methods.* The 53 females (and similar number of males) will be reared by adult FGSP pairs in captivity following a captive breeding for immediate release program (as described by Lieberman and Kuehler 2009). In addition, head-starting of salvaged eggs and nestlings will be used to supplement captive breeding and to help us meet or exceed our annual production goals.
3. *Location of captive-rearing facilities.* The captive-rearing facilities will remain distant to the wild site (*ex situ*) rather than constructing a new facility at a wild population site (*in situ*), unless the results from the DRA, release trials, or the mid-point program evaluation suggest an *in situ* option should be considered.
4. *Magnitude of captive effort.* At least 14 FGSP breeding pairs will be necessary to meet our annual release target of 53 captive-reared females (see Appendix A). Captive FGSP will be housed and cared for in outdoor aviaries, and facilities will follow the protocols proven to successfully produce parent-reared fledglings (Appendix D). We currently have enough FGSP in captivity to meet this target, but additional funding is needed to expand breeding facility space and support operations.
5. *Age at release.* Captive-reared birds will be released as both independent juveniles and as second-year adults within an adaptive framework to test the survival and recruitment probabilities of these two developmental stages (Appendix B).
6. *Quarantine.* FGSPs targeted for release will be quarantined in outdoor aviaries *ex situ* and will undergo one or more pre-transfer health screening exams (Appendix C) prior to being cleared for transfer and release. The details of quarantine and pre-transfer screening will be further developed during and after the DRA in November 2018.

7. *Acclimation with pre-release observation.* Parent-reared FGSP will spend up to 5 days in field aviaries prior to release to allow for adjustment to radio transmitter harnesses, recovery from human handling, and recovery from transport (Appendix B). Shorter acclimation periods within this time frame or direct release of parent-reared birds may occur, if warranted, to mitigate risks to the birds (e.g., to eliminate intraspecific aggression from males). For hand-reared birds, a longer acclimation in field aviaries will be used (e.g., 8 weeks; see rationale in Appendix A). Adjustments to the length of field acclimation will be made based on the results of the DRA and observations of birds pre- and post-release.
8. *Location of release.* The 2019 experimental release trials (Appendix B) of captive-reared FGSP will take place at TLWMA, with the intent to release birds at other sites as soon as feasible (see Appendix A: Release Location). We will evaluate release sites for 2020 and beyond each fall (Appendix B: Evaluation of Release Methods) using triggers based on outcomes from prior releases as well as the preparation and suitability of alternate release sites to receive birds (Appendix A: Release Location).

The decisions outlined above and in Appendix A assume that the trust resource agencies will decide that it is appropriate to release captive FGSPs in 2019. The following are potential outcomes of the DRA that preclude 2019 releases, presented with their associated contingencies. An evaluation of alternatives to the current captive-rearing effort (e.g., *in situ* facilities, cessation of captive-rearing efforts) will be initiated if any of these outcomes are realized:

- *If additional information about disease is required before releases are warranted.* Birds will be prevented from breeding, either by separation or egg collection, and will be held in a safe and humane manner until the necessary information is obtained. Limited breeding may occur if sufficient funding is identified to expand one or both facilities.
- *If captive birds cannot be released from some but not all facilities.* An evaluation will be made as to whether genetic material can be shared between the two facilities via egg transfer. If possible, genetically valuable pairs will continue to breed at the site at which birds cannot be released and eggs will be transferred to the alternate captive-breeding facility. This would continue until sufficient genetic material was transferred to the active captive-breeding facility. Birds at the non-releasing facility will be retained in a safe and humane manner until they join another collection for display or education or they die naturally. The breeding facility with releasable birds will implement the preferred captive management and release strategy. If the number of release birds per cohort is less than what is identified in the preferred strategy, partners may consider expanding capacity at the releasing facility or exploring the feasibility of alternative options for captive rearing (e.g., *in situ* captive breeding, head-starting, or establishment of a captive flock at a third facility).
- *Captive birds cannot be released from any facility.* Birds will be retained by each facility in a safe and humane manner until they join another collection for display or education or they die

naturally. Partners will explore the feasibility of alternative options for captive rearing, such as *in situ* head-starting or *in situ* captive breeding and release.

Action 10: Rear enough captive FGSP to add at least 8 competent breeding females to the wild population on an annual basis during 2019-2024, in accordance with Appendices A and D. We estimate that this will take release of at least 53 females (plus males) per year (Appendix A).

Action 11: Conduct limited collections of individuals from the wild and transfer individuals between facilities as necessary to maintain genetic diversity of the captive population. Continue genetic management of the captive and wild FGSP populations using PMx kinship analysis software (Lacy et al. 2011; Appendix B).

Action 12: Augment the wild population through release of captive FGSP, in accordance with Appendices A, B, C, and D.

Action 13: Monitor released birds to evaluate success of releases, and test hypotheses regarding release methods that maximize survival and recruitment (Appendix B).

RESEARCH (Objective 5)

Continued research is necessary to identify strategies to stop the decline, grow the population, and reduce and eventually eliminate the continued need of a captive support population. Addressing the following research topics will provide information necessary to guide future actions and to frame the likelihood of meeting population recovery goals.

Action 14: Determine the influence of roller-chopping on nest survival.

Nest survival for sparrows is too low to support a stable or growing population. One hypothesis suggests that nest predators such as snakes may prefer the cover provided by palmettos, so palmetto reduction may improve nest survival by reducing predator densities. Densities and nest survival of FGSPs and Bachman's sparrows (*Peucaea aestivalis*) within existing roller-chopped areas or experimental plots can be compared with untreated control plots.

Action 15: Continue ongoing research into diseases of wild and captive FGSP.

Although recent demographic data point to low nest success as a proximate cause of population declines, one hypothesis is that disease may have played a role in previous observed declines (e.g., at APAFR in the early 2000s) and in the low adult survival observed in some recent years. In addition, disease has been a common cause of mortality of captive FGSP. More information on pathogens in the wild and captive populations is needed to understand the risk associated with release of captive birds and whether any wild pathogens exist that could be responsible for historic or ongoing declines.

Although preliminary data suggest that pathogens observed in captivity are also found in the dry prairie, it is still unknown whether they are novel or endemic to the wild population.

Two active studies focus on describing diseases found in wild and captive FGSP populations. First, FWC and University of Florida (UF) are collaborating to screen blood and fecal samples from birds from all wild sites and WOCH for a suite of vector-transmitted (e.g., filarial nematodes), direct-transmitted (e.g., extra-intestinal coccidia), and virome-based pathogens. Second, University of Georgia (UGA) is conducting necropsies on carcasses from captive and wild populations to identify the specific causes of mortality. UGA is also attempting to sequence the entire genome of the coccidia organisms discovered in captive FGSP to assist with wild sample screening.

Action 16: Investigate how fire history, habitat features, and post-fledging conditions influence survival rates of juvenile birds.

Like most passerines, juvenile survival for FGSPs is lower (ca. 21%) than annual adult survival (ca. 48%; FWC unpub. data). Improving juvenile survival could help compensate, in part, for low nest success and low adult survival observed in FGSP. Research on other passerine birds suggests that habitat features often influence survival during the first few weeks after fledging when most mortality for juveniles occurs, so opportunities may exist to alter management strategies to improve juvenile survival. FGSP juveniles have been observed utilizing habitat with more shrub vegetation than territorial adults and it is possible that juveniles may rely on areas of dense vegetation for predator avoidance during key developmental periods. Fire history and other habitat data (e.g., distance to forest edge, roller chopping history) from natal sites can be used to understand how these factors may influence first year survival. Understanding how other factors such as individual condition (mass at banding), time of year, and severe weather events influence juvenile survival in the wild also will be relevant when planning and evaluating the release of captive-reared birds. Monitoring released captive birds (or possibly wild fledglings) with radio-transmitters will complement this research action by providing information on general habitat use and causes of mortality (Appendix B).

Action 17: Understand the effects of land use changes, alternative management strategies, and climate factors on the decline of FGSP to inform future management actions.

Understanding the factors driving past FGSP declines will provide a retrospective review of contemporary management efforts and shed light on the degree to which large-scale processes have affected the sparrow. This comprehensive analysis will help frame the likelihood of meeting population recovery goals with or without captive supplementation. Twenty years of point count survey trend data (1998-2018) can be used to evaluate competing hypotheses explaining historical declines of FGSP, such as changes in land use (habitat fragmentation, waterflow restoration), management strategies (changes in fire seasonality and frequency, palmetto density), and climate factors (Southern Oscillation Index, rainfall patterns, severe weather events). Data availability, especially with respect to post-fire habitat metrics, may limit the scope of this study.

Action 18: Improve our understanding of FGSP genetic diversity at the subpopulation and subspecies level and develop program goals for genetic management.

Intentional genetic management of captive and wild FGSP subpopulations will be necessary to maximize the health and viability of the subspecies into the future. The FGSP presents a challenge because the pedigree is relatively shallow (2013-2018 data only) and incomplete (i.e., many birds have unknown parentage). However, the available pedigree data can be updated annually and analyzed with population management software to select optimal captive pairings, select individuals for collection or release, and inform optimal release locations. Additionally, existing DNA samples can be used for genomic sequencing of FGSPs. This sequencing data can be used to 1) set goals for genetic integrity of the captive-breeding flocks, 2) refine the captive pedigree analysis, and 3) evaluate the relatedness of the *floridanus* and *pratensis* subspecies using modern genomic techniques.

The actions listed above represent the highest priority research questions over the next five years. Given sufficient time, partners, and resources, other avenues for future research might include the effect of fire intensity and scale on nest predation risk, the predator-prey dynamics that drive abundance of known nest predators (i.e., spotted skunks and snakes), identifying limited resources for FGSP in winter, and exploring factors that drive FGSP territory movement and dispersal. Some of these questions are expected to be logistically difficult to answer but may be worth exploring if time and resources allow.

OUTREACH (Objective 6)

Continued outreach efforts are important to increase public knowledge about the plight of the species, to engender support for conservation efforts, and to inform the public about how they can assist these efforts. The Fish and Wildlife Foundation of Florida's [Florida Grasshopper Sparrow Fund](#) provides an opportunity for the public to support conservation actions in the vision.

Action 19: Ensure partners and agency staff coordinate with each other and with FWC and FWS community relations staff to deliver responsible, consistent messages that support the vision goal.

Action 20: Produce and distribute outreach products (e.g., news releases, popular articles, social media posts) designed to increase awareness and provide avenues for the public to support conservation actions identified in the vision.

CHECK POINTS AND CRITERIA FOR SUCCESS

The working group and its Implementation and Coordination Team will meet as necessary to make minor adjustments to project methods (e.g., habitat management techniques, nest protection methods, husbandry techniques). There also will be check-ins each fall, and in mid-June of 2020 and 2021, to adjust release methods (e.g., the optimal age class for releases), as outlined in Appendix B. We expect that check-ins will also provide FWS and FWC an opportunity to review the general progress and success of the program, and, if appropriate, adaptive management may extend beyond minor adjustments. A briefing of FWS and FWC leadership on the status of the program will occur annually at the end of each year.

Additionally, it is imperative to have measurable criteria to gauge success of the program and trigger discussion and decisions. Formal checkpoints will occur in years 3 and 5 to evaluate progress according to the criteria below and to make decisions regarding the future of the program. The criteria below will guide and focus actions and will be revisited as appropriate based on new information and needs of the program.

January 2022

A thorough program evaluation will occur after three years to assess whether captive rearing of FGSP is contributing enough to the conservation effort to merit continued agency support. Success of the captive-rearing program at this point will be defined as:

1. Released birds exhibit reproductive behaviors (e.g., song patterns, territory establishment, pair formation) comparable to wild birds.
2. Demonstration that at least one of the released age classes in at least one year can achieve a recruitment rate of $\geq 15\%$ for females.

If measures of success are achieved, the captive-rearing effort will continue through the next evaluation period and recommended improvements to methodology will be implemented. If measures of success are not achieved, the approach to captive breeding outlined in the vision will be revised based on the best available data and agency leadership will determine whether continued support is warranted.

January 2024

Another program evaluation will occur after five years to assess the impact of all management and research actions to improve wild FGSP population growth. At this check-point, the measures of programmatic success will be defined as:

1. Released birds exhibit reproductive behaviors (e.g., song patterns, territory establishment, pair formation) comparable to wild birds.
2. Demonstration that at least one of the released age classes can achieve a mean recruitment rate of $\geq 15\%$ for females during 2022–2023.

3. All properties with known wild FGSP populations have met their habitat management goals.
4. FWC staff and partners have continued monitoring and nest protection activities.
5. The wild FGSP population is extant and has remained stable or increased following the release of captive-reared birds.
6. Priority research projects have been completed, and research results indicate promising avenues for arresting and reversing the decline of wild populations without the need for continued captive breeding.

If measures of success are achieved at the end of the five-year period, then another vision document will be drafted to inform agency leadership of future goals for the FGSP program. If measures of success are not achieved, the captive-breeding program will be re-evaluated with FWS and FWC leadership and the agencies will decide whether continued support is warranted. If program cessation is warranted, the specific timeline and details will be determined at that time in consultation with captive facilities. Options include release of birds into the wild or retention of birds in captivity in a safe and humane manner until they join another collection for display or education or they die naturally.

BUDGET

We estimate the average annual expenditures required to fulfill the actions of the vision to be \$1,212,500, which includes ongoing land management activities provided by FWC, Florida Department of Environmental Protection, and Department of Defense (DOD) that specifically benefit FGSPs. Table 1 summarizes the annual budget and anticipated shortfalls by activity. We intend to address the budget shortfall in three primary ways:

- FWS and DOD have made variable but substantial annual contributions to FGSP conservation efforts in recent years. FWS staff will allocate \$150,000 toward FGSP needs and will continue to pursue FWS funding throughout the duration of the strategic vision.
- We expect that continued fund-raising by RSCF, WOCH, the Fish and Wildlife Foundation of Florida, Audubon Florida, and other NGOs will contribute to captive-breeding costs.
- FWC's internal discretionary funding and FWC's Section 6 grants can help fund outstanding information needs.

TABLES

Table 1: Strategic vision budget

Action	Average annual program costs		Anticipated annual shortfall	
			2019	2020-2023
FGSP specific land management ^{1,2}	\$	457,000	\$ 52,000	\$ 52,000
Field monitoring and nest protection ²	\$	454,000	\$ 38,000	\$ 38,000
Captive rearing & studbook management	\$	230,000	\$ 230,000	\$ 230,000
Release and post-release monitoring ³	\$	56,000	\$ 21,000	\$ 56,000
Research ³	\$	47,500	\$ 50,000	\$ 35,250
Total	\$	1,244,500	\$ 391,000	\$ 411,250

¹Estimate of FGSP-specific management costs.

²Shortfall is specific to monitoring and protection at a private ranch.

³A portion of the shortfalls may be covered via FWC discretionary funding and Section 6 grants.

Table 2: Timeline

Action	2019	2020	2021	2022	2023
Land management					
TLWMA					
KPPSP					
APAFR					
Ranch					
Other private lands?					
Field monitoring + nest protection					
TLWMA					
KPPSP					
APAFR					
Ranch					
Other private lands?					
Captive rearing					
RSCF					
WOCH					
SF (holding only)					
Studbook management					
Release and post-release monitoring					
TLWMA					
Other sites					
Research					
Roller-chopping and nest survival					
Juvenile survival					
UF disease project					
UGA disease project					
Historic trends					
Genetic diversity					
Probable funding and resources available Funding source or resources uncertain Pending results from January 2022 check-in					

APPENDIX A. Rationale for Captive Management and Release Strategy

INTRODUCTION

The purpose of this Appendix is to provide the rationale and decision-making process behind the captive management and release presented in the FGSP 5-Year Strategic Vision (vision). When developing the vision, we sought to revisit and redirect the objectives of the FGSP captive-breeding program to maximize the probability of meeting the vision's conservation goal. As described in the vision, the FGSP captive program is expected to produce birds that will serve as reinforcements (*sensu* Seddon et al. 2014) for wild FGSP populations. The overall goal of the captive-breeding program is to reduce the probability of extinction while we identify management actions that can stop the decline, grow the population, and reduce and eventually eliminate continued need for a captive support population. The success of a captive-breeding and release program can be influenced by many factors, including the species' life history characteristics; the age, sex, and number of individuals released; the timing and number of release events; and the release protocols that are used (Wallace 1994, Batson et al. 2015). We summarized available demographic data, reviewed the captive-breeding literature, and sought expert opinion to address the following primary questions:

- 1) How many captive-reared birds need to be produced and released each year to maintain at least a stable wild population, while solutions to wild population declines are discovered?
- 2) How should these "reinforcement" birds be produced (reared from eggs laid by captive pairs [captive breeding], or collected as wild eggs or nestlings and reared in captivity [head-starting])?
- 3) What is the appropriate location for the captive facility relative to the wild sites (*in situ* or *ex situ*)?
- 4) What is the required magnitude of the captive-rearing effort?
- 5) At what age should captive birds be released?
- 6) What pre-release health screening or quarantine protocols should we follow?
- 7) How should we acclimate birds to their wild habitat prior to release?
- 8) Where should birds be released?

The captive management and release portions of the vision will be executed within an adaptive framework, and all initial decisions described below are subject to evaluation and revision as new information becomes available. Recommendations from a Disease Risk Analysis (DRA) in November 2018 will inform quarantine and release decisions. This Appendix focuses on what we consider to be the most likely outcome of the DRA. The vision presents contingency plans for other potential DRA outcomes.

1. DETERMINATION OF MINIMUM ANNUAL COHORT SIZE

The first step in determining the appropriate design for a captive FGSP program was to determine how many captive-reared birds would need to be released each year to meet our goal of stabilizing and growing the wild population, with “stabilizing” meaning maintaining a similar number of breeding females as the 2018 season (estimated at ca. 23 pairs). We estimate that 9 of the 23 wild females from 2018 will survive to 2019 based on recent annual survival rates that average 40%. Wild recruitment rates vary annually, but, based on recent data, we estimate that the 2018 females will recruit 6 new females into the 2019 population. We therefore estimate that a minimum of 8 captive-reared females would need to be recruited into the breeding population each year to stabilize the wild population at a modest 23 breeding females. As noted in the Captive Management and Release section, we hope to exceed the minimum presented here, but the captive program must at least produce enough recruits into the wild population to keep the population stable while ways to improve wild vital rates are sought.

Predicting the number of released birds necessary to achieve annual recruitment of at least 8 captive-reared females is challenging, given the highly variable outcomes of prior release programs. Decades of translocation research with wild birds in New Zealand indicate that the cost of release (i.e., mortality attributable to stress from translocation and initial release) ranges from 0-80% and averages about 30% (D. Armstrong, pers. comm.). Although data are lacking from captive-reared birds, the cost of release also appears to be high. Mean survival during the first month following release was 36–80% for three captive-reared passerine species (Table A1). Survival for captive-reared individuals over the first-year post-release is unknown for many projects, although some data suggest it can be comparable to survival of wild individuals (e.g., Loggerhead Shrikes [*Lanius ludovicianus*]; Lagios et al. 2014). Mean survival of independent juveniles to the following breeding season ranged from 26–52% for three species (Table A1). Recruitment values in the literature for captive-reared passerines ranged from 3-48% but tended to be below 10% (Table A1). The Mauritius Fody, which was released on a predator-free island, had the highest recruitment rate (Table A1). Low recruitment rates appear to have been in part a function of circumstance. For example, Mangrove Finches were released during a drought, when survivorship and breeding rates for many Galapagos finch species are low (Boag and Grant 1981, Grant and Grant 2006). In contrast, the low recruitment rates for captive-bred Loggerhead Shrikes exceeded that of wild juveniles (Nichols et al. 2010), highlighting the importance of referencing wild vital rates when evaluating the relative success of release programs.

Decision

Considering the high cost of release and low recruitment rate of past release programs, we chose a minimum recruitment rate of 15% of released birds, which is roughly 1/3rd the recruitment rate of wild juvenile birds (43%), when setting a target for the annual cohort size. Our intention is to achieve a recruitment rate greater than 15%, but we needed to choose a realistic minimum starting point to establish initial estimates for minimum cohort size. We will refine the recruitment rate (and hence the minimum cohort size) once we have data from releases. At this rate, at least 53 captive-reared females (as well as any males not needed for captive breeding) would need to be released per year to maintain a

stable wild population. Fifteen percent recruitment is somewhat higher than most previous passerine programs (Table A1), but we think it may be realistic biologically given the adaptive design of our release effort and the high site fidelity of wild juveniles. Furthermore, it is important to note that even incremental reductions in recruitment rates below 15% would require substantially greater captive-breeding capacity than currently exists (e.g., 80 and 160 females would need to be released if the recruitment rates were 10% and 5%, respectively). As such, a 15% minimum target represents an objective that is both biologically and logistically feasible in the near future.

2. CAPTIVE REARING METHODS

To reach our target for annual production of released individuals, we considered two captive-rearing alternatives: captive breeding and head-starting. Captive-breeding programs use breeding pairs held in captivity to produce and rear chicks (Lieberman and Kuehler 2009). In contrast, head-starting programs involve intentionally collecting eggs or nestlings from the wild to rear in captivity (typically by hand; Lieberman and Kuehler 2009, Jeffs et al. 2016). Head-starting has been selected in cases where adults cannot be successfully housed or bred in captivity, or the probability of nest or fledgling survival in the wild is exceptionally low. For example, Mangrove Finch eggs were collected as part of a head-start program and raised in captivity until they reached nutritional independence to avoid a lethal parasite affecting wild nestlings (Cunninghame et al. 2015).

We considered several relevant factors when assessing which approach (or combination of approaches) is most appropriate for the FGSP program. The following bullets compare the advantages of captive breeding versus head starting as the *primary* means of producing birds for release.

Advantages of captive breeding for FGSPs:

- *The infrastructure and expertise are in place.* We are currently housing 10 FGSP breeding pairs in outdoor captive-breeding facilities, and substantial investments have resulted in major improvements in husbandry techniques resulting in high productivity (e.g., 9.3 fledglings/breeding pair at WOCH in 2017).
- *Few or no wild collections need to occur each year.* As noted above, the *ex situ* FGSP flock is already established. In the future, only minimal collection efforts of partial clutches will be required to maintain the genetic integrity of a captive-breeding population. In contrast, head-starting programs require removing eggs and nestlings from the wild each year. We estimate that, on average, 21% of wild dependent fledglings (and 19% of wild 4-day-old nestlings) would survive and breed if not collected (FWC, unpub. data). It is important to account for the number of nestlings collected from the wild when calculating the total recruitment (captive plus wild) that can be achieved through head-starting. Nestlings that are high risk of death from flooding or other events (salvaged nestlings) are an exception because none would have survived in the wild to be recruited into the population. Box A1 demonstrates how the three strategies (captive breeding using an existing captive flock, head-starting nestlings with average recruitment probabilities, and head-starting salvaged nestlings) contribute to overall recruitment and what

the post-release recruitment rates would need to be under each scenario to accomplish the same goal. As demonstrated in Box A1, captive breeding is more efficient than head-starting as the primary means to produce birds for release, because few or no wild collections need to occur.

- *Retention of wild breeding pairs may be higher.* Another less quantifiable cost to head-starting nestlings with average risk relates to the propensity of nesting passerines to disperse from a breeding site within (Powell and Frasch 2000) or between (Hoover 2003) breeding seasons following repeated nest failure. Disruption from nest collection might cause dispersal, reducing the probability of renesting, or making the renest attempts difficult to find and protect.
- *Productivity is not reliant on availability of wild nests.* Captive FGSP have already demonstrated high reproductive potential. Woch produced 9.3 fledglings per breeding female (28 fledglings by 3 females) in 2017 and 6 fledglings per breeding female (24 fledglings by 4 females) in 2018 when reproduction was halted mid-season because of capacity constraints. In contrast, head-starting is limited by the number of wild nests that are discovered and survive to collection age. We estimate that at least 42 wild nests would need to be collected each year to produce the 8 “reinforcement” female target if head-starting were the only method used to supplement wild populations. For reference, only 46 wild FGSP nests were discovered by field crews across all sites in 2018.
- *Nestlings are housed with and raised by adult birds.* Young birds may benefit by regular interactions with adults (Nichols et al. 2010). For example, young birds in outdoor enclosures responded to alarm calls from adult birds when a raptor flew overhead (A. Schumann, pers. obs.), which is an important natural behavior. Furthermore, parent-reared birds may be more proficient breeders (Myers 1988), though further study is required to understand whether this is the case with FGSPs.

Advantages of head-starting for FGSPs:

- *It may be less expensive.* Captive-breeding programs require year-round facilities and staff, whereas head-starting programs may only need to operate for 4-6 months if birds are released as independent juveniles.
- *Potential for pathogen avoidance.* Hand-rearing wild nestlings has the theoretical potential to reduce exposure to high coccidia loads during the dependent period, because the young birds are kept indoors in cages that are easier to clean. However, in 2018, neither outdoor parent-reared nor indoor hand-reared FGSP juveniles suffered from coccidia-related mortality in captivity, suggesting that alternative husbandry methods have provided some relief to this serious challenge to the health of captive birds.
- *It can take advantage of salvaged eggs and nestlings.* Adult mortality, high water events, and other unexpected circumstances can lead to nest failure in the wild. Head-starting programs are well positioned to accept and rear salvaged eggs or nestlings that would otherwise be lost in the wild.

Decision

The advantages listed above indicate that a program focused primarily on captive-breeding techniques will be more likely to meet our objectives in the next 5 years than use of head-starting as the primary means of producing captive reared individuals. However, head-starting of salvaged eggs and nestlings is planned to supplement captive breeding to help us meet or exceed our annual production goals. As noted in Appendix B, limited head-starting also could help achieve sufficient sample sizes to compare survival and recruitment of parent-reared and hand-reared birds. This proposed combination of both captive breeding and limited head-starting efforts is provisional, pending results from the DRA that will occur in November 2018. Our selected strategy is represented in Box A2 using the values presented in Determination of Minimum Annual Cohort Size of this appendix.

3. LOCATION OF THE CAPTIVE PROGRAM

Captive-rearing programs can occur within or very near the wild site (*in situ*) or distant to the wild site (*ex situ*). Initial FGSP captive-rearing efforts were established *ex situ* to leverage the facilities and expertise of RSCF and WOCH. Subsequent concern about disease at rearing facilities has led to a reexamination of the most appropriate location of continued captive-rearing efforts.

Advantages of an *in situ* captive program for FGSPs:

- *Exposure to natural conditions.* This would allow captive-reared birds to learn and acclimate to wild climates and prey items prior to release.
- *Reduced exposure to novel pathogens.* By maintaining FGSP at or near the collection and release site, the introduction of novel pathogens would be limited to those transferred accidentally on husbandry equipment, supplemental food, or staff. This is contrary to animals produced at *ex situ* breeding facilities which present the highest risk of novel pathogen exposure and post-release transfer (Kock et al. 2010).
- *Proximity to release site.* Lengthy transport times can result in weight loss, increased stress, and even death of birds prior to release (Kuehler et al. 2000, Jakob-Hoff et al. 2014). Rearing birds on site reduces these risks.

Advantages of an *ex situ* captive program for FGSPs

- *Established infrastructure and staff.* *Ex situ* programs benefit primarily from established infrastructure and expertise and as such can be less expensive and achieve program goals more rapidly than a new *in situ* facility. *Ex situ* infrastructure and captive populations already have been established.
- *Reserve against disaster.* While an *ex situ* facility is in operation, it serves as a reserve population if a natural disaster or other catastrophe eliminates the remaining wild population(s).

Decision

The preferred alternative includes *ex situ* rather than *in situ* captive rearing. Off-site captive rearing has already produced a substantial population of captive FGSPs that demonstrate extremely high survivorship and productivity. Assuming continued success of *ex situ* captive rearing and the likelihood that the risk of release is acceptable (see Quarantine section below), we will proceed with an *ex situ* program rather than invest substantially in an *in situ* program at this time. We recognize the potential benefits of *in situ* facility, and the DRA will inform us as to whether a transition to *in situ* captive rearing is a necessary precursor to releases. We will periodically re-evaluate the need for captive rearing *in situ* at check points outlined in the vision.

4. THE MAGNITUDE OF THE CAPTIVE EFFORT

The captive-breeding effort must produce enough young to meet our target of releasing at least 53 captive-reared females as well as replace breeding adults that die in captivity. Our target is specific to females because they are the limiting sex, but any males not needed to support the captive population will also be released. We used the following assumptions to generate the target number of breeding pairs for the FGSP captive-breeding program:

- The sex ratio of dependent fledglings in the captive flock averages 50:50.
- Captive annual fecundity will match 2017 estimates from WOCH (4.67 female fledglings/breeding female/year).
- First-year survival in captivity will remain high (90%) because of improved husbandry.
- Using these parameters, 14 captive-breeding pairs could produce 59 females of breeding age each year.
- Approximately 3% (n=2) of captive-reared females will be retained each cohort-year to replace breeders (based on 85% captive adult survival).
- This leaves 57 females available for release each year (4 more than our target).

Decision

Our annual release target of 53 captive-reared females should be met if we have at least 14 FGSP breeding pairs in captivity, provided they are housed and cared for in outdoor aviaries matching the conditions proven to successfully produce parent-reared fledglings (Appendix D). Currently 4 and 6 breeding pairs reside in outdoor pens at WOCH and RSCF, respectively. Another 2 and 7 unpaired adult males reside at WOCH and SF, respectively. There are currently 23 and 22 juveniles (sexes pooled) at WOCH and RSCF, respectively. Therefore, at a minimum, only 4 captive-reared second-year females would need to be retained in 2019 to create the target 14 captive-breeding pairs. However, optimal pairings will be determined by pedigree analysis (see Appendix B), and the housing location of the

breeding pairs will be determined based on the criteria established in Appendix D and in coordination with captive facilities.

5. AGE AT RELEASE

Success of release programs is in part a function of the age of released birds. Younger animals are often more capable of adapting to a new environment than older ones (e.g., VanderWerf et al. 2014) perhaps because their brains are still developing (Krochmal et al. 2018). But there are also costs associated with releasing younger birds, the most substantial being reduced overwinter survivorship for wild versus captive animals. Current FGSP data demonstrates that annual survival for independent juveniles is 79% in captivity but only 43% in the wild. However, many captive programs report behavioral deficiencies in captive-reared birds post-release and these deficiencies may be exaggerated for captive-raised animals prevented from associating with conspecifics in wild environments during critical learning periods (Snyder et al. 1996).

Programs typically release birds as independent juveniles or as adults. The relative advantages of each age class are summarized here:

Advantages of releasing independent fledglings:

- *May increase learning of wild behaviors.* Learning and matching the songs of future neighbors may be important for territory establishment (Beecher et al. 1994). In the late summer, juvenile FGSP have been observed practicing song while in juvenile flocks as well as visiting the territories of unrelated adult males still singing in late summer (FWC unpub. data). It is unknown how valuable these visits are to their social and song learning experience, but FGSP males with atypical songs may fail to attract mates (Hewett Ragheb et al. 2015). Exploratory movements by newly independent wild FGSP juveniles also may be important for surveying suitable habitat at a regional scale or the development of habitat-specific preferences.
- *May provide birds better conditions in which to acclimate.* Releasing birds in the summer of their hatch year also allows them to acclimate to the wild during warmer temperatures when insect prey may be more abundant. Post-release survival for hand-reared juvenile Cirl Buntings was higher for birds that were released in June or July compared to August (Fountain et al. 2017). Preliminary analysis on FGSP indicate that nestling mass and first-year survival is highest for wild birds that fledge in mid-summer (30 June) suggesting improved resource availability or favorable weather conditions during that period (FWC unpub. data). Releasing FGSPs in the summer would allow acclimation several months prior to the fall arrival of migratory Northern Harriers (*Circus hudsonius*; the most reported predator of overwintering FGSPs; Dean 2001). Additionally, releasing birds soon after they reach nutritional independence reduces the amount of time individuals are exposed to potentially novel pathogens *ex situ* (S. Citino, pers. comm.).
- *Birds released as independent young require fewer resources from captive-breeding facility staff.* If all captive-reared young were released as independent fledglings, the size of the captive flock would decrease during winter months, reducing annual operational expenses.

Advantages of releasing second-year adults

- *Greater proportion of captive flock would survive to breeding age.* As noted above, survivorship of birds in captivity is substantially greater than that in the wild.
- *Releases would not co-occur with molt.* FGSP pre-basic molt lasts approximately 38 days (starting in early July for first clutches; P. Reillo, unpub. data), is energetically demanding, and reduces flight capabilities in passerines (Swaddle and Witter 1997), and as such can restrict movement (Vega Rivera et al. 1999).
- *May increase site fidelity.* Male FGSPs appear to leave their breeding territories in the fall and wander to points unknown (Dean 2001). Release of captive birds just prior to the start of the breeding season would coincide with environmental, social, and hormonal cues that encourage pairing and breeding (i.e., residency) and thus potentially reduce the opportunity for dispersal. Conversely, it also is possible that releases just prior to the breeding season may reduce site fidelity because of intraspecific aggression from territorial birds (R. Bowman, pers. obs.).
- *Monitoring in spring is more effective.* The fate of released birds can be more effectively determined during spring and summer when sparrows are more conspicuous and full-time field crews are in place.

Decision

Given the uncertainty surrounding the appropriate age to release birds, we will conduct releases within an adaptive framework (Kemp et al. 2015). Appendix B outlines releases of both independent juveniles and second-year adults, with release of second year birds beginning in Spring 2019, pending the outcome of the DRA. We set a minimum goal of 15% recruitment (see Determination of Minimum Annual Cohort Size) for both release age classes because we do not yet know which age class will have a higher *combined* rate of 1) survival to breeding age, and 2) recruitment. Our hypothesis is that independent fledglings will have lower survival to breeding age, but higher recruitment rates compared to released second-year birds.

6. QUARANTINE

Quarantine protocols are implemented to minimize risk of spreading novel diseases to wild populations from captive birds and to ensure that birds are in good health before they are released. Quarantine protocols often include a period of separation from other individuals and a series of relevant health screenings. However, the need for screening samples and health evaluations must be balanced with the detrimental effects of handling and confinement stress for the birds (Kock et al. 2010, Sangster and Vogelnest 2016). DRAs are often implemented to inform quarantine protocols (and release programs more generally) because they provide a structured approach to describing the risk to wild populations associated with releasing captive-born or translocated animals (Leighton 2002, Jakob-Hoff et al. 2014, Jeffs et al. 2016). We have scheduled a DRA in November 2018 to assess the risk to the wild population of releasing captive FGSP and to inform quarantine protocols for release candidates.

A minimum quarantine period of 30 days has been broadly recommended for wildlife translocations (Kock et al. 2010), however, the quarantine duration and protocols for passerine captive rear-and-release programs vary substantially. Quarantine for Cirl Buntings and Mangrove Finches required indoor facilities dedicated for hand-rearing nestlings that were insect-proof and separate from other species (Cunninghame et al. 2015, Jeffs et al. 2016). Once independent, juveniles were directly transferred to *in situ* field aviaries for acclimation, but no pre-release screening was performed once outdoors (Cunninghame et al. 2015, Jeffs et al. 2016). Captive hand- and parent-reared `Alala and Puaiohi underwent an initial health screening prior to transfer to an *in situ* acclimation aviary and a second health screening prior to release (Kuehler et al. 1995, Kuehler et al. 2000, Switzer et al. 2013). The DRA for Regent Honeyeaters found that there was no need for a quarantine at the breeding facilities to manage disease risk, but an extensive pre-release screening was performed at a central facility (Jakob-Hoff et al. 2014).

Decision

Release decisions and quarantine length and protocols are preliminary and will not be finalized until they are informed by the November DRA. However, our *a priori* hypothesis is that the risk of release to wild and released birds will be acceptable, and we propose the following logic and methods for the aviary conditions, location, and duration of FGSP quarantine based on a review of the literature and consultation with wildlife veterinarians.

- The purpose of the quarantine period for FGSP will be to ensure that symptomatic, unhealthy birds are not released. Therefore, pre-release health screenings will occur at the *ex situ* facilities as described in Appendix C.
- Any quarantine involving captive, parent-reared FGSP will be conducted in outdoor aviaries because confining these birds to small indoor spaces may increase stress leading to immunosuppression or increased susceptibility to infection (Kock et al. 2010). In addition, confining birds in small indoor spaces will likely be detrimental to our goal of promoting wild-like behaviors.
- Outdoor quarantine will consist of spatial separation of a small group from other captive species and conspecifics. Strict adherence to quarantine protocols for human and equipment movement between aviaries will be followed. However, it is still possible that birds may be exposed to vector-borne pathogens or the feces of other birds flying over or perching on the aviaries.
- Because outdoor aviaries cannot entirely prevent the flow of pathogens to or from the aviary, quarantine pens should be located at the *ex situ* facilities to reduce the risk of exposing wild populations to disease. Furthermore, *ex situ* quarantine will facilitate timelier visual and clinical health monitoring by wildlife veterinarians (Kock et al. 2010) compared to remote *in situ* locations.
- The length of quarantine depends on the pathogens of concern. In the case of FGSP, pathogens of most concern include microfilaria and extra-intestinal coccidia, pending the results of the DRA. Preliminary data indicate that the same genera (and possibly the same species) of microfilaria found in the captive flock are also present in the wild population (J. Austin, J. Eells, and J. Wellehan, unpub. data). Additional identification of the coccidian pathogen is pending,

although many rear-release and translocation projects consider this pathogen low-risk since it is usually host specific (McGill et al. 2010, Ewen et al. 2012). Quarantine decisions depend in part on whether the pathogens of concern can (or should) be cleared from birds scheduled for release. Coccidia can be reduced but not eliminated, and filarial worms often cannot be eliminated without endangering the bird. Some exposure to native parasites may improve post-release survival for captive-bred animals (Faria et al. 2010). The Cirl Bunting program aimed to control, but not eliminate coccidia parasites during pre-release quarantine (McGill et al 2010).

- The length of quarantine will be minimized to the extent practicable because birds may be at increased risk of pathogen infection while held in aviaries at higher stocking densities than they would be in the wild. Lengthy quarantines may also impair our ability to release birds to the wild as independent juveniles during a developmental period that may be critical to learning.

FGSP disease research continues, and we will modify our proposed path forward when new information suggests it is warranted. In addition, the DRA will provide recommendations to guide quarantine procedures.

7. ACCLIMATION WITH PRE-RELEASE OBSERVATION

The decision to include an acclimation period before release is an important consideration of release programs. Expert reviews (Jones and Merton 2012, Batson et al. 2015) recommend direct releases for wild birds and delayed releases for birds raised in captivity. Delayed release may help ease the transition of naïve birds raised in captivity to the wild (Batson et al. 2015). For example, captive-bred gamebird species benefit (e.g., greater survival, greater site fidelity) from an acclimation period before being released into the wild (Combreau and Smith 1998, Lockwood et al. 2005). *In situ* acclimation periods are commonly seen in captive-rearing programs where birds are hand-reared in indoor “clean” spaces and no long-term *ex situ* holding space for independent juveniles is available or desired (‘Oma’o [6-9 days] and Puaiohi [8-14 days], Kuehler et al. 2000; Cirl Buntings [5-9 days], Fountain et al. 2017; Mangrove Finches [4-6 weeks], Cunnigham et al. 2015). Other programs retain birds in *in situ* field aviaries to allow them to acclimate to novel temperatures (Regent Honeyeater [1-3 days], Jakob-Hoff et al. 2014), to learn to forage on native food items (‘Alala [3-5 months], Kuehler et al. 1995), to regain weight lost during lengthy transport (Jakob-Hoff et al. 2014), or to provide structures that also serve as supplemental feeding stations post-release (Kuehler et al. 1995, Kuehler et al. 2000). However, some animals with critical developmental periods may not learn about their new environment effectively even with *in situ* acclimation periods if the acclimation occurs too late in life (Krochmal et al. 2018). Long durations may not always be warranted. For example, Menkhurst and colleagues (2010) did not find a survival advantage sufficient to justify the costs of extended *in situ* acclimation for captive-reared helmeted honeyeaters. The rearing method (parent- vs. hand-reared), age of release, similarities in climate between source and release sites, and the duration of *ex situ* acclimation should be considered when deciding if captive-reared birds will benefit from an extended on-site acclimation period.

To test these ideas, the FWS started a collaboration with WOCH and Tall Timbers Research Station to breed wild Eastern Grasshopper Sparrows (GRSP; *A. s. pratensis*; the migratory, non-listed subspecies) in captivity. During the summers of 2016-2018, 38 captive-raised GRSPs (36 independent fledglings and two second-year males) were transported from WOCH and released with radio transmitters to the wild Bainbridge population (J. Cox, unpublished data). Twenty were held in small field aviaries for 2-5 days prior to release, whereas 18 were released directly (16 by hand and two by passive release from transport box). Supplemental food was provided near the field aviaries for ca. 3 days, but none of the released birds was observed returning to the aviaries to feed. Of the 20 birds released after a short acclimation, six (30%) were confirmed settled on the landscape, nine died prior to leaving the aviary (five cage-related mortalities [flooding and ants] and four were too young), three went missing after release, and two dropped their transmitters and escaped from the aviary (J. Cox., unpub. data). Of the 18 birds that were released directly, 12 (67%) settled on the landscape and six went missing after release (J. Cox. unpub. data). Survivorship beyond the first month was unknown because very limited systematic searching was conducted after the radio-telemetry period ended and migratory passerines often disperse to new locations in their first year of life.

Several unanticipated cage related mortalities occurred in the group that had 2-5 days of acclimation resulting in higher post-release settlement for the direct-release group. However, some birds released directly by hand made long initial flights after release (potentially leaving the study area). This was not the case for the two birds that were allowed to exit the transport box on their own timing (passive direct release) suggesting that maintaining a calm environment during release may improve direct release settlement rates. The fact that so many birds survived the 3-4 weeks post-release suggests they were able to successfully forage on wild prey items and avoid predation. However, additional research is needed to determine optimal release protocols for FGSPs (Appendix B).

Advantages of direct release or short *in situ* acclimation periods (≤ 5 days)

- The following cage-related risk factors must be addressed when birds are kept in *in situ* enclosures. Despite measures taken to address these sources of stress and mortality, the probability of these risks occurring increases the longer birds are held in enclosures, therefore, short acclimation periods should have reduced risk associated with:
 - Predation from predators that gain access to aviaries
 - Conspecific aggression due to unnatural stocking densities or holding birds during periods when they would normally exhibit territorial aggression (this aggression has resulted in injury and death of FGSP in captivity).
 - Disease-related risks associated with stocking densities higher than wild sparrows (e.g. coccidiosis often occurs in captive situations when the birds are at higher than natural densities or are stressed; risk of coccidia exposure would be especially high around communal food bowls and would have to be managed; mosquitos may congregate inside aviaries increasing risk of vector-borne diseases)
 - Harassment from predators perching on or near aviaries
 - Unanticipated injury from cage structure
 - Malicious human disturbance (if the release site is on public property)
 - Insufficient nutritional supplementation (especially if birds in large aviaries will not drink from water bowls)

- Keeper error
- Would not require subsequent capture events to apply radio transmitters
- Reduced in situ husbandry staffing requirements and therefore annual programmatic costs
- Reduced aviary size requirements because captive-reared birds can be released in stages over a period of several weeks and rotated through the same facility space
- More flexibility to schedule acclimation during periods of optimal weather conditions

Advantages of longer acclimation periods (>5 days)

- Additional time to recover from handling or transport stress
- Additional time to get familiar with native prairie habitat and climate
- Potential for increased site fidelity once released
- Sick birds could be captured and treated or removed from aviary
- Increased potential to find carcasses of birds to identify disease-related mortality

Decision

The optimal *in situ* acclimation length for captive-reared FGSP remains unknown but, some *in situ* acclimation period for FGSP is likely warranted based on the benefits noted for most captive-born animals (Batson et al. 2015). However, many of the reasons provided for holding birds in acclimation cages in other captive-rearing programs do not apply in our system. We intend to initially evaluate two acclimation scenarios. First, parent-reared birds will be held in field aviaries for a brief acclimation period (up to 5 days). The objectives of the acclimation period for parent-reared FGSP are to allow for adjustment to radio transmitter harnesses, recovery from human handling, and recovery from transport (e.g. hydration). A short holding time seems appropriate for parent-reared birds because 1) the climates between the source and release sites are similar, 2) captive-reared FGSPs will be acclimated to outdoor conditions and natural prey items *ex situ* prior to release, and 3) preliminary trials with parent-reared GRSP showed wild behaviors and relatively high immediate post-release survival for birds held in field aviaries for only 2-5 days (J. Cox, unpub. data). A shorter acclimation period within the 5-day time frame or direct releases for some parent-reared birds may occur, if warranted, to mitigate risks to the birds (e.g., to eliminate intraspecific aggression from males). Second, hand-reared birds will be held in field aviaries for 8 weeks, unless the DRA suggests that a shorter acclimation period is sufficient or unless risks encountered during acclimation warrant reducing time spent in enclosures. Hand-reared birds may require additional time to develop natural behaviors relative to parent-reared birds. Additionally, this extended acclimation period may provide additional insight on disease-related mortality events that occur because of the stress of transport, stress of new environment, or illness related to exposure to wild pathogens they may not have been exposed to in captivity. The extended acclimation may increase the probability that only physically robust hand-reared birds are released. The extended acclimation period will not be considered a second quarantine period because the permeable aviaries will not prevent the spread of pathogens from the captive birds to wild birds and because the birds that survive the acclimation period may be resistant carriers. The risks of introducing pathogens to the wild population will be assessed at the DRA, and acclimation approaches may be adjusted based on the results of the DRA and using an adaptive framework after observation of FGSP pre- and post-release.

8. RELEASE LOCATION

The goal of many captive-rearing programs is to establish new populations at sites not currently occupied by the target species (e.g., Kuehler et al. 2000, Jeffs et al. 2016), whereas others aim to support existing populations (e.g., Cunnigham et al. 2015). The “best” release location for captive birds may not necessarily be the location where birds were most recently located or are currently located (A. Lieberman, pers. comm.). Release sites may be selected based on predator control programs (Cristinacce et al. 2008), flood risks, availability of nest sites, native food abundance (Kuehler et al. 2000), avoidance of pathogens (Tweed et al. 2003), or amount of contiguous habitat or management support by local landowners (Jeffs et al. 2016). Other considerations include accessibility and the ability to construct acclimation pens in difficult terrain (Kuehler et al. 2000).

Translocation projects often suffer from dispersal away from the release site (Tweed et al. 2003; Clarke and Schedvin 1997), and a lack of conspecifics at new release sites has been proposed as a factor leading to increased dispersal behavior (Mihoub et al. 2011). Observations of FGSP territory placement and site fidelity in the wild suggest they may also prefer to be near conspecifics (FWC unpub. data). Releasing captive-reared birds into larger extant populations may result in higher initial fidelity to the release site and facilitate pairing with wild FGSP. Alternatively, although site fidelity could be lower, releasing birds into small populations could bolster the populations most at risk of extirpation.

Decision

Initially, captive-reared FGSP will be released into extant subpopulations rather than attempting to establish new subpopulations. TLWMA was selected for the first year of release trials (2019; Appendix B) because it 1) has the largest extant FGSP population, suggesting conditions may be more suitable for survival or reproduction, 2) nest predation by red imported fire ants is rare, 3) we anticipate the larger resident population will promote post-release retention via conspecific attraction or pairing with wild birds, 4) it has an existing grid of fire lines which is amenable to systematic radio-telemetry tracking using an all-terrain vehicle, 5) year-round staff, funding, and equipment are available for post-release monitoring, 6) no additional authorizations are necessary, and 7) there are no military activities, private permissions, or seasonal high-water levels that would make certain areas inaccessible during key monitoring periods. Releasing captive-reared birds at one site first will allow for an initial evaluation of transport, acclimation, release, and post-monitoring techniques without adding a site effect. We will revisit our release location strategy based on recommendations from the DRA, if necessary.

Nevertheless, we recognize the urgency of bolstering smaller populations with captive-reared recruits in the near future to prevent extirpation. To balance the need to develop successful release methods with the urgency of supporting small populations, triggers will be developed (with consultation by the FGSP working group) and evaluated in fall 2019 to establish if, when, where, and how many birds should be released at alternate sites the following spring. Annual check-in meetings will be held each fall to revisit release site goals in relation to the most current data (see Appendix B: Evaluation of Release Methods). Example triggers may include:

1. Recruitment rates of captive-reared birds observed to date.
 - Example: If recruitment is <10%, this suggests that more work is needed to test and improve release techniques, and answering remaining methods questions may take priority over releasing birds at multiple sites. Also, if recruitment rates are very low, expanding the program to a second site may not be justified relative to the level of conservation benefit.
 - Example: If recruitment is >15%, this suggests that release methods are performing better than the minimum acceptable rate. Improvement may still be desired but supplementing vulnerable small populations may take priority over improving release methods, and/or the addition of a site covariate would have less impact on the evaluation of release techniques.
2. Preparation and suitability of alternate release sites to receive captive-reared birds.
 - Example: Depending on the recommended release strategy, field aviaries may need to be constructed, telemetry equipment purchased, and additional staff hired and trained. Organization and agency approvals for the required activities would need to be in place prior to the release of birds. An evaluation of known threats (e.g. nest predators) and availability of management solutions at that site may also be considered.
3. Availability of birds not necessary or otherwise suitable for testing pilot release techniques.
 - Example: If the genetic management of the captive flocks determines that several three-year old adult males are genetically redundant and not high priority for breeding, these males may be selected for release at an alternate site to make space for breeders with higher genetic value. Because these males fall outside of the target age classes for the initial release trials, releasing them at an alternate site would not detract from our ability to evaluate release methods, but may provide great value to the small population through increased conspecific attraction and genetic diversity at the site.
 - Example: More captive birds are produced than required for initial release trials and these birds are scheduled for release at an alternate site.

Table A1: Summary of demographic rates of released captive-bred passerines from published literature and expert opinion.

Metric	Common name	Scientific name	Mean	Citation
1st month survival	Cirl bunting	<i>Emberiza cirius</i>	59%	Jeffs et al. 2016
	Puaiohi	<i>Myadestes palmeri</i>	36%	Switzer et al. 2013
	'Oma 'o	<i>Myadestes obscurus</i>	80%	Kuehler et al. 2000
Survival to 1st breeding season	Cirl bunting	<i>Emberiza cirius</i>	28%	Jeffs et al. 2016
	Mangrove finch	<i>Camarhynchus helibates</i>	26% ¹	Charles Darwin Foundation 2018
	Mauritius fody	<i>Fouda rubra</i>	52% ²	Cristinacce et al. 2009; A. Cristinacce, pers. comm.
Recruitment rate (proportion of released birds that bred)	Mangrove finch	<i>Camarhynchus helibates</i>	5%	Charles Darwin Foundation 2018
	Canadian loggerhead shrike	<i>Lanius ludovicianus</i>	3%	Nichols et al. 2010
		<i>Lichenostomus melanops</i>		
	Helmeted honeyeater	<i>cassidix</i>	23%	Menkhorst et al. 2010
	Puaiohi	<i>Myadestes palmeri</i>	9%	Switzer et al. 2013
	Mauritius fody	<i>Fouda rubra</i>	43% ²	Cristinacce et al. 2009; A. Cristinacce, pers. comm.

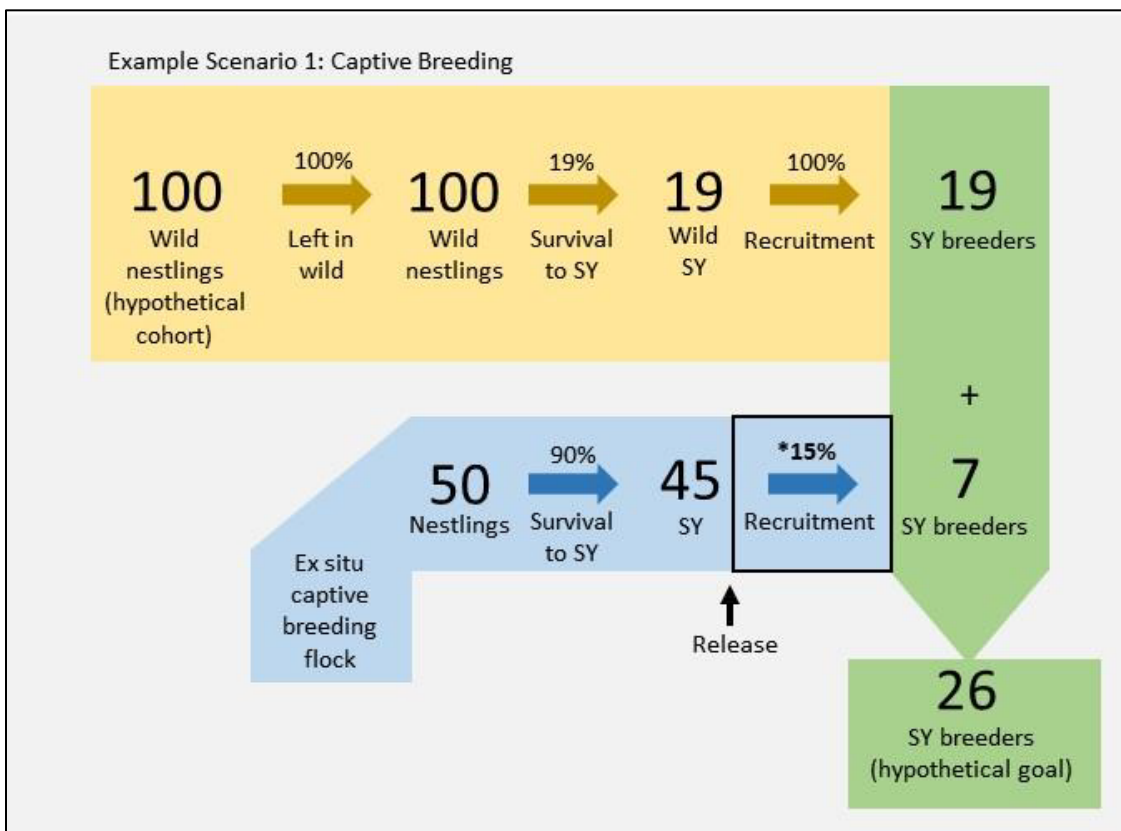
¹Reported as "long-term post release" rather than survival to breeding.

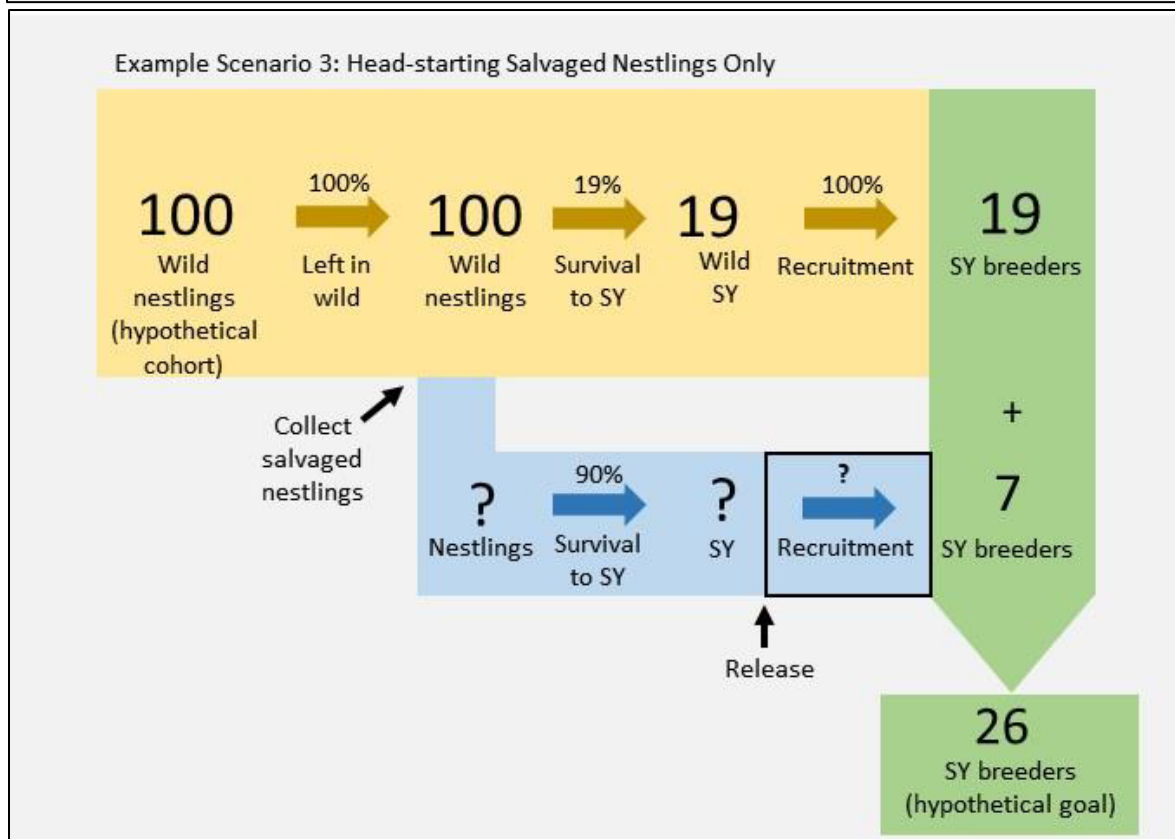
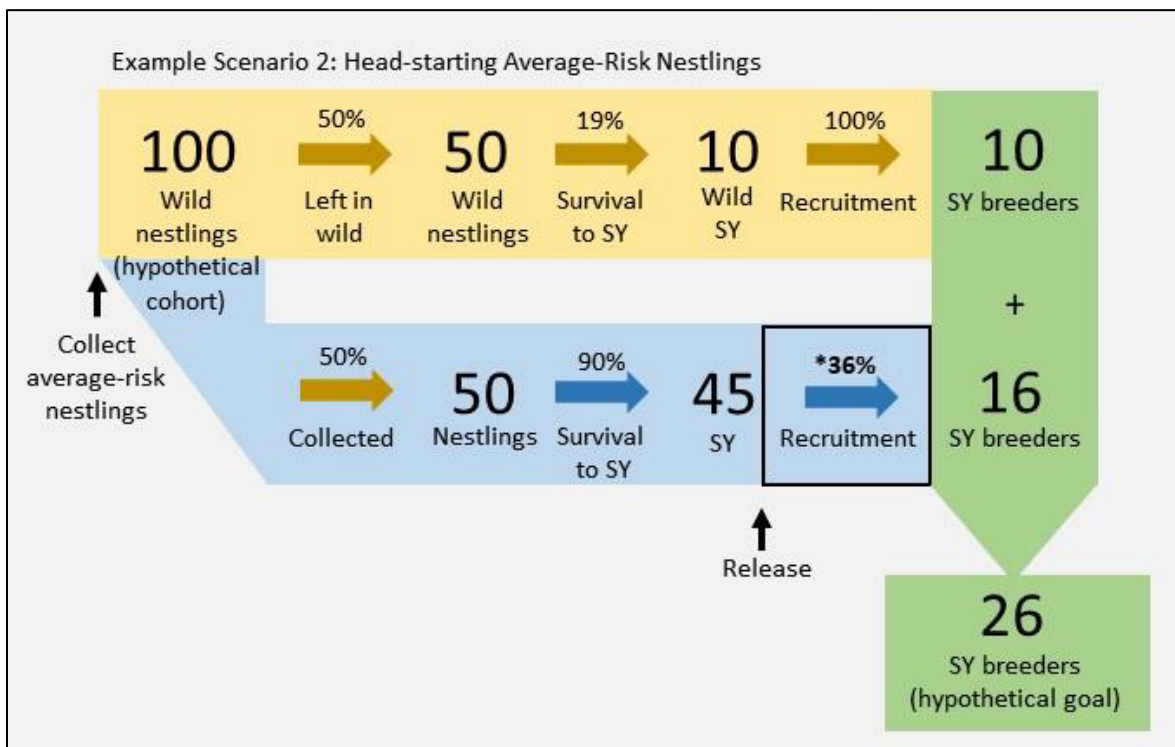
²Releases occurred on a predator-free island

Box A1: Comparing the efficiency of alternative captive management options for FGSP.

Introduction: The flowcharts in this section represent three alternative captive management scenarios for FGSP: 1) traditional captive breeding, 2) head-starting average-risk nestlings, and 3) head-starting salvaged nestlings only. The hypothetical goal for all scenarios is to produce a wild population with 26 second-year (SY) breeding females starting with a hypothetical cohort of 100, 4-day old nestlings. The post-release recruitment rates of captive-reared birds required to accomplish the goal are highlighted in the black boxes.

Methods: All scenarios use 4-day old nestlings as the starting point because this is the youngest age nestlings are eligible for collection and head-starting (P. Reillo, pers. comm.). We assumed a 19% probability of surviving the period between 4-days old to SY based on an 89% probability of surviving the period between 4 and 8 days of age in a fenced nest at TLWMA (0.97 daily survival rate; 2015-2017) and a 21% probability of surviving the period between fledging and the following year (first-year survival; FWC unpub. data). We assumed that 100% of uncollected females that survive to SY age are viable breeders because unpaired SY females are not detected. For collected or captive-bred nestlings, we assumed that all 4-day old nestlings in captivity will fledge and 90% will survive to breeding age. For Scenario 3, we assumed that collecting an unknown number of salvaged nestlings (certain to die if left in the wild) would not decrease the number of wild SY breeders produced. We back-calculated the post-release recruitment rate (black boxes) required to meet the hypothetical goal by calculating the number of SY breeders from captive-reared sources needed to reach 26, then dividing this number by the number of captive-reared birds surviving to SY age (45 in Scenarios 1 and 2).

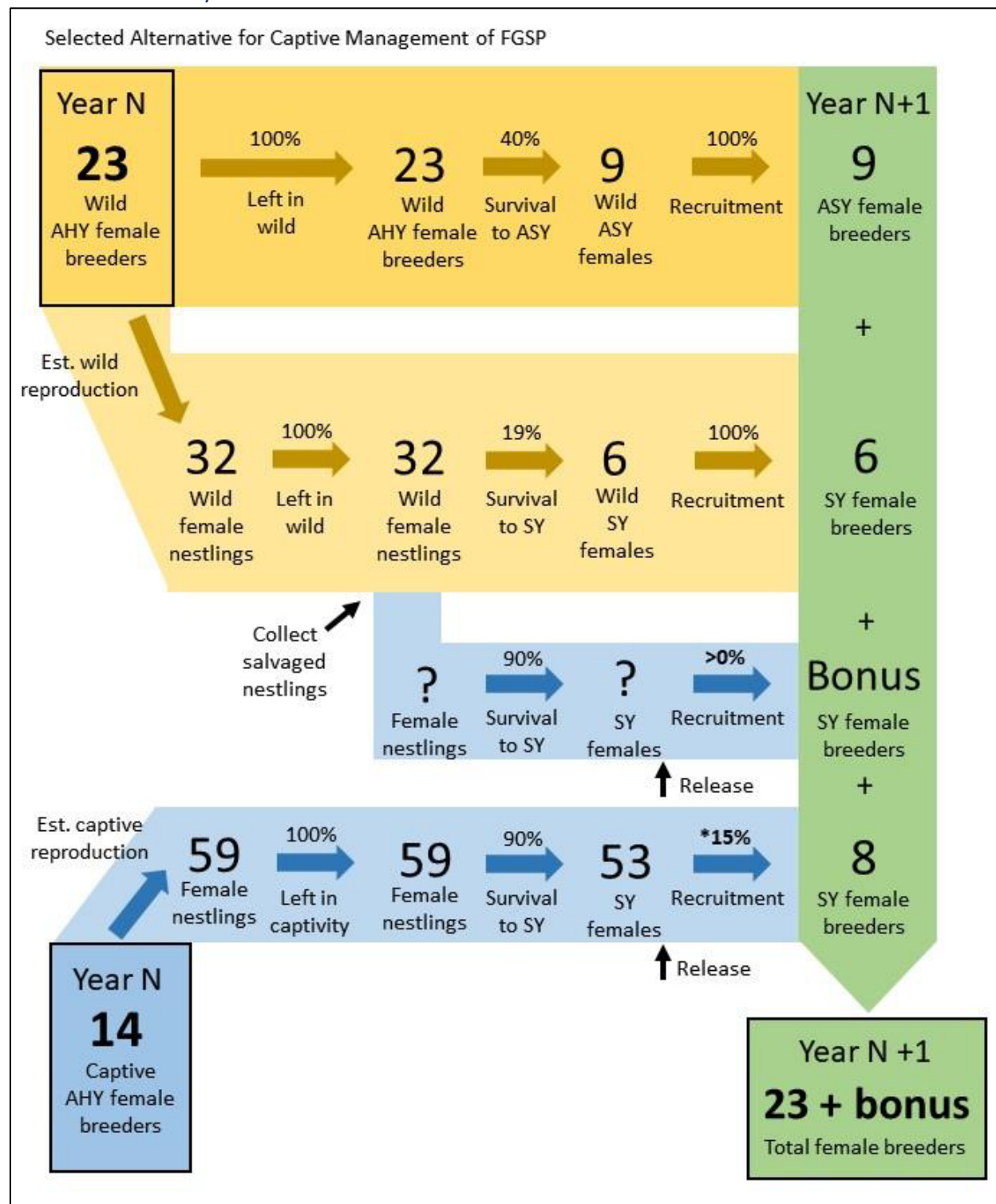


Box A1: continued

Box A1: continued

Conclusion: Traditional captive breeding (Scenario 1) is more efficient than head-starting average risk nestlings (Scenario 2) because it requires a lower post-release survival and recruitment rate. The post-release survival and recruitment rate required to meet the goal using only head-started salvaged nestlings (Scenario 3) is unknown, because the number of salvaged nestlings available each year is unknown. Therefore, using a traditional captive breeding strategy as the primary means to accomplish wild population goals may be the most effective, but collecting salvaged nestlings for head-starting has minimal impact to the wild population and may be used as a source of “bonus” SY recruits.

Box A2: Flowchart depicting the preferred alternative for captive management of FGSP as described in the 5-year vision document.



Box A2: continued

Methods: The starting number of after-hatch-year (AHY) adult females in Year N (23) is based on the estimated number of females in the wild in 2018. The minimum goal set by the vision for the captive program is to supplement the wild population with enough second-year (SY) female breeders to maintain at least 23 total female breeders in the wild. We estimate that 9 of 23 AHY females will survive to the following year (after-second-year; ASY) based on a mean annual survival rate of 40% (Three Lakes Wildlife Management Area population [TLWMA]; 2013-2017; FWC unpub. data). We assume that 100% of uncollected females that survive to breeding age are viable breeders because unpaired females are rarely detected. We estimate that the 23 females in Year N will produce 32, 4-day old female nestlings based on a fecundity rate of 1.39, 4-day old female nestlings per breeding female (TLWMA; 2013-2017; FWC unpub. data). We estimated a 19% probability of surviving the period between 4-days old to SY based on an 89% probability of surviving the period between 4 and 8 days of age in a fenced nest at TLWMA (0.97 Mayfield daily nest survival rate; TLWMA; 2015-2017) and a 21% probability of surviving the period between fledging and the following year (first-year survival; TLWMA; 2015-2017; FWC unpub. data). The number of salvaged nestlings collected for head-starting varies each year and is unknown but because there is little impact to the wild population, any salvaged female nestlings that survive to breeding age are considered bonus females. We estimate that 14 adult female breeders in a traditional captive breeding program in Year N will produce 59, 4-day old female nestlings based on an observed fecundity rate of 4.67, 4-day old female nestlings per breeding female (White Oak Conservation Holdings population; 2017). We assume all 59 will survive to fledge, and 90% will survive to the following year (Year N+1) while still in captivity. Using the minimum threshold for post-release survival and recruitment (15%) outlined by the vision, these captive-reared females will contribute 8, SY female breeders to the wild population resulting in a total of 23 female breeders in Year N+1 plus any recruits produced by head-starting. The numbers and rates presented in this figure represent the best available data and understanding of FGSP biology. However, the small FGSP populations are vulnerable to annual stochasticity and mean demographic rates are refined each year as additional data are collected.

APPENDIX B. 2019-2020 Adaptive Release Plan

OBJECTIVES

Rigorous experiments are warranted to address questions about the effectiveness of animal reintroductions. However, the ability of researchers to carry out true experiments in reintroductions is usually limited by low numbers of source individuals and difficulties associated with conducting synchronous releases at multiple experimental sites (Armstrong et al. 2007). Consequently, an adaptive management framework has been recommended (Kemp et al. 2015). We propose to assess the feasibility of releasing male and female FGSPs when the birds are at different stages of development (independent juvenile vs. pre-breeder). If sample sizes allow, we also will assess the relative effect of two rearing types (hand-reared vs. parent-reared) and acclimation lengths during the first year of release. Proposed objectives include:

1. Releasing enough captive-reared FGSP to meet the FGSP vision's objective of stabilizing or growing the wild population (i.e., maintaining at least 23 wild breeding females) each year.
2. Tracking released birds using radio telemetry to assess basic habitat use, movement patterns, and causes of death when possible.
3. Estimating the survival of released birds according to age at release and sex.
4. Resighting released birds and monitoring their nests during the breeding season to estimate pairing and nest success rates.

METHODS

Composition of Captive Releases

Release candidates

FGSP reared or housed at any *ex situ* facility will be identified for potential release in coordination with captive facilities, based on genetic value to captive or wild populations, sex, and age. FGSP pedigree data will be updated each fall with all captive and wild progeny surviving to banding age. This pedigree data will be evaluated with PMx demographic and genetic analysis software (Lacy et al. 2011) to evaluate the male-female pairings that would result in the highest genetic diversity in the captive flock. PMx will provide kinship parameters to assess relative genetic uniqueness and birds with fewer relatives in captivity or the wild will be prioritized for retention as breeders in the captive flock. The sex ratio of the birds targeted for release will depend on the sexes of the surviving captive birds and capacity constraints.

Birds will be released as part of a formal release experiment when they are either independent fledglings or pre-breeding second-year birds (see *Release Methods* below). Captive birds older than one year of age will be retained in the captive flock as breeders as necessary, transferred to holding facilities, or released.

Ex Situ Quarantine and Pre-transfer Screening

**NOTE: The health screening protocols presented here and in Appendix C will be modified based on the outcome of a formal disease risk analysis (DRA) workshop scheduled for November 2018.*

All captive-reared birds will be banded as nestlings when 5 days old. During this handling period they will be weighed, color-banded, vaccinated for West Nile/WEE/EEE, and a buccal swab will be collected for genetic sexing. Once juveniles reach nutritional independence (30-40 days post-hatch) they will be separated from their parents and undergo their first health screening examination (Appendix C). After the examination, juveniles will be placed in a separate, naturally vegetated, outdoor *ex situ* quarantine aviary with other birds from their eventual release cohort. Juvenile release cohorts often will be sibling groups to reduce the number of times birds will need to be handled. However, groups to be released as adult pre-breeders may contain unrelated birds depending on optimal pairings in captivity or wild flocks or other husbandry needs. While in the quarantine aviary, the juveniles will remain separate from other captive birds at the facility but may be exposed to wild birds that fly over or perch on the pen. Human movement to and from each *ex situ* aviary will follow strict quarantine procedures (Appendix C). Preventative health measures such as frequent observation, routine blood draws, and vaccination will be conducted year-round such that sick animals can be identified and treated quickly.

Birds selected for release as independent juveniles will remain in their quarantine pens until all results from their health exam have been reviewed (number of days pending DRA) and they are at least 40 days post-hatch (younger birds may fail to thrive as observed during GRSP release trials; J. Cox, unpub. data). Birds selected for release as second-year pre-breeders will remain in their *ex situ* quarantine pens until the following January-February. Prior to their scheduled release, they will be screened again following the same examination protocol (Appendix C). A decision tree will be produced after the DRA to provide guidance when determining which birds pass their health screening and are eligible for release. No intermediate or *in-situ* quarantine facility will be used.

Translocation and Release

Release location

Release trials in 2019 will take place at the Route 60 dry prairie unit of the TLWMA in Osceola County (see Appendix A for rationale). Releasing birds at additional sites will be evaluated each fall (see Appendix A and Evaluation of Release Methods).

Age of release

Sparrows will be released at two different seasons when the birds are at different developmental ages (Fig B1). First, juveniles will be released soon after they reach nutritional independence (≥ 40 days post-hatching) and have passed their pre-release quarantine exams. Although the exact month of releases is uncertain, we anticipate that fledglings (≥ 40 days old) may be available for release from June to September, which matches the natural timing of increased wild fledglings on the landscape. The second release group will be second-year pre-breeders released during mid-Feb, just prior to the development

of aggressive territorial behavior (mid-Feb; A. Schumann, personal observation) and pre-dawn singing in the wild (mid-Feb; FWC unpub. data).

Hand-reared and parent-reared

If sample sizes allow, we also will assess the relative effect of two rearing types (hand-reared vs. parent-reared) during the first year of release. Releasing approximately 15 hand-reared birds (from a combination of captive-bred and head-started individuals) per year over two years would likely be sufficient to address whether or not hand-reared birds are effective breeders in the wild. If there are fewer than 15 hand-reared birds available by August 1, additional wild nestling collections may be considered in the first year of releases as a way to ensure sample sizes are sufficient to test the efficacy of this strategy. This comparison early in our program will provide information for future programmatic planning.

Transport

On the morning of transport, captive birds will be captured within 2 hours of sunrise using mist nets or hand nets and placed in separate transport boxes in climate-controlled vehicles. Transport time is ca. 3 hrs 30 min from WOCH and 1 hr 45 min from RSCF. Whenever possible, birds will be transported to the field site in batches (tentatively 4-8 birds per group) and captive staff will meet field staff at a halfway point to transfer birds to reduce transport time for staff. Birds will be provided with fresh insects and seed during transport. For reference, the total transport time (including a stop for radio harness attachment) was 6 hours for the GRSP release trials during transfer (J. Cox, pers. comm.).

Color-band verification

All birds scheduled for release will be banded with a USGS numbered aluminum band prior to entering the field aviary. Band combinations will be confirmed in advance with the captive facilities and changed if needed to ensure that no captive-reared birds are accidentally released with band combinations that match wild birds.

In situ acclimation

In 2019, we plan to pursue and compare two acclimation length strategies that both require the use of field aviaries (see Appendix A for rationale): First, birds that have been parent-reared in captivity will be placed in field aviaries for up to 5 days. Birds will be transported in group sizes consistent with release occurring over 2-4 weeks, depending on quarantine and acclimation constraints to be determined at the DRA. Our goal is to release all males prior to late February to avoid aggressive behavior in the field aviaries and allow released males to establish territories at the same time as wild males. Females may also be released prior to late-February or up to a few weeks later. A shorter acclimation period during the 5-day time frame or direct release of some birds may occur as warranted to mitigate risks to the birds (e.g., to eliminate intraspecific aggression from males). Second, birds that were hand-reared in captivity will be placed in field aviaries for 8 weeks, unless the DRA suggests that a shorter acclimation period is sufficient, or unless risks encountered during acclimation warrant reducing time spent in enclosures. The start of the acclimation period will depend on the construction of the field aviaries, but our goal is to transport birds to field aviaries by mid-January so they can be released by mid-March.

Acclimation periods of different lengths (including possible direct release) will be considered based on the results of early releases to maximize the safety of the birds and the effectiveness of the program.

The field aviaries will be placed in dry prairie habitat not scheduled for prescribed fire during the holding period or prone to severe seasonal flooding. Field aviaries will be constructed to provide sufficient space (ca. 60 sq ft per bird) depending on quarantine and acclimation constraints to be determined at the DRA. For example, if an 8 week acclimation period is required for all birds, two, 20 x 56 ft aviaries will be required to hold all birds simultaneously, but a single aviary may be sufficient if a shorter acclimation is selected because birds can have staggered entries and releases.

While in the field aviary, FGSP will be provided with ample seed, insects (crickets and waxworms), water, and shelter from severe rain. They will be monitored by local staff twice a day during daylight hours. Field cameras may be placed at the feeding stations inside the cages to monitor general behaviors, census the flock, and possibly detect any injury or illness. Field staff will attend 1-2 weeks of training at a captive facility prior to the transport of birds to the field aviaries. A detailed *in situ* husbandry protocol document will be drafted prior to the arrival of birds. Veterinarians will be contacted to serve as on-call resources in the event of injury or other health concerns.

Release

We will monitor the weather forecast with the intent to release birds when at least two consecutive days have <30% chance of rain. We will also delay the scheduled release of birds if high winds or severely cold temperatures (<32 deg F) are predicted. On the day of release, the food dish will be moved outside the cage and the release door(s) of the field aviary will be left open to allow the birds to leave on their own. Releases will be conducted mid-morning to increase the likelihood that birds have an opportunity to visit the food and water bowls prior to release. The door will be shut once all birds have left. Any birds that have not exited the aviary three hours prior to sunset on the release day will either be returned to the captive facility (if health or behavioral issues are presented) or encouraged to leave the pen by gentle flushing. Supplemental food (seed) will be provided outside the field aviary for up to one-week post-release unless camera evidence suggests the food is not being used.

Post-release Monitoring

Radio-tracking

We will fit parent-reared birds (short-acclimation) with a radio transmitter while still at the captive facility on the day of transport. This will allow for one additional examination period prior to placement in field aviary to ensure harness fit. We will capture hand-reared birds (long acclimation) and apply their radio telemetry harnesses within the field aviary 2-days prior to their scheduled release date.

Transmitters will weigh ca. 0.6g or less (ca. 4% of a 15.0 g sparrow; tentatively Ag379 Lotek Brand transmitters) attached with a hip-harness using stretchable sewing thread that degrades relatively quickly so tags will eventually be dropped (Streby et al. 2015). No transmitter related injury was observed during the release of 38 captive-reared GRSP (2016-2018; J. Cox, unpub. data).

Approximately 25 radio transmitters will be deployed for each age class (50 per year). In 2019, radios will be divided among both sexes and acclimation length treatment groups. Tracking males may allow us to monitor the effectiveness of the transmitters (e.g. males that lose signal may be detected singing confirming survival and transmitter failure rather than dispersal). We may also be able to observe and respond to more transmitter related injuries (should they occur) with males than females. Because females are rarely observed incidentally, transmitters will be the only way to monitor their survival and movement during the 3-4 week period post-release. However, it may be conservative to leave some females without transmitters until we can verify the safety and effectiveness of the devices.

On the day of release, we will monitor released birds as frequently as possible during the first 48 hours following release, with a goal of acquiring 2-5 locations per bird per day. On the first two days of tracking, released birds will be given sufficient physical space to avoid flushing or forcing movement by the observer. Observers will get close enough to retain a strong signal when volume dialed down to 3-4, but not much closer (J. Cox, pers. comm.). By tracking birds intensively during the first and second days post-release, we hope to better distinguish mortality from dispersal. For birds that do perish shortly after release, intensive tracking may allow us to locate the carcass to assign probable cause of death through visual evidence (predation) or necropsy. Any carcasses without obvious cause of death will be kept cold and immediately shipped to a diagnostic laboratory for necropsy.

We will attempt to obtain at least one morning and one evening location on days 3-7 following release. We will then track the individuals once daily until transmitter signal is lost or the bird perishes. We expect transmitter batteries to reliably last up to 28 days (J. Cox, pers. comm.). For every detection event, we will collect a GPS location as well as data on flush distances and general behavior (wariness) upon discovery. If we cannot obtain a location for the individual, we will conduct a standardized search by driving ATVs (or a field truck with a mounted antenna) and stopping periodically across a standardized grid of firebreaks transecting the entire study area. Occasional airplane flights may be available to assist with detection of missing birds that may have left the study area. The mean daily distance traveled for wild independent GRSP juveniles was 146 m (range = 5-966 m; Small et al. 2015). The number of detections per day may vary depending on the number of staff available in relation to the total number of birds released. Movement patterns and distances traveled will be summarized for each released bird using GIS spatial analysis. We will summarize the fates, daily distances, and total number of tracking days for each released individual.

Post-release monitoring

After the radio transmitter battery dies, we will systematically search for males released as second-year pre-breeders during routine spring point count surveys at TLWMA (3 replicates; mid-Mar through early June). We will use a spotting scope to record the band combination and collect one or more GPS locations for each FGSP detected during point count surveys or opportunistically. Females released as second-year pre-breeders will not be easily detectable during standard point count surveys. Instead, we will use male behavioral information to assist with pairing status assignment and nest searching. We will then resight females in the field, on nest camera, or by capture to confirm identification of each female associated with active nests each year (Apr-Aug). We will add nest cameras to nests of captive-reared females to observe breeding behaviors. If abnormal behaviors are observed (e.g. infanticide, low

provisioning rates) the field crews will consult the agencies to discuss emergency actions (collection of female or young).

For birds released as independent juveniles (both sexes), late-summer point count surveys are unlikely to result in detections. Once transmitter batteries die, juveniles will be resighted opportunistically during routine visits to occupied territories or areas where fledglings have been observed congregating. It is possible that juveniles with radio-tags will inform the field team of new fledgling congregation areas increasing the probability that banded juveniles will be incidentally resighted. Juveniles that survive their first winter will be included in routine monitoring (described above) to assess survival and reproductive status.

We will use mark-recapture modeling (R-mark; Laake 2013) to estimate interval survival and detection probabilities for birds released as second-year pre-breeders (Mar-Sep) and independents (Jun-Sep). Apparent annual survival of birds released in 2019 and 2020 will be assessed by their detection on point count surveys or opportunistic sightings in 2020 and 2021 respectively. Partners at sites where birds will not initially be released (KPPSP, APAFR, and a private ranch) will report any dispersal movements of released birds detected during routine point count and band resighting surveys (Apr-Aug).

CRITERIA FOR SUCCESS

1. Released birds exhibit reproductive behaviors (song patterns, territory establishment, pair formation) comparable to wild birds.
2. Demonstration that at least one of the released age classes can achieve a recruitment rate of $\geq 15\%$ for females.

EVALUATION OF RELEASE METHODS

We will evaluate release locations, the success of the two release age classes, and modify future release methods at specific timepoints.

Fall 2019: Evaluate transport, acclimation, release, and post-release monitoring methods and make recommendations for improvement. Also evaluate the post-release survival and recruitment of second-year adults released in Feb 2019 and the post-release survival of juveniles released in summer 2019. This preliminary information will be used to develop triggers for the potential release of captive birds at KPPSP, APAFR, or other sites. If triggers are met, then actions will be taken to expand the release program to multiple sites.

Mid-June 2020: Evaluate age of release. All juveniles produced in captivity in 2020 will be retained overwinter and released as pre-breeders in 2021 if the survival and

reproductive rates of the 2018 and 2019 pre-breeders exceed those of the 2019 juveniles. If the two groups are not statistically different, then half of the 2020 cohort will be released as juveniles and half retained in captivity for release in 2021.

- Fall 2020: Review results to date. Repeat evaluation of release location triggers and make recommendations to modify release methods (if needed). These fall evaluation meetings will be repeated each year.
- Mid-June 2021 Evaluate age of release. All juveniles produced in captivity in 2021 will be retained overwinter and released as pre-breeders in 2021 if the survival and reproductive rates of the 2018-2020 pre-breeders exceed those of the 2019-2020 juveniles. If the two groups are not statistically different by this time, then we would release all birds as independent juveniles to allow for increased captive production and reduced *ex situ* operational costs.
- January 2022: Review results to date. Repeat evaluation of release location triggers and make recommendations to modify release methods (if needed). This evaluation will be combined with the 3-year program checkpoint (see Vision 'Checkpoints and Criteria for Success')
- Fall 2022: Review results to date. Repeat evaluation of release location triggers and make recommendations to modify release methods (if needed).
- January 2024: Review results to date. Repeat evaluation of release location triggers and make recommendations to modify release methods (if needed). This evaluation will be combined with the 5-year program checkpoint (see Vision 'Checkpoints and Criteria for Success')

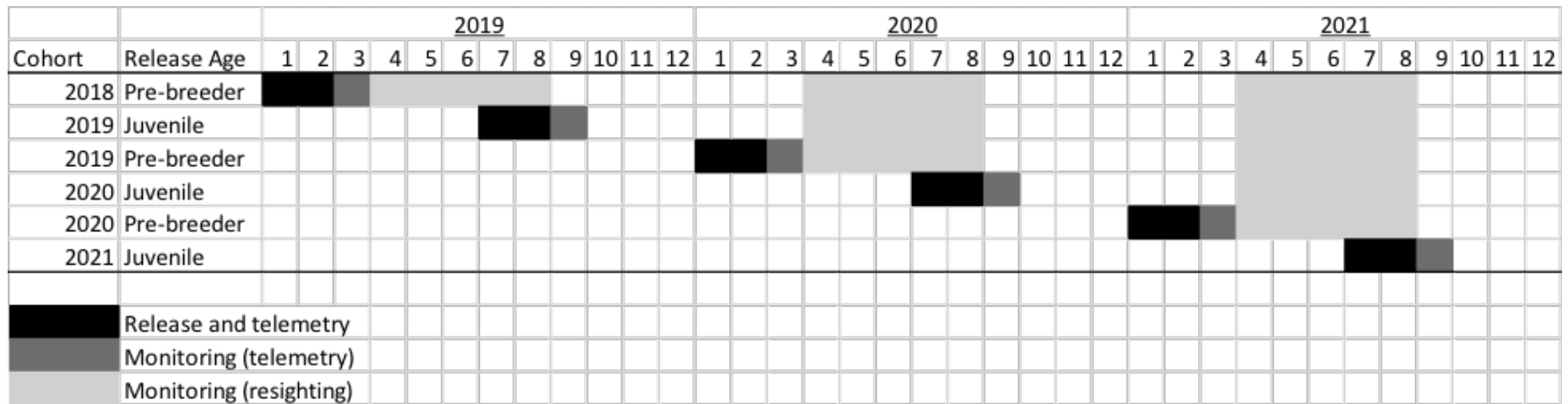


Figure B1: Proposed schedule of release and monitoring for captive-reared juvenile and pre-breeding second-year FGSP at TLWMA (2019-2021).

APPENDIX C. Pre-Transfer Health Screening Protocol

**NOTE: The details of this section will be refined during the IUCN DRA workshop (Nov 2018). This version is a strawman intended to guide discussion.*

All newly independent fledgling FGSP at the captive facilities will undergo a health screening on the day they are removed from their parents (ca. 21-23 days post-hatch) and transferred to juvenile holding pens. Keepers should monitor the sparrows closely during the pre-release period for abnormal behavior, weakness, flight abnormalities, feeding characteristics, or other abnormalities that could indicate a health problem that requires further evaluation and veterinary contact. The same screening will be repeated for birds held overwinter prior to their scheduled release date (TBD based on DRA). However, the imperative for sample collection and examination must be balanced with the potential detrimental effect on acclimatization, health, and welfare of the birds (Sangster and Vogelnest 2016). The following procedures will be conducted during each health screening event:

- Complete physical veterinary exam
 - A veterinarian will examine the eyes and conjunctiva, the periorbital area and head, the nares, the beak and oral cavity, all joints of the wings and legs should have normal range of motion and function, the skin of the feet and legs and cloaca, and the uropygial gland. The pectoral musculing and amount of subcutaneous fat should be evaluated. The weight of the bird should be compared to previous weights.
 - The overall strength of the bird will be evaluated during catching and handling.
 - Feathers will be examined for parasites, excessive wear that might hinder flight, or other abnormalities.
- Sexing
 - If not already completed, a buccal swab, blood sample or feather sample will be used to collect DNA required for genetic sexing.
- Replace or apply colored leg bands
 - All birds will be assigned unique color-band combinations prior to transfer if not done so already. A single numeric USGS aluminum band will be applied at the release site.
- Fecal sample collection
 - Samples will be collected opportunistically for coccidia screening.
 - Fecal flotation will be used to determine presence and degree of shedding.
 - A portion of fecal or swab of float meniscus should be saved for PCR and sequencing.
- Venipuncture
 - Blood smear analysis will be conducted to examine the distribution and morphology of white blood cells, the morphology of red blood cells, and to screen for extracellular coccidia, microfilaria, or other blood parasites.
 - Whole blood will be collected for PCR screening of target pathogens identified during DRA. Any remaining blood (or DNA) will be preserved for future uses.
- Oral Toltrazuril dose (for coccidian treatment)
 - Liquid Toltrazuril (0.01 ml/dose) will be given as treatment for coccidia during handling of the birds in addition to routine ESB dusting of insect food.

- Dusting with 5% carbaryl Sevin dust
 - Birds will be dusted with Sevin dust to reduce ectoparasite load.
- EEE/WEE/West Nile vaccination booster
 - Birds will receive this vaccine three times: once during nestling banding, once on the day of removal from parents, and once prior to transfer.
 - The vaccine used is West Nile Innovator + EW (Zoetus) at 0.10 ml per dose subcutaneously in the inguinal leg fold.

Disinfectant footbaths will be used prior to entering *ex situ* quarantine aviaries. Separate tools, nets, bags, and food bowls will be used for each aviary. Aviaries should be screened to reduce exposure to vectors. To avoid cross contamination between aviaries and quarantine facilities, free roaming mammals should be excluded from these areas.

Birds will either pass or fail their pre-transfer evaluation based on behavioral signs of illness and/or any abnormalities in the screening. The presence of pathogens confirmed to be found in the wild FGSP populations will not necessarily disqualify an animal for release, however, excessive pathogen loads indicating severe infection or other health issues will be retained for treatment. One product of the DRA will be a decision tree clearly outlining the conditions required to pass pre-transfer screening.

APPENDIX D. Captive Breeding Protocols

FWS and FWC, with guidance from the working group, have supported adaptive management at the captive-breeding facilities to allow for discovery of the captive-rearing protocols (e.g., aviary size and configuration, husbandry and health screening, etc.) that result in the greatest successes in productivity and survival. The cooperative nature of the captive breeding effort to date has resulted in substantial improvements in husbandry techniques and disease management at both captive facilities. The captive breeding effort will continue to be a cooperative effort, as evidenced by continued rapid data sharing among partners and a commitment to joint authorship of peer-reviewed scientific products that arise from program activities. Moving forward, the FGSP recovery program will adopt the following practices, which are those associated with consistently positive outcomes, to optimize the success of the captive-breeding program. These best practices will be adopted by all *ex situ* facilities housing adult breeding pairs and juveniles (2019-2024). Novel husbandry issues and new information may require adaptive modifications to the listed protocols pending consultation with FWS and FWC.

Breeding Aviaries

- No more than two adults occupy a breeding aviary (birds are considered adults by March 1 of the year following their hatch year), unless otherwise authorized by FWS and FWC (one male and two females may be considered on a case-by-case basis).
- Breeding aviaries at least 8' x 24' x 7' in size have proven sufficient to support successful breeding and rearing of young FGSP. Canadian Loggerhead Shrike and Mauritius Fody programs both experienced improved behaviors and reproductive success following increase in aviary size (Cristinacce et al. 2008, Steiner et al. 2013). To maximize captive reproductive success, all breeding aviaries will be ≥ 192 square feet.
- All existing outdoor breeding pairs remain in outdoor aviaries unless they need to be moved indoors temporarily for health or other emergency reasons.
- Any indoor adult birds are transitioned to outdoor aviaries when and if safe to do so.

Juvenile Aviaries

- Independent fledglings occupy an outdoor aviary that provides a minimum of 30 square feet per individual bird.
- Hand-reared juvenile birds are transitioned to outdoor aviaries upon reaching nutritional independence (21-23 days; post-hatch).
- Parent-reared juvenile birds are separated from their parents and transferred to juvenile aviaries when they reach nutritional independence (21-23 days; post-hatch).
- Any wild-caught independent juvenile or adult FGSP collected for captive breeding is directly placed into an outdoor aviary.

Pairing and Genetic Management

- Captive facilities submit studbook and nest data each fall.
- The sex of all juvenile FGSP hatched or reared in captivity is assigned using genetic techniques.

- FGSP pairs are established based on efforts to maximize and preserve genetic diversity of the captive flocks (see details in Appendix B).
- Breeding pairs are established prior to March 1 of each year.
- FGSP pairs may be split and repaired in cases of behavioral or reproductive incompatibility.
- Breeding males are monitored for signs of aggression towards young and separated from family group after fertilization of eggs if necessary.

Rearing

- Breeding pairs in outdoor aviaries parent-rear their young unless the nestlings need to be removed from their parents and hand-reared because of health or safety reasons.

Conditioning

- All outdoor birds are provided with live (preferably wild) insects several times weekly to encourage or maintain natural foraging behaviors.
- All outdoor pens have natural substrate designed to prepare birds for wild conditions.
- Husbandry staff reduce interaction time with birds to prevent habituation.

Predation and Flood Management

- All field aviaries are constructed or reinforced with metal hardware cloth to prevent damage by rodents or other predators.
- The screen size for aviaries does not exceed 0.25 x 0.25 inches.
- Field aviaries have doors that fit tightly, with no gaps to allow entrance by snakes or other predators.
- Field aviaries are inspected regularly for damage.
- All newly constructed aviaries are thoroughly searched for snakes or other predators prior to adding FGSP.
- If present, red imported fire ant mounds in or near the aviaries are treated.
- All field aviaries have sufficient elevation to prevent nest flooding. Nests are lifted using established protocols (FWC, unpub. data) if at risk of flooding.
- Rain shelters are provided in each field aviary.
- All captive facilities have a hurricane-preparedness plan approved by FWS and FWC.

General Husbandry

- All FGSP are housed separately from other species including during incubation and hand-rearing, and FGSP eggs and nestlings have dedicated incubators, hatchers, and brooders.
- All FGSP food, food preparation spaces, and equipment are housed separately from that of other species.

Health Screening

- All captive facilities conduct routine and opportunistic health screening of FGSP in their care as outlined in Appendix C (pending Disease Risk Analysis [DRA] in November 2018).
- All independent juvenile FGSP undergo health screening shortly after they are removed from their parents (Appendix C).

- All adult FGSP held for captive breeding undergo at least one annual exam. This can be done opportunistically or when birds are being handled for other reasons.
- Opportunistic health screening occurs whenever birds are captured for moving or other non-health or non-emergency-related reasons unless the bird is exhibiting signs of stress (e.g., labored breathing, lethargy, squinty eyes).
- Opportunistic fecal sample collection occurs to monitor for coccidia.
- Whenever enough blood, tissue, or other material exists, captive facilities share it with designated labs at the request of the FGSP Health Team coordinator.
- Captive-breeding facilities notify the FWS about mortalities resulting from suspected disease, trauma, predation, or other unusual circumstance via email within 48 hours of the event.
- Copies of necropsy and pathology reports with notable findings are forwarded to the FWS so that they can be shared with the FGSP Health Team.

Transfers

- Pending the results of the DRA, birds may need to be moved between captive and holding facilities to alleviate capacity issues, or to meet genetic pairing and release goals.
- All captive facilities participate in pre-transfer screening exams (Appendix C) and engage in giving and receiving egg, nestling, juvenile, or adult FGSP that qualify for transfer during the health screening exam.

Procedure

- No FGSP or their resulting progeny in the care of the captive facilities are transferred or disposed of without prior written approval from the FWS.
- The captive facilities do not deviate significantly from the FGSP 5-Year Strategic Vision or any of its appendices (A - E) without first consulting the trust agencies (FWS and FWC).

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Appendix VII

Workshop Participant Dissenting Statement

[E-mail sent to P. Miller on 4 February 2019]

Dear Phil,

Thanks for your draft report. Please find attached here my edits and extensive comments for inclusion (see track changes and embedded comments). I appreciate the daunting task of coalescing the discussions and challenges from this workshop, especially in light of the agencies' expressed desire to leverage the DRA for immediate decision-making purposes. An accurate alignment of perceptions and realities is imperative, as the many unknowns severely constrain confidence in FGSP release strategies. In addition, the late date for convening the workshop, the long federal shutdown, delays in preparing pre-release screening, handling and habituation protocols, lack of on-prairie facilities, etc., etc. cast a long shadow over proposed actions for this month or next.

A principal criticism is how respondents' opinions and views were tallied and used to validate position statements. As you will glean from my review, this meeting was not assembled from a vetted list of experienced pathogen or captive-breeding specialists, and representation from stakeholders was neither balanced nor stratified. Therefore, the range of views expressed, rather than the number of people supporting each view, is what should be reported. For example, FWS, FWC, UF and White Oak had multiple participants, all with previously expressed views on diseases and release, yet RSCF and UGA had only one delegate each.

My first extended comment on the first page of the report reads as follows:

Appropriate to discuss composition of participants here. Participants were not a juried pool of independent disease specialists, captive-breeding specialists, etc., but rather an ad hoc group of representatives from FWS, FWC, WOC, RSCF and UGA, and a few others. There was no attempt to balance representation from the agencies or organizations (e.g., 4 each from FWS, FWC, RSCF, WO, etc.) and therefore the participants do not define an independent review panel or jury. Reference to decision making in this DRA report should be restricted to presenting the range of inputs and positions, NOT the number of participants agreeing on any issue or decision. Participants at the DRA all had previously expressed positions regarding the relevance of FGSP diseases and their influence on releases and how releases should be implemented. All participants engaged in prior discussions about the DRA topics and had repeatedly debated all talking points presented at the DRA. The DRA presented no new findings or materials. It was offered as a framework to help resolve disparity in views and opinions.

A priori bias is important to document in this report because any claim of consensus or plurality, or majority/minority support for decisions or issues is flawed by biases inherent in the group's composition. If the DRA were intended to provide a neutral, objective framework with which to evaluate potential disease impacts upon release options and methodology, a different group composition, providing balance from independently vetted participants, would have been required.

For transparency, the above deserves considerable discussion, along with the fact that the DRA did not yield any formal recommendations to the agencies regarding release timing, procedures, protocols—nor did it resolve the question of how to address pathogens altogether. I trust that you are aware that the agencies released a written decision to the working group (19 Dec.), stating that releases would

commence early this year, even though your draft DRA report had not been circulated. This written decision contradicts the agencies' previous, signed correspondence in which the agencies made clear that no decisions regarding FGSP releases would be made until after the DRA report was completed.

As you know, our organization's positions, and those of Dr. Ritchie and UGA, have long been documented and are clear, corroborated by science and FGSP-specific data. The overwhelming conclusion from the facts is that far less is known about FGSP pathogens than what is known, and the impacts of these pathogens on released birds and wildlife with which they may interact are utterly unknown. Our collective position has long been to take a very conservative, careful approach to pilot FGSP releases and to develop a sustainable release/recovery strategy over time. Releases should not be a management tool, which is currently the principal motivator for the agencies' rush to release birds before essential disease investigations are completed. We are equally concerned that unless the wide data and knowledge gaps-- and the manner in which the DRA group was assembled and ideas discussed-- are openly presented, the DRA may be seen from outside reviewers and the media as a means to justify *a priori* agency decisions. Your efforts, integrity and CPSG's mission are vital to ensuring objectivity.

Please let me know if I can elaborate on any of the above, or clarify anything. I look forward to receiving the revised report and to important next steps to save the FGSP.

Thank you for your energy and diligence.

Best regards,

Paul

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