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Uncharacterized Isosporoid Parasite in the Florida Grasshopper Sparrow (Ammodramus savannarum floridanus)

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UNCHARACTERIZED ISOSPOROID PARASITE IN THE FLORIDA GRASSHOPPER SPARROW (AMMODRAMUS SAVANNARUM FLORIDANUS*)*

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Matthew Morris

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To: Dean Michael Heithaus
College of Arts, Sciences and Education

This thesis, written by Matthew Morris, and entitled Uncharacterized Isosporoid Parasite in the Florida Grasshopper Sparrow (*Ammodramus savannarum floridanus*), having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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The thesis of Matthew Morris is approved.

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ABSTRACT OF THE THESIS

UNCHARECTERIZED ISOSPOROID PARASITE IN THE FLORIDA GRASSHOPPER SPARROW (*AMMODRAMUS SAVANNARUM FLORIDANUS)*

by

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Florida International University, 2021

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Professor Alessandro Catenazzi Major Professor

A novel and potentially fatal isosporoid parasite was discovered within a captive population of Florida Grasshopper Sparrows, kept at the Rare Species Conservatory in Loxahatchee, Florida. The purpose of my thesis was to (1) to ascertain the prevalence of the *Isospora* sp. in the captive population of FGSPs; (2) to show that the pathogen can cause both morbidity and mortality in the FGSP; (3) to use population modeling as a management tool to show the potential effects of the disease on the wild population; and (4) recommend mitigation and management strategies informed by our research. Overall, histopathology and necropsy reports reveal the *Isospora* sp. has the ability to cause and/or contribute to morbidity and mortality in FGSP. Projection models demonstrate any level of impact of *Isospora* sp. would have severe negative impacts on the estimated growth rate of the wild population. My study considers *Isospora* sp. to be a pathogen of significance and recommends that the organism should be considered in all current and future management approaches for the recovery of the FGSP.

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I. INTRODUCTION

The scale and rate at which biodiversity is disappearing is only rivaled by the Earth's last five extinction events, leading some to believe the natural world is currently entrenched in the sixth major extinction event (*1*). The erosion of the Earth's biosphere will have rippling effects through all aspects and scales of human society and the loss of the associated "ecosystem services" will extend far beyond a dollar evaluation (*2*). The increase in frequency of natural disasters, such as brush fires and hurricanes, rapid climate change, mass species extirpation, extinction events, pandemics, etc. are startling evidence of an Earth whose ability to function has begun to falter.

An unfolding environmental disaster within the ongoing biodiversity crisis is the precipitous global reduction of avifauna, including the loss of 3 billion North American birds since 1970 (*3-6*). Avian management practices have shifted from preventative to reflexive and ascertaining drivers of decline is an important research topic to inform applied conservation management and recovery approaches. Drivers of decline rarely work alone and are typically a combination of human-driven factors working in concert (*7*).

As an assemblage, grassland or savanna occurring birds have been disproportionally disappearing and are becoming top conservation priorities in North America (*6, 8-15*). Since 1960, grassland bird abundance has declined an estimated 50%; a fraction of the blink of an eye in ecological time and the cause for major concern (*16*). Without applied conservation management, grassland bird populations will continue to

decline and the associated loss of ecosystem services, integrity, and stability will devastate an already imperiled biome (*5, 17, 18*).

Emergent infectious disease (EID) is one of many threats that drive grassland bird declines. The World Health Organization (WHO) defines EID as a disease "that has appeared in a population for the first time, or that may have existed previously, but is rapidly increasing in incidence or geographic range" (*19*). Today, EIDs are becoming a major threat to global biodiversity and principal driver causing decline. However, the true magnitude of their involvement in past species extinctions is likely underestimated because of a lack of focus and technological capacity to study them (*20*).

Many human or wildlife EIDs are parasitic diseases. Parasitic disease is an inherent component of any wild population and plays an important role in host population biology. In declining populations, parasites, especially from pathogens to which hosts may be immunologically naïve, can be an additional stressor depressing population growth (*21-23*). Parasites can also play other roles: they maintain genetic diversity, interact with sexual selection, protect the host population against other pathogens and act as keystone species (*23-27*).

Systemic Isosporosis

Coccidia (Protista: Apicomplexa: Eimeriidae), especially Isospora, are one of the most common, and species rich protist parasites found in vertebrates and are known to infect a variety of passerine species (*28*). Despite their ubiquity, little is known about coccidian parasites in wild populations and their role in driving avian population decline (*23, 29*).

Isospora are transmitted orally, with coccidial infection starting upon the ingestion of a sporulated oocyst. Sporulated oocysts contain infective sporozoites, which are released into the avian gut by excystation (*30*). Once in the gut, the sporozoites infect neighboring epithelial cells and asexually replicate themselves via merogony. After merogony, gametogenesis occurs, followed by the fusion of male and female gametes and the subsequent production of un-sporulated oocysts, which pass with the feces and enter the exogenous face of development (*30*). Un-sporulated oocysts sporulate and become infective, in the presence of proper environmental conditions that include, time, temperature, and moisture (*31*). Sporulated oocysts are highly environmentally resistant and can remain viable and latent exogenously for years. The most virulent and least understood clinical manifestation of coccidial infections is the development of systemic isosporosis, or atoxoplasmosis, which is characterized by an extra-intestinal proliferative phase of infection (*23, 32-36*).

In contrast to a traditional intestinal coccidian infection, in a systemic infection the coccidian agent, at its asexual stage, proliferates through the hosts duodenum and infects macrophages, lymphocytes, and natural killer cells (*32*). The agent is then transported in the bloodstream to other organs where it causes lesions and visceral damage to the soft tissues. The agent often causes legions in the liver, but can also harm the spleen, lungs, and the intestines (*37*). Since the systemic infection occurs at the asexual stage of replication, the extra-intestinal phase of infection can occur independently of the sexual intestinal cycle. Therefore, hosts that are not shedding oocytes, it may still be infected with systemic isosporosis (*37*).

There are many clinical symptoms of systemic isosporosis that are non-specific, including: hyperoxia, bristly feathers, loss of balance, pectoral muscle wasting, weight loss, diarrhea, abnormal distention of the stomach, etc. (*37*). Adults are commonly asymptomatic, however, many juveniles may die in captivity (*37*).

The Florida Grasshopper Sparrow

The most endangered bird in North America is the non-migratory Florida Grasshopper Sparrow (*Ammodramus savannarum floridanus* hereafter FGSP), a central-Florida grassland endemic (*38-40*). Floristically, FGSP's mesic-sandy, prairie habitat consists of pyrogenic vegetation, mostly grasses and shrubs, which is maintained by natural and prescribed burns that typically occur every 2-3 years (*38, 41, 42*). Over the past half-century, the central prairie system of Florida has been intensively modified to accommodate large-scale intensive agriculture, resulting in concomitant declines in endemic prairie species that rely on this now fragmented ecosystem (*15, 43, 44*).

Edward Mearns first described the FGSP in 1901, and noted their widespread distribution across the Florida prairie (*45*). Since then, their abundance on the prairie has precipitously and severely declined. The FGSP was first listed as federally endangered in 1986, in response to the rapid reduction of its distribution within its historical range (*46*). Since then, all FGSP sub-populations have declined dramatically. In 1999, the total wild population was estimated to be ~ 1,000 individuals, spread across six populations; as of the 2018 breeding season, less than 23 breeding pairs might occur in the Three Lakes Wildlife Management Area (*38, 47*).

The FGSP has high reproductive and dispersal ability (*48*): on average, it produces clutches of four eggs that hatch in approximately 14 days, each pair attempting

multiple nests over the course of the breeding season (**Table 4)**. Despite their high reproductive ability, all sub-populations declined rapidly since their federal listing in 1986. Potential drivers of decline include: cattle grazing, too infrequent burning regimes, invasive species, changes in vegetation and secondary predator effects. Decades of intensive monitoring and intervention by the Florida Fish and Wildlife Conservation Commission (hereafter FWC) and the United States Fish and Wildlife Service (hereafter FWS) failed to produce a unifying explanation for the FGSP decline (*49-52*).

The Captive Breeding Program and Discovery of A Novel Coccidian Pathogen

By 2014, it was clear that without reflexive management, the FGSP would rapidly and unavoidably go extinct. Therefore, in 2014, governmental and non-governmental organizations initiated a recovery effort, that included a collaborative captive-breeding program, and formed the FGSP working group. From 2015 to August 2019, the captive-breeding program established an *ex-situ* population of FGSP, using field collected nestlings, fledging and adults, at The Rare Species Conservatory (hereafter RSCF) in Loxahatchee, Florida. The goal of establishing this *ex-situ* population was to create a pool of individuals to be released back onto the prairie via population reinforcement and reintroduction.

The RSCF received its first group of seven FGSP in 2015, comprised of four-day-old nestlings collected from at-risk field nests and several independent, hatch-year birds. The RSCF established a captive population with these seven founders. New enclosures were built by RSCF and they designed clean environments to encourage the safety of the birds and to foster breeding. Within weeks, RSCF quickly developed a hand-feeding

formula and feeding regimen for nestling FGSP that proved successful for rearing, through independence; all FGSP that are parent-reared for at least four days of age.

Husbandry breakthroughs were quickly overshadowed by a clustered mortality event within the first group of captive-bred, hatch-year birds, triggering the launch of a pathogen investigation. Carcasses and tissue samples from deceased birds were sent to the University of Georgia Infectious Disease Laboratory for full necropsy and histopathological analysis. Sequencing of the whole mtDNA-genome indicates that the pathogen is a genetically novel, emergent extra-intestinal coccidian *Isospora* sp.. In addition to its genetic novelty, there is no documentation of systemic isosporosis in a Florida endemic, grassland bird.

Disease-Risk Assessment

The novel *Isospora* sp. discovered at RSCF, known to cause systemic isosporosis, was brought to the immediate attention of the FGSP working group. In November 2018, all the stakeholders involved in the recovery program (notably, White Oak Conservation, U.S Fish and Wildlife Service, FWC, and RSCF) conducted a disease risk analysis (*53*). Overall, the level of risk *Isospora* sp. posed to the overall recovery approach was considered low, however, the report admits "the presence of significant data gaps which add to the uncertainty of this assessment" (*53*).

A survey of wild birds was carried out to compare pathogen prevalence in the captive vs. wild population of FGSP (*54*) . Researchers found generic coccidia in 2 of 44 wild FGSP and in three other migratory species found on the prairie. However, the prevalence and impact of the extra intestinal *Isospora* sp. (the form found at RSCF and associated with the mass mortality event) within the wild population is unknown, and a

major gap in the research surrounding this pathogen. As a result of differing opinions regarding the recovery approach and the relative risk of *Isospora* sp., RSCF and FWC ended their partnership in 2019 (*55*). The FGSP continue to be the recipient of applied conservation management, and the stakeholders involved in the sparrow recovery program, other than RSCF, continue to release captive-bred and reared sparrows back onto the prairie (*56*).

Study Objectives

Using FGSP and *Isospora* sp., we sought to understand the role that emergent parasitic disease plays in conservation management and how adaptive management can be reflexive upon the discovery of a novel, emergent pathogen.

The disease risk assessment working group defined a "high-risk pathogen" 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" (*53*). We hypothesized that *Isospora* sp. is a high-risk pathogen and that it should be considered in the recovery strategy. The objectives were: (1) to ascertain the prevalence of the *Isospora* sp. in the captive population of FGSPs; (2) to show that the pathogen can cause both morbidity and mortality in the FGSP; (3) to use population modeling as a management tool to show the potential effects of the disease on the wild population; and (4) to recommend mitigation and management strategies informed by our research.

II. METHODS

The Study Population

Husbandry and The Study Population

Appendix A describes all detailed husbandry procedures, including hand-rearing, feeding, housing and handling protocols.

Briefly, from 12 February – 29 August 2019, RSCF housed a population of 26-28 FGSP in 14 outdoor, soft-meshed enclosures in Loxahatchee, Florida. **Table 1** summarizes the 14 enclosures' numbers of individuals and sex composition per cage.

The housing composition was ad hoc and mandated by FWS to minimize breeding, not a result of a randomized block experimental design to examine density, gender, age and cage effects.

Discovery of Isospora sp.

Histopathology

The RSCF sent carcasses of captive FGSP to The University of Georgia Infectious Disease Laboratory (hereafter IDL) for postmortem analysis. The IDL pathologists performed full body necropsies, including gross and histopathological examination of tissues from the brain, lung, liver, kidney, spleen, intestine. Pathologists also collected and froze choanal and feet swabs for future diagnostic tests.

Coccidiacide

Upon the discovery of an uncharacterized *Isospora* sp., RSCF adapted a treatment protocol that includes a prophylactic dose (150 mg Toltrazuril/gallon drinking water once per week, all birds) and a treatment dose (250 mg Toltrazuril/gallon drinking water for

two consecutive days, started whenever high oocyst loads are detected by fecal floatation; see next paragraph).

Fecal Floatation

To monitor oocysts shedding prior to the development of the PCR assay, we collected fecal samples for fecal flotation. We transferred fresh fecal samples from their 15ml collection tubes to 50ml glass test tubes and manually agitated in Fecasol or Fecatect solution using a sterile stir stick. We added additional Fecasol or Fecatect solution to the test tube until a positive meniscus formed on top, and then placed a 22 mm×22 mm coverslip atop the meniscus. After 15-20 minutes, we moved the coverslip to a clean microscope slide and examined for oocysts at 10X-40X. Whenever we encountered a fecal slide with more than 30 oocysts visible (Figure 1), we treated all birds in the corresponding enclosure for two consecutive days with 250 mg/gallon Toltrazuril as the exclusive drinking water.

Isospora sp. *Pathogen Investigation*

Fecal Samples for PCR Detection Assay

From 12 February – 29 August 2019, we collected 3,224 pooled fecal samples, from each of the 14 RSCF sparrow enclosures, during routine morning feedings (approx. 8:00AM-9:30AM). We collected samples using standard 15ml fecal-collection tubes, with a built-in spoon for collecting samples, and labeled with the corresponding enclosure. We transferred samples into 1.5ml centrifuge tubes, labeled with the date and enclosure, and stored them at 1.6° C until shipment to IDL for PCR analysis.

PCR Detection Assay

We extracted DNA from fecal samples using the ZymoBIOMICS DNA Miniprep Kit. We added at least 125mg of the feces to the bead bashing tube and mixed on speed 5 for 300 seconds on the VWR® 4-Place Mini Bead Mill. We followed the manufacturer standard protocol for fecal samples and eluted DNA with 50µL of water.

We used eluted DNA samples (2 µL) from the extraction in a PCR reaction with primers designed to amplify a (339 bp) segment of *Isospora* sp. cytochrome oxidase I. The PCR cycles included one cycle at 95°C for five minutes, 34 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72° for 30 seconds.

We ran 5.0µL of PCR product on a 2% agarose gel, visualized with Sybr gold and photographed using Biorad Gel Doc XR. We considered a sample as positive when it displayed appropriately sized DNA bands (339 base pairs) of the *Isospora* sp. mitochondrial DNA (cytochrome oxidase I) amplicon. Each gel was run with a positive control (a known sample of *Isospora* sp. used to sequence its genome) and a negative control (containing only DI water).

Analyzing Factors that Influence Isospora sp. *Detectability*

The Data

Factors within the FGSP captive environment were chosen opportunistically to search for and identify factors that influence *Isospora* sp. detectability, including: whether or not it was the breeding season, the sex, and number of individuals per enclosure, month and cage (**Table 2)**.

Univariate Binomial Logistic Regression

We performed univariate logistic regression models to evaluate the importance of the five factors that might influence the detection of *Isospora* sp., and to build our multivariate model. We used the packages "lme4" and "lme' for the open-source program R (Version 4.0) to construct statistical models.

Multivariate Binomial Logistic Regression

Out of five total factors, the three significant factors, revealed by the univariate comparisons of the regression variables, were used to create multivariate models, which were then compared using Akaike Information Criterion (AIC) to determine the best-fit model. The resulting model is a mixed effect generalized logistic regression model with a binomial distribution. **Table 3** details the characteristics, and their respective influence on the best-fit model.

Modeling demographic effects of Isospora sp. *infection*

Constructing the Base Model

To model the effects of *Isospora* sp. on the wild population's growth rate (λ), we used the population model developed by the U.S Fish and Wildlife Service (Ricklefs 1973, Windsor and Bowman 2019). The U.S Fish and Wildlife Service model calculated λ based upon a modified two-stage model approach. Figure 2 shows a two-stage population growth model with: (1) a non-reproducing Hatch-Year stage and (2) a Reproducing After-Hatch-Year Stage (HY and AHY, respectively).

We obtained life history data for FGSP wild populations from the literature and from technical reports (**Table 4**). Whenever multiple values were available for the

different life history data, we bootstrapped the data 500 times with replacement, to obtain averages and variances. **Table 3** summarizes the bootstrapped life history data.

Calculating λ

We simulated six demographic projections to model the potential effects of *Isospora* sp. on the wild population of FGSPs. We ran both deterministic and stochastic models for each demographic projection.

We used the following equation to calculate the finite rate of population growth λ, from average annual adult and juvenile survivorship data obtained during mark and recapture studies (*57*).

$$\lambda = AHY\varphi + HY\varphi \times (\beta), \qquad (I)$$

where AHYφ = average annual adult survival, HYφ = average annual juvenile survival, and β = [(F/2) × p× n], where F = fecundity measured as brood size, p = daily nest survival probability, and n = number of nesting attempts (Windsor and Bowman 2019). The model assumes equal fecundity across all breeding adults and a sex ratio of 50:50. We modified the model into five demographic projections (see next paragraph/section) to examine the effects of *Isospora* sp. on λ. We used the averages on Table 5 for deterministic models. For stochastic models, we used R (Version 4.0) to randomly sample each life history value from the pool of all observed values, resulting in a random combination of values each time. We looped each demographic projection 1000 times to estimate average and variance of λ.

Demographic Projections

Demographic Projection 1

Projection 1 assumes that *Isospora* sp. disproportionately impacts young birds. Prior to Toltrazuril treatment, juvenile mortality within the captive population was as high as 83%. Since this high mortality impact was in captivity, the assumed impact of the disease is relaxed to a 50% rate of juvenile mortality when infected (= 50% HY mortality). On the basis of the observed prevalence of *Isospora* sp. in captivity (100% of cages tested positive), projection scenario 1 also assumes a high proportion of infected juveniles (75%). Again, the assumed prevalence is relaxed because of the observed rate (100% of cages infected) being in a captive setting. The impacts of the first projection scenario are incorporated into the model via Π and can be seen below in equation II:

$$\lambda = AHY\varphi + (HY\varphi \times \Pi) \times (\beta) \qquad \text{(II)}$$

where AHYφ = average annual adult survival, HYφ = average annual juvenile survival, Π = Hatch Year disease impact (1-[pj × Di)]), where pj is the proportion of infected juveniles and Di is the proportion of individuals who die from infection, and β = [(F/2) × p× n], where F = fecundity measured as brood size, p = daily nest survival probability, and n = number of nesting attempts.

Demographic Projection 2

Projection 2 assumes that *Isospora* sp.-positive adults are impacted minimally, whereas juveniles are impacted more severely. Projection 2 assumes high virulence in juvenile birds (50% mortality rate) and low virulence in adults (10% mortality rate).

Prevalence values are 75% for juveniles and 37.5% for adults. All the assumptions of the base model remain intact. The impacts of the disease in projection scenario 2 are incorporated into the base model as Σ and Π, as seen below in equation III,

$$\lambda = (AHY\varphi \times \Sigma) + (HY\varphi \times \Pi) \times (\beta) \qquad \text{(III)}$$

where AHYφ = average annual adult survival, HYφ = average annual juvenile survival, Π = Hatch Year disease impact (1-[$p_j \times D_i$]), where p_j is the proportion of infected juveniles and D_i is the proportion of individuals who die from infection, Σ = After Hatch Year Disease Impact (1-[$p_a \times D_a$]), where p_a is the proportion of infected adults and D_a is the proportion of adults who die from infection, and β = [(F/2) × p× n], where F = fecundity measured as brood size, p = daily nest survival probability, and n = number of nesting attempts.

Demographic Projection 3

Projection 3 assumes that *Isospora* sp. impacts females 1.5 times more than males. The assumption is derived from the sex skew seen in the wild population of FGSP, the true populations does not have a sex ratio of 50:50 and is skewed towards males. Adult prevalence is assumed to be 50%, following the assumption that the wild prevalence will be half of the observed captive prevalence. The mortality rate is assumed to be 25% in males and 37.5% in females. The increased mortality in females is caused by higher energetic costs associated with breeding and the wild population is male skewed, therefore, female survivorship is assumed to be 25% less than adult male survivorship (AHYφ – (.25* AHYφ). Juveniles again are assumed to have a 50%

mortality rate when infected and 75% of juveniles are assumed to be infected. The equation for projection scenario 3 is:

$$\lambda = (((FAHY\varphi \times \Sigma_f) + (MAHY\varphi \times \Sigma_m)) *.50) + (HY\varphi \times \Pi) \times (\beta) \qquad (IV)$$

where FAHYφ = average annual female adult survival, MAHYφ = average adult male adult survival, HYφ = average annual juvenile survival, Π = Hatch Year disease impact (1- [$p_j \times D_i$]), where p_j is the proportion of infected juveniles and D_i is the proportion of individuals who die from infection, Σ_f = Female After Hatch Year Disease Impact (1-[$p_{af} \times D_{af}$]), where p_{af} is the proportion of infected female adults and D_{af} is the proportion of female adults who die from infection, Σ_m = Male After Hatch Year annual survivorship (1-[$p_{am} \times D_{am}$]), where p_{am} is the proportion of infected male adults and D_{am} is the proportion of male adults who die from infection and $\beta = [(F/2) \times p \times n]$, where F = fecundity measured as brood size, p = daily nest survival probability, and n = number of nesting attempts.

Demographic Projection 4

Projection 4 shares all the same assumptions that projection scenario 3 has, with the addition of a single assumption; *Isospora* sp. causes morbidities in females that impact their fecundity, not their survivorship, so male and female survivorship are again assumed to be equal. The assumed sex ratio of the population will be 70:30 male to female, and to reflect female morbidity, fecundity will be reduced by 10%. Projection scenario 4 is represented by Equation V below:

$$\lambda = (((FAHY\varphi \times \Sigma_f) + (MAHY\varphi \times \Sigma_m)) *.50) + (HY\varphi \times \Pi) \times (\beta) \qquad (V)$$

where FAHYφ = average annual female adult survival, MAHYφ = average adult male adult survival, HYφ = average annual juvenile survival, Π = Hatch Year disease impact (1-[$p_j \times D_i$]), where p_j is the proportion of infected juveniles and D_i is the proportion of individuals who die from infection, Σ_f = Female After Hatch Year Disease Impact (1-[$p_{af} \times D_{af}$]), where p_{af} is the proportion of infected female adults and D_{af} is the proportion of female adults who die from infection, Σ_m = Male After Hatch Year annual survivorship (1-[$p_{am} \times D_{am}$]), where p_{am} is the proportion of infected male adults and D_{am} is the proportion of male adults who die from infection and $\beta = [(F*.40) \times p \times n]$, where F = fecundity measured as brood size, p = daily nest survival probability, and n = number of nesting attempts.

Population Projection 5

Projection 5 is the "minimum effects model," which assumes a "minimum" impact of 20% infected individuals in either age class, and 10% mortality for infected individuals. Females and males will be assumed to be impacted equally. All the other assumptions of the reference model stand. Population scenario five is represented in equation VI below:

$$\lambda = (AHY\varphi \times \Sigma) + (HY\varphi \times \Pi) \times (\beta) \qquad \text{(VI)}$$

where AHYφ = average annual adult survival, HYφ = average annual juvenile survival, Π = Hatch Year disease impact (1- [$p_j \times D_i$]), where p_j is the proportion of infected juveniles and D_i is the proportion of individuals who die from infection, Σ = After Hatch

Year Disease Impact (1-[$p_a \times D_a$]), where p_a is the proportion of infected adults and D_a is the proportion of adults who die from infection, and $\beta = [(F/2) \times p \times n]$, where F = fecundity measured as brood size, p = daily nest survival probability, and n = number of nesting attempts.

Population Projection 6

Projection 6 takes projection scenario 1, the juvenile impact model, and considers head-starting and reintroduction as applied management strategies (Figure 5). Population scenario six is represented in equation VII below:

$$\lambda = AHY\varphi + (((HY\varphi + IHY\varphi) \times .50) \times \Pi)) \times (\beta) \quad \text{(VII)}$$

where AHYφ = average annual adult survival, HYφ = average annual juvenile survival, IHYφ = HYφ in captivity, Π = Hatch Year disease impact (1-[$p_j \times D_i$)]), where p_j is the proportion of infected juveniles and D_i is the proportion of individuals who die from infection, and $\beta = [(F/2) \times p \times n]$, where F = fecundity measured as brood size, p = daily nest survival probability, and n = number of nesting attempts.

Population Viability Analysis

We ran population viability analysis on any deterministic projection scenario with $\lambda > 1$ in the open source program R (Version 4.0), using the package "pvaclone." A classical Ricker model was used, with demographic stochasticity, a carrying capacity, and chance catastrophe. Models were projected over a 25-year period.

Demographic stochasticity was incorporated into the model as the standard deviation of λ, obtained from the stochastic demographic projections. We selected a carrying capacity from a previously published PVA analysis for FGSP conducted in 2006

(*49*). For a quasi-extinction threshold, field count estimates from the 2019 Three Lakes Wildlife Management Area breeding season were used.

III. RESULTS

Histopathology and Necropsy

A subset of six histopathology reports were randomly sampled from the deceased individuals that tested positive for the specific *Isospora* sp. PCR array. *Isospora* sp. has the ability to cause morbidities and subsequent mortalities in the FGSP, disproportionately impacting juvenile hatch year birds. The sex, age, accession number, and key findings for the six reports are summarized on **Table7**.

Within the random subset of necropsy reports selected, 6/7 of the individuals displayed hepatitis of the liver or lymphoplasmacytic infiltration. Intracytoplasmic merozoites, part of the lifecycle of the extra intestinal coccidia, were found in the liver of IDL18-1955 **(Table 7).** Asides from the liver, the small intestine and spleen were the two next most commonly impacted organs.

PCR Dataset Analysis

Detection:

From February 12th, 2019 to June 20th 2019, an average of 49% (SD 22%), of the captive population tested positive for *Isospora* sp. Daily frequencies of enclosures with infected birds ranged from 0% to 82%. **Figure 3** displays the daily percentage of the population that tested positive for *Isospora* sp. through time. Periods of high frequencies (65–80%) alternate with periods of low frequencies (0-10%), separate by intervals of five to seven days.

Factors that Influence the Detection of *Isospora* sp.

The number of individuals per enclosure, cage, and sex predict a positive PCR results in univariate analyses (**Table 6**). Determined by AIC, the best fit multivariate predictive model included sex, the number of individuals per enclosure and cage as a random factor **Table 10**.

Population Modeling

Deterministic Projection Models

Overall, none of the deterministic models for population projection scenarios 1–5 yielded a positive model. **Table 9** summarizes the results for the deterministic models created for projection scenarios 1–5.

Projections scenario 5 yielded the highest λ value of 0.77, which is still a declining population. Projection Scenario 4, the female morbidity impact model, resulted in the lowest λ value of 0.44, demonstrating the detrimental effects of *Isospora* sp. on population growth rate even in the form of morbidities.

Stochastic Projection Models

Stochastic projection models looped 1000 times; the resulting averages, variances, minimum and maximum values for λ for each projection scenario, and the percentage of $\lambda > 1.0$ are detailed on **Table 8**.

Overall, Projection scenario 5, the minimum effects model, yielded the second highest average λ value of 0.77 with a SD of 0.12. Within this projection scenario, the minimum calculated λ was 0.54 and the maximum was 1.1. Out of 1000 stochastic

projections of population projection scenario 5, the population grew in only 6.3% of projections.

The projection scenario with the highest average λ value was Projection Scenario 6, which considers applied conservation management. The average λ value, out of 1000 projections, was 1.20 with a SD of 0.15. Within this projection, the max λ was 1.75 and the minimum λ was 0.64; 45% of the projections had a lambda above 1.00.

Population Viability Analyses

Out of all the deterministic demographic projections run, only projection scenario 6 yielded a $\lambda > 1.00$.

Overall, using the demographic parameters predicted by projection scenario 6, population is viable past a 25-year window and has the population above 1500 individuals after 25 years (**Figure 5).** The chance of extinction within the first 12 years is negligible, at only 2.00%, however, after 25 years that probability reaches 8.00%. Additionally, the PVA for projection scenario 6 shows the probability of falling below the extinction threshold (50 AHY individuals) at 10% for the first two years, however, this value falls to 6.25% after 12 years and then to 6.00% at 25 years (**Figure 8)**. Once past the first two years, the population becomes more robust and less susceptible to stochastic events.

IV. DISCUSSION

Overall, histopathology and necropsy reports reveal *Isospora* sp. has the ability to cause and/or contribute to morbidity and mortality in FGSP. Projection models demonstrate any level of impact of *Isospora* sp. would have a devastating effect on the already imperiled wild population. My study recommends that *Isospora* sp. should be considered in all current and further management approaches.

On the basis of the histopathology reports, *Isospora* sp. has the ability to cause severe damage to the Florida Grasshopper Sparrow. The most virulent stage of the organism is the proliferative, extra-intestinal phase, which causes soft tissue damage as the parasites embed and consume the host's viscera. In the necropsy reports, the examiners made notice of soft tissue damage and hemorrhaging in key organs of the FGSP, including but not limited to the spleen, small intestine, lungs and heart. The organism also occurred in various life stages in the tissues of the FGSP, for example, intracytoplasmic merozoites were found in the liver of IDL18-1955 **(Table 7).**

Through PCR analysis, we detected *Isospora* sp. in every enclosure within the captive population and testing period (**Figure 3**). This demonstrates the ability of the treatment to ameliorate the mortalities suffered in the first clustered event but failure to eradicate the organism from the enclosures.

All enclosures composed of captive-born sparrows, who were born in clean spaces and never lived in the prairie, eventually tested positive for *Isospora* sp. While sparrows are young, their parents feed them by bringing them food directly, or regurgitating food stored in the crop (*58*). Parental regurgitation and subsequent direct

feeding of young is one suspected way the organism spreads so effectively amongst the FGSP.

To understand the factors that affect detectability of *Isospora* sp., we constructed univariate and multivariate logistic regression models, using month, cage, sex, and the number of individuals per enclosure as regressors. Overall, we found that cage, the number of individuals per enclosure, and sex significantly affected detection in the univariate comparisons, with cage being the strongest individual model per **Table 6.**

Per our statistical models, having four or five individuals per enclosure significantly affected detection. Even though it might be a logistical necessity, housing individuals at densities above 4 birds/cage might enhance transmission of *Isospora* sp., because enclosures with fewer than three birds had a lower chance of yielding a positive detection. Density-dependence of *Isospora* sp. detectability is consistent with results from previous studies (*59*).

Additionally, all female enclosures significantly affected detection. This significance might suggest that *Isospora* sp. impacts females to a higher degree than males, which is consistent with the male-dominated skew seen in in the wild population.

Parasitism is an inherent component of any population, but the morbidities and mortalities seen in the FGSP, in addition to its *mt*DNA sequence variation from known *Isospora* spp., suggest the disease is novel to FGSP. The captive population of FGSP was founded by wild collected sparrows, making a strong case for the organism coming in with the founding population.

A sampling of wild birds on the prairie was conducted by FWC to investigate if *Isospora* sp. was a normal, chronic illness in FGSP, or if the severe effects of the

organism seen were a consequence of their captive condition. If the latter were true, *Isospora* sp. should be detectable among living, wild FGSP; however, the wild collection failed to find extra intestinal *Isospora* sp. in any living FGSP more than once and only consistently detected it in three migratory, over-wintering species (*54*).

Isospora sp. was detected in all captive RSCF individuals, who in addition to carrying the disease, had been receiving weekly prophylactic Toltrazuril treatments. Using drugs to control parasitic organisms commonly leads to the development of drug resistance (*60*). Since *Isospora* sp. is the causative organism, if we lose the ability to control it with Toltrazuril, we lose the ability to control the disease in the captivity and in the wild. Drug modification can cause pathogens to adapt, ultimately affecting, sometimes dramatically increasing, their pathogenicity and virulence.

Because of the danger and uncertainty surrounding *Isospora* sp., we used demographic modeling and population projections to show the potential effects of *Isospora* sp. on the wild population of FGSP. Theoretical models can be useful, especially in conservation settings, because they allow for researchers to explore various outcomes before making any potentially detrimental management decisions.

The FWC reported a lambda of 1.09 for the 2019 FGSP ranch wild population (*57*). The FWC value was the second reported lambda value 1 > and is a result of intensive management practices, including: nest-monitoring, predator fencing, and reintroductions. Even with these mitigation strategies, a population with a lambda of 1.09, and very low population numbers, is highly susceptible to stochastic events.

Using the same model and previously published vital rates, we show that even the most conservative estimates of impacts of *Isospora* sp. on the wild population of FGSP will cause λ to fall below 1.0 and the wild population to suffer further declines.

As seen in both the stochastic and deterministic projections of population projections 1-5, even in its most conservative of impact estimates *Isospora* sp. has the ability to decimate the wild population. Projection scenario 5, the minimum effects model, was designed to show even if *Isospora* sp. caused 2.5% mortalities in either age class, the population would still suffer greatly and continue to decline. The deterministic projection of this model has the population declining to zero abundance within a 25-year period and the chance of extinction as 100%. The chance of extinction within the first ten years of this 25-year projection is under 30%, however, following that period the population declines severely. A population that is down to the size of the FGSPs, is extremely vulnerable to perturbation in general and does not have the genetic material available to survive a mass infectious disease event, like *Isospora* sp. (*61*).

Recommending Mitigation Strategies

To conclude, my study recommends the management strategies of: head-starting, an *in-situ* two-week release period on the prairie, or to hold off on reintroducing birds until more data are available.

Head-starting is an applied conservation management strategy where individuals are raised in captivity, past some identified critical life history stage, and subsequently released into the wild to augment the wild population (*62*). In settings of disease management, it can be especially useful and help grow immune resistance in the captive flock.

For FGSP, the first year of life is when the highest rate of mortalities occur (57), so by raising them past the first year in captivity, you can release breeding adults, with a much higher chance of survival, into the wild population **Table 4**. **Figure 5** displays a conceptual model for a two-stage population, being augmented by captive AHY birds. Instead of the growth rate λ being a function of only AHYφ and HYφ **(Equation I)**, it is a function of AHYφ, HYφ, and IHYφ **(Equation VI)**.

Hatch-year survivorship in the wild is as low as 27.99% (**Table 4**), however, after adapting the coccidiostat treatment in captivity at RSCF, HYφ was as high as 95%. Prior to treatment, the mortalities seen in the captive flock were amongst HY birds, which is what led to the discovery of *Isospora* sp.. The disproportionate mortalities seen in juveniles compared to adults would suggest they are more vulnerable to the associated morbidities and mortalities caused by *Isospora* sp., which is also consistent with trends seen in other parasitic infectious disease (*63-65*).

Population projection scenario 6 considers head-starting and reintroduction in the population growth model. Scenario 6 Yields a λ of 1.21 in the deterministic projection (**Table 9)** scenario 6 and an average λ of 1.20 in the stochastic models (**Table 8)**. Out of all the projection scenarios, including the FWC'S estimated of 1.09 for the 2019 season, projection scenario 6, the head-starting model, gives the FGSP the best chance of surviving the next 25-year period (**Figure 5)** and 36% of the stochastic model projections had the population growing in a positive direction (**Table 8)**. For the next 25 years, projection scenario 6 has under an 8% chance of falling below the quasi-extinction threshold.

It is important to acknowledge the caveats and limitations of the demographic models 1-6. Firstly, none of the models, nor the ongoing current recovery effort, are in direct response to the known effects of *Isospora* sp. in the wild population, they are premised upon the pathogen's putative impact, therefore, there are certain limitations to our conclusions and specific management guidance. Additionally, my study recognizes the influence of *Isospora* sp. is modeled as a constant pressure in their growth model, which is unlikely to be a biological reality in the wild.

Captive breeding affords researchers with the opportunity to closely monitor and manipulate their environments, making it an ideal setting to study novel infectious wildlife disease. The first question asked of an expert panel of infectious disease experts assembled by FWC is, what will happen to the FGSP once their prophylactic dose of coccidiostat is removed? The panel of disease experts' comments were compiled in a peer review to inform the Disease Risk Analysis. In their review, the expert panel expressed many concerns regarding *Isospora* sp. and the removal of Toltrazuril. Doctor Barbon, DVM at the Durrell Wildlife Conservation Trust, expressed concerns regarding treatment resistance in the captive flock and recommended the drug treatment be removed under *ex-situ* settings before the release into the wild. Dr. Peeter Hõrak, Ph.D. with the Animal Ecology Unit at University of Tartu, stated: " theoretically, resistant birds would probably be the worst outcome from the epidemiological point of view, as they will carry the infection they survived but others might not", he recommended if it is at all dangerous to release the carriers to stop all medication (*66*). Dr. Ewen, Ph.D with the Dept. of Conservation New Zealand and Member of IUCN SSC Reintroduction Specialist Group, shared Dr. Horak's novel disease concerns and recommended " If you

are truly concerned the birds have a novel parasite, then you should not release any of them" (*66*). Overall, the experts brought in to comment on *Isospora* sp. expressed many different concerns regarding the disease and its risk to both the captive and wild populations. Most of them ultimately recommended to either wait, attain more data, or to delay the release to garner more information on the disease in a captive setting.

My study shares many of the concerns of the experts and also would recommend additional time to remove the coccidiostat in a controlled environment. The risk of introducing a novel extra intestinal coccidia into the extant population is unacceptable, especially when that pathogen could have disease resistance and becomes unmitigable, even in a controlled setting.

In 2019 and 2020, FWC and partners released 150 captive-reared FGSP, some of which are known to carry *Isospora* sp., into the largest existing portion of the wild population (*67*). This release included 88 hatch-year (HY) and 62 after-hatch-year (AHY) individuals, who were introduced onto the "Three Lakes Wild Management Area" (TLWMA) to " support existing wild populations while new management solutions are discovered and implemented" (*67*). Of the first 150 individuals, a total of two adults and 16 juveniles recruited into the existing population, for recruitment rates of 10.6% and 1.3% respectively (*inlit)*.

Since the initial 150 sparrows, almost 100 more individuals have been released in late 2020 and early 2021, making the total number of birds released almost 250 (*56*). There is no recruitment rate information on the later 100 birds released yet. However, in an article in National Geographic, the head of South Florida's FWS office reports that

some of the released sparrows have died as a result of *Isospora* sp., confirming that it can be a mortality factor for released sparrows (*56*).

Despite releasing 250 critically endangered FGSP, there are still many missing critical data gaps and uncertainty regarding wild FGSP, including: cause of death data, mortality rates, and a unifying explanation of their decline. Until the primary driver of decline is eliminated in the system, pouring birds onto the prairie will not be sufficient to reverse the massive declines suffered within their population. As seen in our analysis, any additional mortality factors introduced into the extant population will have devastating effects. The management recommendations made by this study are within the context of the current reintroduction to be relevant and comparable to the real time recovery effort, however, this study questions the viability of *ex-situ* breeding and reintroduction as an appropriate conservation management approach.

V. CONCLUSION

The Florida Grasshopper Sparrow has a long way to go in terms of population stability. Overall, our study made recommendations in the wake of the discovery of a novel emergent extra intestinal coccidia, and purely from the standpoint of science. All crises, epidemiological or not, have the same unsettling backbone of truth; they are all avoidable in hindsight. Emergent disease is a very real concern and threat to wildlife, and as seen by COVID-19 and the current ongoing pandemic, once a disease is prolific enough to come to attention, it can be too late. Management decisions involving emergent disease, like the extra intestinal *Isospora* sp. this study focused on, cannot be made off resting assumptions and data gaps. Infectious disease is too dangerous to wildlife and humans alike.

The FGSP is one of the many species imperiled by cumulative, direct and indirect anthropogenically induced pressures within an intensively modified central-Florida grassland ecosystem. Understanding the processes, like emergent wildlife disease, that drive avian declines is fundamental to conserving critically endangered species and proposing effective recovery measures. Many species of birds will, or have been, the focus of applied conservation management, and a recovery effort. As avifauna populations continue to decline on a global scale, the necessity for applied conservation management grows exponentially and it is important to use science to improve upon methods for recovering critically endangered species.

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Supplemental Material

Appendix A: Rare Species Conservatory Florida Grasshopper Sparrow Husbandry-Protocol

VI. Tables

Table 1: FGSP Enclosures' Sex Composition and Number of Individuals

Enclosure	Sex	Number of Sparrows
SS1	*Female*	*5*
SS2A	*Male*	*1*
SS2B	*Male*	*1*
SS2C	*Male*	*1*
SS3A	*Male and Female*	*2*
SS3B	*Female*	*3*
SS3C	*Female*	*3*
SS4	*Female*	*4*
SS5A	*Male*	*1*
SS5B	*Female*	*3*
SS5C	*Male*	*1*
SS6A	*Male*	*1*
*SS6B**	*Male*	*1*
*SS6C**	*Female*	*1*

** SS6A and SS6B were added outside March 12th*

Table 2: Description of PCR Data Set

Variable	Type
PCR Result	*Response*
Density	*Independent*
Cage	*Independent*
Sex	*Independent*
Month	*Independent*
Breeding Season	*Independent*

Table 3: Description of Variables Used to Construct the Best Fit Multivariate Model.

Variable	Range	Type	Effect on Model
Fecal PCR Result	*1: Positive* *2: Negative*	*Binomial*	*Response Variable*
Density	*1-5*	*Discrete*	*Fixed*
Sex	*1: Female* *2: Male* *3: Female and Male*	*Categorical*	*Fixed*
Breeding Season	*1: Yes* *2: No*	*Categorical*	*Fixed*
Month	*1: February* *2: March* *3: April* *4: May* *5: June*	*Categorial*	*Fixed*
Cage	*1-14 **	*Categorical*	*Random*

** Full summary of cage composition is described in **Table 1***

Table 4: Previously Published Demographic Characteristics Used to Construct the Reference Model

Nest Survival Probability	**Year**
7.50%	*2016*
17.40%	*2017*
33.10%	*2018*
27.20%	*2019*
† R. Bowman, R. L. Windsor, Ed. (U.S. Fish and Wildlife Service, 2019).	
Average Nests Per Pair	**Year**
1.45	*2019*
2.7	*2018*
2.6	*2017*
† R. Bowman, R. L. Windsor, Ed. (U.S. Fish and Wildlife Service, 2019).	
Annual Adult Survivorship	**Source**
63.6%	*R. Bowman, R. L. Windsor, Ed. (U.S. Fish and Wildlife Service, 2019).*
59.8%	*B. Pranty, J. W. Tucker Jr, in Land of fire and water (2006)*
48.2%	*D. W. Perkins, P. D. Vickery The Wilson Journal of Ornithology , (2001)*
53.3%	*D. W. Perkins, P. D. Vickery The Wilson Journal of Ornithology , (2001)*
Juvenile Survivorship	**Source**
21-22%	*R. Bowman, R. L. Windsor, Ed. (U.S. Fish and Wildlife Service, 2019).*
35.10%	*B. Pranty, J. W. Tucker Jr, in Land of fire and water (2006)*
43%	*USFWS. (2019)*
Fecundity (Measured as Average Clutch Size)	**Source**
3.71	*B. Pranty, J. W. Tucker Jr, in Land of fire and water (2006)*
3.47	*B. Pranty, J. W. Tucker Jr, in Land of fire and water (2006)*
3.56	*B. Pranty, J. W. Tucker Jr, in Land of fire and water (2006)*
3.75	*B. Pranty, J. W. Tucker Jr, in Land of fire and water (2006)*
3.93	*R. Bowman, R. L. Windsor, Ed. (U.S. Fish and Wildlife Service, 2019).*

Table 5: Bootstrapped Demographic Parameters for Reference Model

Demographic Parameter	Value
Average Annual Adult Survivorship (AHYφ)	56.26% ± 2.97
Average Annual Juvenile Survivorship (HYφ)	33.14% ± 5.15
Fecundity (F) *	3.68 ± 0.07
Daily Nest Survival Probability (p)	21.32% ± 4.89
Number of Nesting Attempts per Pair (n)	2.24 ± .38

**** Measured as Clutch Size***

Table 6: Univariate results of logistic binomial regression examining the effect of independent variables on disease detection*

Variable	*df*	χ^2	p	ΔAIC
Number of Individuals	*4*	*66.92*	*< .05*	*36.418*
Cage	*13*	*121.34*	*< .05*	*0*
Sex	*2*	*64.911*	*< .05*	*34.426*
Month	*4*	*4.7965*	*0.3088*	*98.54*

**All reported χ^2 and p values are from liklihood ratio tests.*

*** Number of individuals per enclosure*

Table 7 Summarized Subset of Histopathology and Full Body Necropsy Results

Accession Number	Sex	Age	*Isospora* sp. PCR	Key Findings
IDL18-1737	*Female*	*HY*	*Positive*	*1. Liver: Hepatitis, lymphoplasmacytic, multifocal, subacute, moderate with rare, intracellular merozoites* *2. Small intestine: Enteritis, lymphoplasmacytic, multifocal, subacute, moderate to severe with rare intracellular merozoites* *3. Coelom: Coelomitis, lymphoplasmacytic, multifocal, subacute, moderate* *4. Kidney: Nephritis, lymphoplasmacytic, multifocal, subacute, moderate* *5. Skeletal muscle: Myositis, lymphoplasmacytic, multifocal, subacute, mild with single meront* *6. Spleen: Splenitis, histiocytic, multifocal, subacute with severe plasmacytosis* *7. Thoracic air sac: Airsacculitis, heterophilic, focal, subacute, mild with embedded plant material*
IDL18-1738	*Female*	*HY*	*Positive*	*1. Small intestine: Enteritis, lymphoplasmacytic, multifocal, subacute, moderate to severe with rare intracellular merozoites* *2. Liver: Hepatitis, lymphoplasmacytic,*

				multifocal, subacute, moderate *3. Coelom: Coelomitis, lymphoplasmacytic, multifocal, subacute, moderate* *4. Cecum: Typhlitis, lymphoplasmacytic, multifocal, subacute, moderate* *5. Skeletal muscle: Myositis, lymphoplasmacytic, multifocal, subacute, minimal* *6. Spleen: Splenitis, histiocytic, multifocal, subacute, mild with plasmacytosis*
IDL18-1955	*Unsexed*	*HY*	*Positive*	*1. Liver: Moderate, diffuse, lymphocytic peri-portal hepatitis with intralesional merozoites* *2. Lung: Moderate, diffuse, lymphocytic interstitial pneumonia, with intracytoplasmic merozoites* *3. Brain: Mild, multifocal, acute encephalitis with orange pigment deposition (unknown origin)* *4. Bone with bone marrow and skin, unknown location: Moderate, multifocal, heterophilic and granulomatous osteomyelitis with intralesional bacilli* *Mild multifocal subacute to chronic, heterophilic and lymphoplasmacytic dermatitis* *5. Small intestine: Diffuse, severe, chronic,*

				lymphoplasmacytic, histiocytic and eosinophilic enteritis with intraepithelial coccidian parasites
IDL19-6081	*Male*	*AHY*	*Positive*	*1. Coelom: Granuloma, focal, severe with intralesional gram-negative bacilli* *2. Small intestine: Enteritis, lymphoplasmacytic, multifocal, moderate with intraepithelial microgamonts and oocysts* *3. Spleen: Lymphoid hyperplasia, multifocal, moderate* *4. Liver: Hepatitis, lymphocytic and heterophilic, multifocal, mild with one suspect intraciliary cestode* *5. Pineal gland: Adenitis, lymphoplasmacytic and granulomatous, focal, moderate*
IDL19-5167	*Male*	*AHY*	*Positive*	*1. Periorbital tissue: Granuloma, focal with intralesional bacterial coccobacilli* *2. Heart: Atherosclerosis, multifocal, mild* *3. Periesophageal adipose: Steatitis, granulomatous and heterophilic, focal, moderate with embedded keratin* *4. Spleen: One macrophage contains 5 µm, oval, basophilic*

				objects (suspicious of protozoal zoites).
IDL19-1310	*Male*	*AHY*	*Positive*	*1. Large intestine: Multifocal to coalescing lymphoplasmacytic infiltration into the lamina propria Liver: There is a focal area of lymphoplasmacytic infiltration with some heterophilic presence 3. No significant histologic findings were present in the brain, pancreas, skeletal muscle, skin, lungs, ventriculus, proventriculus, crop, and kidney.*
IDL18-0493	*Female*	*HY*	*Positive*	*1. Within the interpretive limits imposed by autolysis in many tissues (see below), all tissues show multiple, discrete, intravascular bacterial colonies of Gram negative bacilli, which are of monomorphic appearance (interpreted as Gram negative bacillary sepsis) 2. All GI tissues, and large expanses of liver, are too severely autolyzed (postmortem autolysis) and have too much (presumed postmortem) bacterial contamination, to allow detailed histological*

	evaluation and diagnostic conclusions.

† All Histopathology and Full Body Necropsies were conducted by The University of Georgia Infectious Disease Laboratory within the University's College of Veterinary Medicine.

Table 8: Summary of Stochastic Population Projections for Projection Scenarios 1-5.

Projection Scenario	1	2	3	4	5	6
Mean λ	*0.67*	*0.66*	*0.53*	*0.45*	*0.77*	*1.20*
SD of λ	*0.11*	*0.12*	*0.10*	*0.08*	*0.12*	*.15*
Min λ	*0.46*	*0.42*	*0.36*	*0.30*	*0.54*	*.64*
Max λ	*0.97*	*0.98*	*0.82*	*0.64*	*1.10*	*1.75*
λ > 1.0	*0%*	*0%*	*0%*	*0%*	*6.8%*	*45%*

Table 9: Summary of Deterministic Population Projections for Projection Scenarios 1-5.

Projection Scenario	λ
1	*0.68*
2	*0.66*
3	*0.55*
4	*0.44*
5	*0.78*
6	*1.21*

Table 10: AIC Multivariate Model Comparisons and Model Equations

Model Equation	AIC
Detection ~ Sex + Numbers of Individuals + (1 | Cage)	*0*
Detection ~ Sex + Numbers of Individuals	*51*
Detection ~ Sex + (1 | Cage)	*58*
Detection ~ (1 | Cage) + Numbers of Individuals	*86*

VII. FIGURES

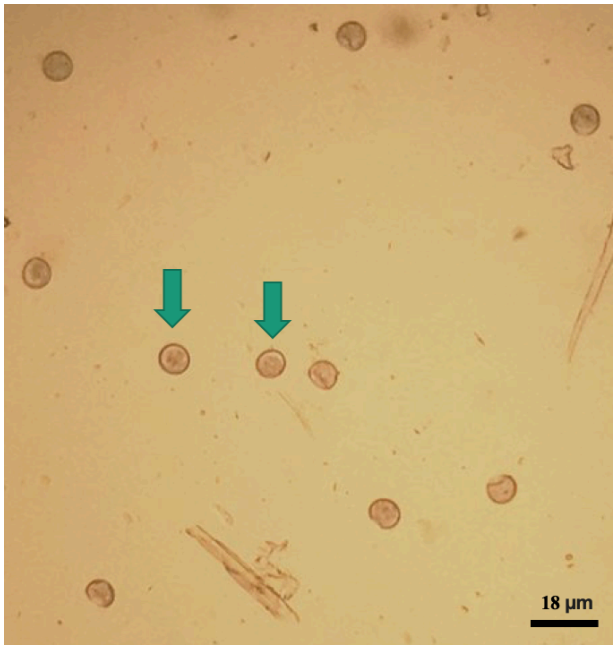

Figure 1: Image of Fecal Slide with High Concentration of Oocysts (Indicated by Green Arrow)

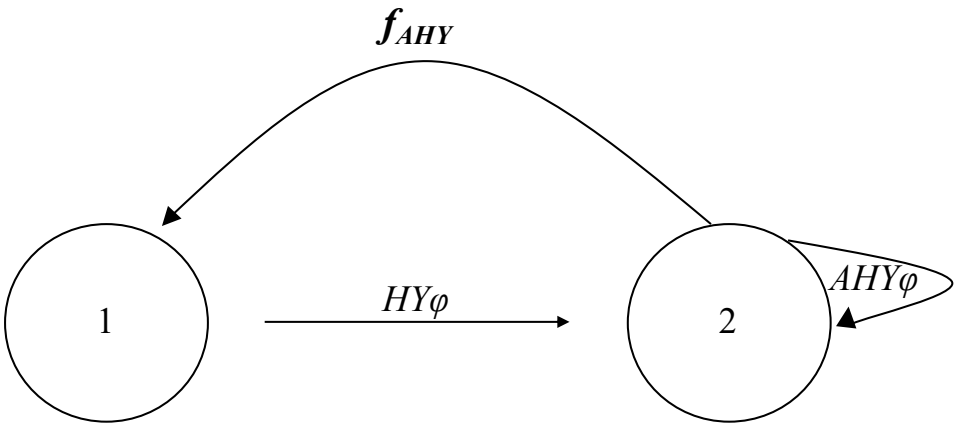

Figure 2 General conceptual model for FGSP population with two stages

The nodes represent (1) Hatch Year and (2) After Hatch Year stages. HYφ represents the first transition probability from Hatch Year to After Hatch Year and AHYφ represents After Hatch Year survivorship. *fAHY* indicates After Hatch Year Fecundity.

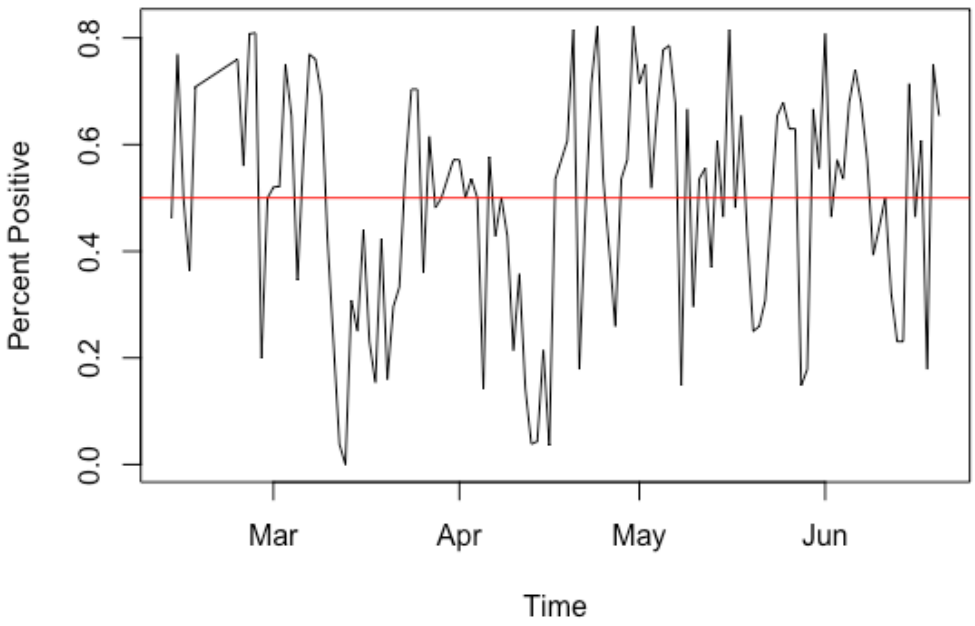

Figure 3: Trend of Positive PCR assay through time. The percent positive represents the weighted frequencies of positives for the entire flock.

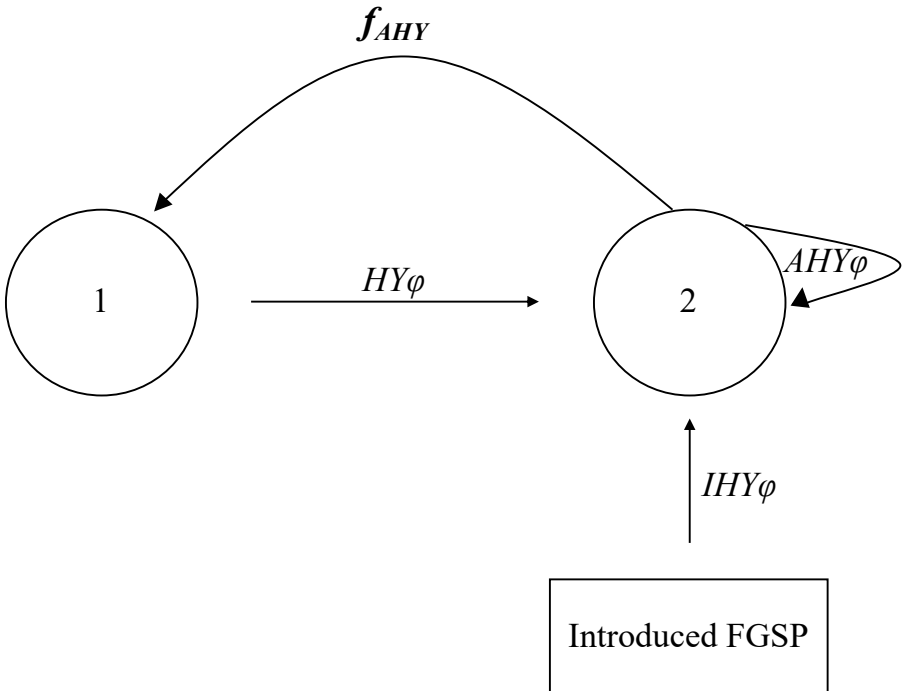

Figure 4 Two Stage FGSP Considering Introduced, Head Started Birds

The circular nodes represent (1) Hatch Year and (2) After Hatch Year stages. HYφ represents the first transition probability from Hatch Year to After Hatch Year and AHYφ represents After Hatch Year survivorship. *IHYφ* represents the head started bird's survivorship in captivity. *fAHY* indicates After Hatch Year Fertility.

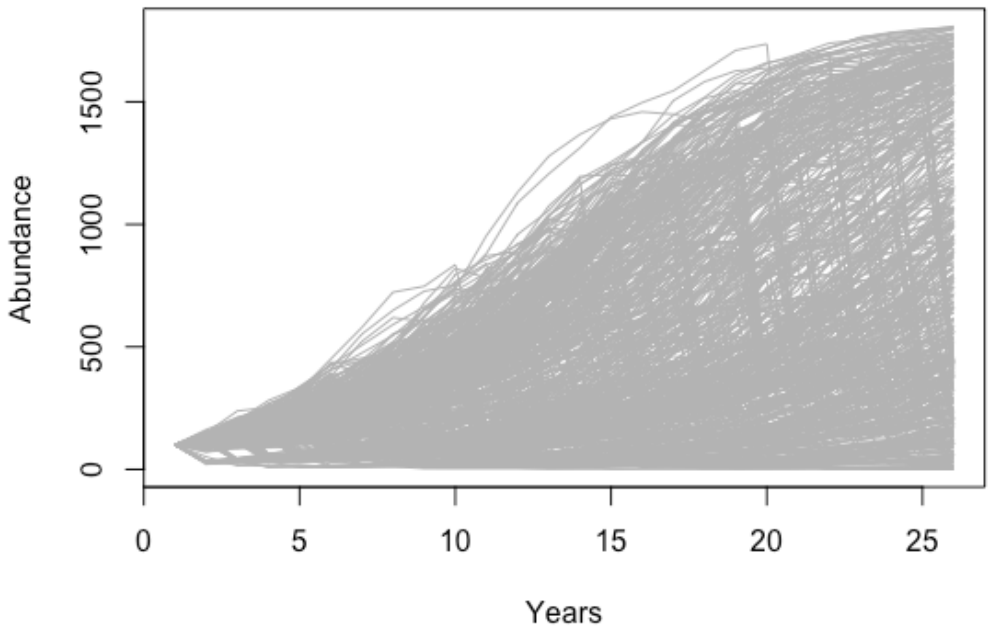

Figure 5: Years Vs. Abundance for Projection Scenario 6

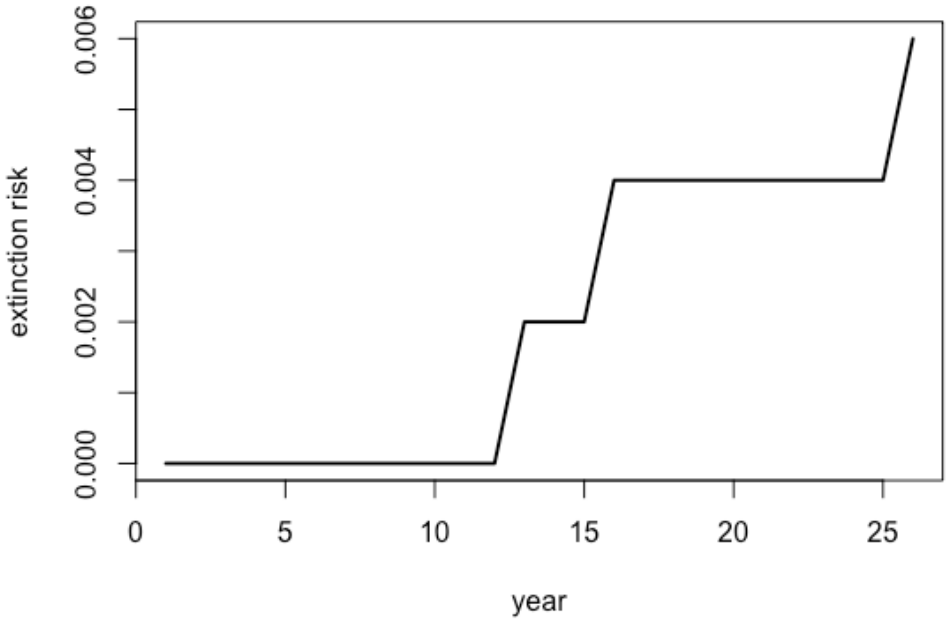

Figure 6: Extinction Risk vs. Years from Projection Scenario 6 PVA

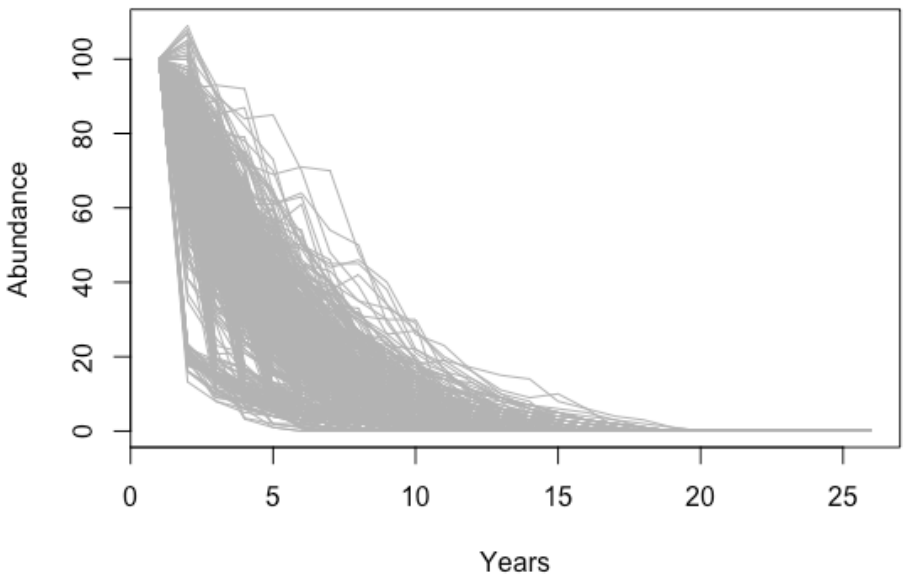

Figure 7: Years Vs. Abundance for Projection Scenario 5

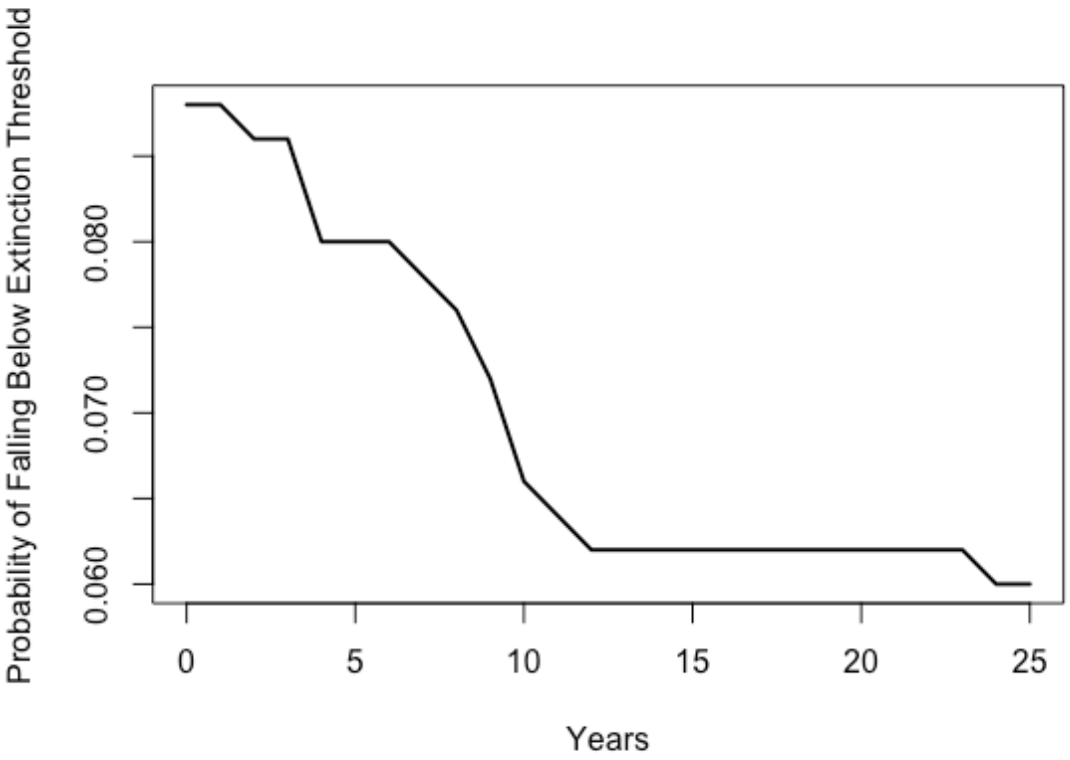

Figure 8: Probability of Falling Below the Extinction Threshold vs. Time for Projection Scenario 6