

Florida Scrub-jay Disease Analysis

**Glen E. Woolfenden
Mary C. Garvin**

Archbold Biological Station
P.O. Box 2057
Lake Placid, Florida 33852

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FINAL REPORT

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Principle Investigator: Glen E. Woolfenden, Ph.D. and Mary C. Garvin, Ph.D.,
Archbold Biological Station

INTRODUCTION

The Florida Scrub-Jay (*Aphelocoma coerulescens*) is declining in numbers and distribution because of loss of its native scrub habitat. Small fragments of scrub now support the remaining populations of this bird, which in 1987 was listed by the USFWS as Threatened. Careful management of the remaining populations and habitat fragments, based on detailed information on reproduction and survival (Soule 1987), is necessary to protect the species.

The Florida Scrub-Jay population at Archbold Biological Station has been the focus of an intensive demographic study for over 25 years (Woolfenden and Fitzpatrick 1984, 1990, 1996). This population occasionally experiences sudden catastrophic declines. Although existing survivorship models take into account these catastrophes (Fitzpatrick et al. 1991), they do so only crudely, in part because the causative agents are unknown.

Little is known about the frequency and impact of diseases in wild populations of almost all species. Although small populations may be especially vulnerable to disease (Jackson 1977), infectious agents are rarely considered in ecological studies of natural populations. The lack of knowledge is primarily attributable to two factors: (1) monitoring sick individuals within a natural population is difficult because usually they are quickly taken by predators or scavengers, which often precludes identification of the pathogen (Wobeser and Wobeser 1992), and (2) the impact of pathogens on populations is difficult to measure (Toft 1991), in part because normal variation of demographic parameters prior to an epidemic usually is unknown.

The objective of this study was to determine the relative prevalence and intensity of pathogens present in a Florida Scrub-Jay population at Archbold Biological Station. The results provide baseline data for identification of causative agents of future epidemics and for

future long-term studies of pathogens in this population.

METHODS

The study was conducted at Archbold Biological Station in Highlands County, Florida, from July 1994 through June 1996. This privately owned preserve is within The Nature Conservancy's nominated Lake Wales Ridge Bioreserve.

The Archbold population of Florida Scrub-Jays includes several hundred individuals of known sex, age, social status, and genetic relations. Jays were examined for pathogens as nestlings, as fledglings when about three months old, and on occasions later in their lives.

We searched each captured jay for ectoparasites and collected 0.2 - 0.3cc of blood to survey for viral and parasitic infections. Blood also was evaluated to determine hemoglobin concentration, plasma protein concentration, and packed cell volume, each of which is an indicator of health.

Approximately 0.2cc of blood was collected from the jugular vein of each nestling and 0.3cc from the jugular vein of each juvenile and adult with a 1cc syringe rinsed with EDTA. Samples were temporarily stored in heparinized microhematocrit tubes and analyzed for hemoglobin, pcv and plasma protein in the laboratory. For virus isolation from nestlings, a single drop of blood was placed in a cryopreservation vial containing biological field diluent (BFD), stored on ice until samples were returned to the laboratory and frozen at -70 C in liquid nitrogen. Samples were later shipped to the Florida Medical Entomology Laboratory in Vero Beach and stored in an ultracold freezer. Samples were then shipped on dry ice to the Tampa Branch Laboratory-Virology Section, Florida Department of Health and Rehabilitative Services where viral isolations were attempted. An additional 0.3cc of blood was collected from juveniles and adults, stored in vacutainers containing BF, and placed on wet ice. After returning the laboratory, sera were separated and decanted into cryopreservation vials, refrigerated and shipped to Florida Medical Entomology Laboratory on wet ice. Samples were then shipped to the Florida Department of Health Virology Lab in Tampa for antibody assays. Assays were run for hemagglutination antibodies (HI) to eastern equine encephalitis virus (EEE) and St. Louis encephalitis virus (SLE), indicating recent infections and neutralizing (NT) antibodies, indicating older infections, to EEE, SLE, and Highlands jay (HJ) virus. Blood for hemoglobin concentration, plasma protein concentration, and PCV was placed on wet ice in the field and processed within several hours of collection. Hemoglobin was determined with a hand-held hemoglobinometer (Leica Inc.) and plasma protein concentration was determined with a hand-held refractometer. PCV was determined after hematocrit tubes were centrifuged for five minutes.

Blood smears were prepared from each bird to examine for protozoan parasites. The smears were fixed in absolute methanol, stained with Geimsa-Wright stains, and scanned under 100x oil immersion until an estimated 100,000 red blood cells were observed. Intensity of infection was indexed by quantifying the number of infected cells per 10,000.

Each bird was examined for the presence of mites, lice, and ticks. The location of infestation was noted and a grade of intensity was assigned. Ectoparasite specimens were collected for identification and preserved in ETOH-Glycerine. Samples were grouped into

spring (March, April, May), summer (June, July, August), fall (September, October, November) and winter (December, January, and February) collections for seasonal analysis of ectoparasites. Adult lice were sent to Roger Price at the University of Minnesota for species identification. Mite specimens were sent to J. W. Mertins at the National Veterinarian Services Laboratory of the U.S.D.A. in Ames, Iowa for identification. Ticks were sent to James Keirans, Institute of Arthropodology and Parasitology, Georgia Southern University for identification. Specimens were deposited at each of the respective institutions.

Fecal samples were collected each month from July 1994 through April 1996 from approximately 30 jays per month. Samples were stored in potassium dichromate, aerated for several days prior to examination, and analyzed for protozoan and helminth parasites. Fecal floatation in Sheather's Solution was used to concentrate oocysts, followed by sedimentation in detergent solution to concentrate helminth eggs. Samples were examined under 10X and 40X optics.

Age designations of jays used in this study are consistent with USFWS guidelines and include L (nestlings), HY (first calendar year), SY (second calendar year), and ASY (after second calendar year). Because SY jays are difficult or impossible to discern from ASY jays during October, November, and December, the category, AHY (after hatching year), was used during this period for birds of unknown age. Juvenile refers to HY jays and adult to SY, ASY, and AHY jays.

RESULTS

Blood parasites:

Thin blood smears were prepared from 82 adult, 128 HY and 67 L jays and examined for blood parasites. Of these, three were found to be positive for intracellular sporozoan parasites. All three jays had low intensity infections (less than 1 infected red blood cell per 10,000 red blood cells) of *Haemoproteus danilewskyi*. One of the three was an unbanded HY bird, found north of Archbold Biological Station on SR 70 after being killed by a car. The two others were HY birds, about three months of age, from Archbold's long-term Demographic Tract. The jays were from different territories at the northern and southern ends of the tract.

Encephalitis virus:

In cooperation with Jonathan Day of the Florida Medical Entomology Laboratory, blood samples were collected for encephalitis virus antibody assay from 1994 and 1995 HY birds that survived to approximately three months of age ($n=120$) and adult birds that were captured incidentally from July 1994 through March 1996 ($n=78$). Hemagglutination inhibition antibodies, indicating recent infections, to EEE and SLE were detected. Overall, 13 of 78 adult jays (17%) and 19 of 120 (16%) HY jays sampled were positive for HI antibodies for EEE (Table 1). HI antibodies to SLE were detected in none of the 78 adult jays examined and in one of the 120 (0.1%) HY jays examined (Table 2).

Six of the 74 (8%) HY jays examined for neutralizing antibodies to EEE were positive and 15 of 63 (24%) adult jays examined for NT antibody titers to EEE were positive (Table

1). One of eight (13%) HY jays examined for NT antibodies to SLE was positive and 0 of the 4 adult jays examined for NT antibody titers to SLE were positive (Table 2). Eighteen of 59 (31%) HY jays and 10 of 57 adult jays were positive for NT antibodies for HJ encephalitis virus (Table 3).

In 1995, viral isolation was attempted from 55 nestlings and 31 adults. An isolation of EEE was made from one 11-day old nestling sampled in May 1995. No virus was isolated from adults.

To monitor virus activity more closely one sentinel scrub jay was maintained at Archbold from January 1995 through July 1995 at a site where virus activity was observed in the summer of 1994. A blood sample for antibody assay was collected monthly and a sample for virus isolation was collected weekly. Analysis of these samples did not reveal infection of any of the three strains of virus.

Ectoparasites:

Seventy-six of 279 (27%) jays examined were infested with nits of chewing lice (Table 4). Although the louse nits could not be used for identification, it seems likely that all or most were laid by members of an undescribed species of *Myrsidea* because the 14 adult lice found on these jays were members of this species. None of 68 nestlings sampled at age 11 days was infested; therefore, age had a significant effect on infestation ($\chi^2 = 34.83$, $df = 4$, $p < 0.001$). However, when comparing jay age groups in which lice were present, all had similar proportions of infested individuals ($\chi^2 = 1.00$, $df = 3$, $p = 0.800$). When all infested individuals were pooled, a significant effect of season was observed ($\chi^2 = 8.183$, $df = 3$, $p = 0.042$). The lowest incidence was observed in winter (7%), the highest incidence in fall (50%). Incidence was similar in spring and fall with 21% and 28% of the birds infested, respectively.

Wing feather mites of an undescribed species of *Pterodectes* were detected on the primary and secondary feathers of 69 of 212 (33%) jays examined (Table 5). Older birds were more often infested than HY birds ($\chi^2 = 14.73$, $df = 3$, $p = 0.002$). However, when HY birds were excluded from the analysis, incidence of infection did not vary among ages. When season was compared among birds older than HY, a significant effect of season was observed ($\chi^2 = 7.95$, $df = 3$, $p = 0.047$). The lowest incidence, 8%, was observed in the winter, and the highest, 58%, was observed in the summer. No significant differences were observed between spring, summer and fall.

Other mites identified from jays include two species of chiggers. One adult jay was infested with *Eutrombicula lipovskyana* and two were infested with *E. alfreddugesi*. All three infestations were on the head, of low density, and observed during July.

Ticks were detected on 48 of 263 (18%) jays (Table 6). *Amblyomma tuberculatum* was detected only during October, November, and December. *Ixodes scapularis* was detected only in July and *Amblyomma maculatum* was detected only in November. *Haemaphysalis leporispalustris* was the most common tick species and infested juveniles more often than adults ($\chi^2 = 8.88$, $df = 3$, $p = 0.031$). Because jays are not likely to become infested with ticks until after they leave the nest, nestlings were excluded from the analysis.

Fecal samples:

Unidentified species of intestinal coccidia of the genus *Isospora* were observed in three HY jays. These infections were observed during the months of December, May and July. Six infections were detected in adults. Two of the six were observed during May 1995 and the remaining four were observed during June 1995. No nematode infections were detected from fecal samples.

Hemoglobin, Packed Cell Volume, and Plasma Protein:

Hemoglobin concentration was measured for 61 adults, 113 juveniles, and 70 nestlings (Table 7). Packed cell volume (PCV) was determined for 64 adults, 115 juveniles, and 72 nestlings (Table 7). Plasma protein concentration was determined for 63 nestlings, 48 juveniles, and 47 adults (Table 7).

Field Observations:

Although no apparently sick jays were observed from July 1994 through June 1995, increased breeder mortality and delayed breeding were observed in April and May 1995. Because an infectious agent may have contributed to these patterns, we increased our field effort and sampled 32 adult jays during this period. Analysis of samples from this period did not reveal increased prevalence of any of the pathogens discussed in this study.

During July 1995, a sick HY jay was observed and captured. Overt signs of poor health included droopy wings, roughed plumage, and ataxia. Blood smears did not reveal infection of blood parasites and fecal floatation and sedimentation did not reveal intestinal protozoan or helminth infection. The jay died within 12 hours of being held captive. Because encephalitis virus was previously detected in the jay population, the carcass was immediately transported to the Florida Medical Entomology Lab in Vero Beach for viral assays. Viral isolation attempts on this bird were unsuccessful. The carcass has been retained by the FMEL as a source of tissue for further molecular analysis for viral infection.

DISCUSSION

Of the pathogens evaluated in this study, encephalitis virus is the most likely to cause increased mortality in the Florida Scrub-Jay population at Archbold. From August 1979 through March 1980, the FSJ population at Archbold experienced an unexplained period of severe mortality (Woolfenden and Fitzpatrick 1990). Also notable during that period were outbreaks of EEE in domestic birds in Florida (J. Day pers. commun.). During this study, we confirmed the presence of EEE, SLE, and HJ encephalitis viruses at Archbold. The only virus isolation was made from an 11-day old nestling. This jay did not survive the fledgling phase, however the definitive cause of death could not be determined. Although most of the individuals that were seropositive for virus survived, survivorship may be reduced under the stress of extreme environmental pressures often experienced by threatened species. Unfortunately, the role of inherited disease resistance, viral recrudescence during stressful

periods, and the degree of pathology associated with encephalitis virus infections in birds are poorly understood and should be addressed through future field and laboratory studies.

Prevalence of blood parasites was 0.7% during the two years of this study. Although both *Plasmodium cathemerium* and *Haemoproteus danilewskyi* were detected at low prevalence and intensity during earlier studies (Garvin unpublished data), only *H. danilewskyi* was detected during the two years of this study. Approximately 30% prevalence of *H. danilewskyi* was found during concurrent study of Blue Jays (*Cyanocitta cristata*), which typically inhabit the forested areas at Archbold. Because both jay species are susceptible to infection, more favorable vector breeding conditions of the forest may have contributed to higher prevalence of the parasites in Blue Jays.

An unidentified species of *Isospora*, a coccidial parasite, was identified in fecal material collected primarily during May and June. These observations are consistent with the epizootiology of the parasite (Todd and Hammond 1971). Although dormant oocysts are available in the environment and may be ingested at any time, only when the appropriate moisture, temperature, and oxygen are available do uninfected oocysts undergo sporulation and, thus, become infective. Moisture required for sporulation apparently became available in May and June, which is the beginning of the rainy season in Florida.

The lack of detectable helminth infections in fecal material is difficult to explain because Kinsella (1974) collected 15 species of helminths, including nematodes, trematodes, cestodes and acanthocephalans, from 35 adult and 5 fledgling Florida Scrub-Jays at and around Archbold Biological Station. Except for the 5 nestlings he examined, all jays were infected with at least one species of helminth. Perhaps the paucity of helminth eggs observed in the fecal samples reflected low intensity helminth infections that were unlikely to be detected through fecal floatation and sedimentation.

Baseline data of infectious agents for threatened populations of birds are important for both the evaluation of future disease-related die-offs and for developing survivorship models for management strategies. The data presented in this study provide such baseline information. Furthermore, indices of general health, including hemoglobin concentration, plasma protein concentration, and packed cell volume, provide standards for future studies. The data we gathered will serve as a foundation for future long-term studies of the diseases of the Threatened Florida Scrub-Jay.

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Table 1. Number of Florida Scrub-Jays testing positive for hemagglutination (HI) and neutralizing (NT) antibodies to eastern equine encephalitis virus. Numbers jays positive / number sampled.

Age	Antibody	Month												Total
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
HY	HI							18/102			1/5	0/10	0/3	19/120
	NT							4/60			0/3	1/9	1/2	6/74
SY	HI		0/1	0/3	1/12		0/2	5/17				0/2		6/37
	NT		0/1	0/3	1/12			2/14				1/2		4/32
ASY	HI			0/2	2/11	1/5	1/4	2/9		0/1		1/1		7/33
	NT			2/2	2/10	0/5	0/1	2/3						6/21
AHY	HI										0/1	0/4	0/3	0/8
	NT										0/3	3/4	2/3	5/10
Total	HI		0/1	0/5	3/23	1/5	1/6	25/128		0/1	1/6	1/17	0/6	32/198
	NT		0/1	2/5	3/22	0/5	0/1	8/77			0/6	5/15	3/5	21/137

Table 2 . Number of Florida Scrub-Jays testing positive for hemagglutination (HI) and neutralizing (NT) antibodies to St. Louis encephalitis virus. Number positive jays / number sampled.

Age	Antibody	Month												Total
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
HY	HI							1/102			0/5	0/10	0/3	1/120
	NT							1/8						1/8
SY	HI		0/1	0/3	0/12		0/2	0/17				0/2		0/37
	NT				0/2									0/2
ASY	HI			0/2	0/11	0/5	0/4	0/9		0/1		0/1		0/33
	NT				0/1	0/1								0/2
AHY	HI										0/1	0/4	0/3	0/8
	NT													0/0
Total	HI	0/0	0/1	0/5	0/23	0/5	0/6	1/128	0/0	0/1	0/6	0/17	0/6	1/198
	NT	0/0	0/0	0/0	0/3	0/1	0/0	1/8	0/0	0/0	0/0	0/0	0/0	1/12

Table 3. Number of Florida Scrub-Jays testing positive for neutralizing (NT) antibodies to Highlands jay encephalitis virus. Number positive jays / number sampled.

Age	Month												Total
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
HY							18/59						18/59
SY		0/1	0/3	1/12			6/14				0/2		7/32
ASY			1/2	1/10	0/1	0/1	1/3						3/17
AHY										0/1	0/4	0/3	0/8
Total	0/0	0/1	1/5	2/22	0/1	0/1	25/76	0/0	0/0	0/1	0/6	0/3	28/116

Table 4. Number of Florida Scrub-Jays infested with nits of chewing lice. Number positive jays / number sampled. "A" indicates months in which adult lice of an undescribed species of *Myrsidea* were observed on the jays of each age group.

Age	Month												Total	
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
L				0/3	0/51	0/14							0/68	0%
HY							38/104	0/0 A		3/7	4/10 A	1/9	46/130	35%
SY		0/1	3/3 A	6/12		0/2	1/18				2/2		12/38	32%
ASY		0/1	0/2	6/10	3/4	0/3	3/9	0/0 A		2/3 A	0/1		14/33	42%
AHY									1/1 A	1/2	2/4	0/3	4/10	40%
Total	0/0	0/2	3/5	12/25	3/55	0/19	42/131	0/0	1/1	6/12	8/17	1/12	76/279	27%

Table 5. Number of Florida Scrub-Jays infested with wing feather mites, *Pterodectes* sp. Number infested jays / number sampled.

Age	Month												Total	
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
L				0/2	0/51	0/14							0/67	0%
HY							17/104			4/7	4/10	5/9	30/130	23%
SY		1/1	2/3	2/12		1/2	10/18				0/2		16/38	42%
ASY		1/1	1/2	4/10	0/4	2/2	5/9			3/3	0/1		16/32	50%
AHY									0/1	1/4	3/4	2/3	6/12	50%
TOTAL	0/0	2/2	3/5	6/22	0/55	3/18	32/131	0/0	0/1	8/14	7/17	7/12	68/279	24%

Table 6. Number of Florida Scrub-Jays sampled (n) and infested with larva and nymph phases of five tick species: *Amblyomma americanum* = A. a., *Amblyomma maculatum* = A. m., *Amblyomma tuberculatum* = A. t., *Haemaphysalis leporispalustris* = H. l., *Ixodes scapularis* = I. s., and unidentified = unid.

Age	Month											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
L	(0)	(0)	(0)	(2)	(51)	(14)	(0)	(0)	(0)	(0)	(0)	(0)
HY	(0)	(0)	(0)	(0)	(0)	(0)	(105) H. l. = 17 I. s. = 2	(0)	(0)	(7) H. l. = 2 A. a. = 1	(10) H. l. = 3 A. a. = 1 A. m. = 1 A. t. = 1	(9) A. a. = 1 A. t. = 4 unid. = 1
SY	(0)	(1) unid. = 1	(3) unid. = 1	(0)	(0)	(0)	(15) H. l. = 4	(0)	(0)	(0)	(2) H. l. = 2	(0)
ASY	(0)	(1)	(2)	(11)	(4) H. l. = 1	(3)	(10)	(0)	(0)	(3)	(1)	(0)
AHY	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(1)	(4) H. l. = 2	(3) H. l. = 1 A. t. = 1 unid. = 1

Table 7. Mean packed cell volume, hemoglobin, and plasma protein values for nestling, hatching year (HY), second year (SY), and after second year (ASY) birds (mean $\pm$ SE (n)).

Age group	Packed Cell Volume (%)	Hemoglobin (g/dl)	Plasma Protein (g/dl)
Nestling	39.51 $\pm$ 1.04 (72)	7.50 $\pm$ 0.32 (70)	2.85 $\pm$ 0.07 (63)
HY	47.68 $\pm$ 0.57 (115)	11.76 $\pm$ 0.18 (113)	3.56 $\pm$ 0.08 (48)
SY	48.62 $\pm$ 1.11 (34)	11.86 $\pm$ 0.32 (33)	3.74 $\pm$ 0.11 (21)
ASY	48.27 $\pm$ 1.10 (30)	11.20 $\pm$ 0.35 (28)	3.76 $\pm$ 0.14 (26)