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## Causes of Mortality of Wild Birds Submitted to the Charles Darwin Research Station, Santa Cruz, Galápagos, Ecuador from 2002–2004

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**ABSTRACT:** Necropsy findings were reviewed from wild birds submitted to the Charles Darwin Research Station, Santa Cruz Island, Galápagos Archipelago between 2004 and 2006. One hundred and ninety cases from 27 different species were submitted, and 178 of these cases were evaluated grossly or histologically. Trauma and trauma-related deaths ( $n=141$ ) dominated necropsy submissions. Infectious causes of avian mortality included myiasis due to *Philornis* sp. ( $n=6$ ), avian pox ( $n=1$ ), and schistosomosis ( $n=1$ ).

**Key words:** Galápagos, necropsy, *Philornis*, poxvirus, roadkill, schistosomosis, trauma, wild birds.

The Galápagos Archipelago, located approximately 1,000 km west of the Ecuadorian coast, is renowned for its endemic flora and fauna (Loope et al., 1988). Currently, avian populations of the Galápagos Islands are threatened by invasive species, growing tourism, immigration, human population growth and associated agricultural development (a rapidly expanding poultry industry), and natural resource exploitation (overfishing; Tye et al., 2002). The introduction of exotic diseases into the Galápagos Islands can have severe negative consequences for native wildlife populations, as previously observed in many Hawaiian bird species (Atkinson et al., 2000; Benning et al., 2002). Identifying significant causes of morbidity and mortality in wild birds through monitoring programs is crucial to wildlife management, conservation, and scientific research strategies in the Galápagos archipelago (Wikelski et al., 2004;

Parker et al., 2006). In 2001, the St. Louis Zoo and the University of Missouri–St. Louis, in collaboration with the Galápagos National Park and Charles Darwin Research Station (CDRS), initiated a surveillance program to monitor avian mortality and morbidity. This monitoring program has two components: 1) active surveillance of live wild and domestic bird populations and disease vectors, and 2) passive surveillance through diagnostic pathology of dead or sick animals submitted to the CDRS and to Galapagos National Park. Surveys of endemic Galápagos birds, including Galápagos Penguins (*Spheniscus mendiculus*; Travis et al., 2006a); Flightless Cormorants (*Phalacrocorax harrisi*; Travis et al., 2006b); Waved Albatross (*Phoebastria irrorata*; Padilla et al., 2003); Red-footed Boobies (*Sula sula*), Nazca Boobies (*Sula granti*), Great Frigatebirds (*Fregata minor*), Swallow-tailed Gulls (*Creagrus furcatus*) (Padilla et al., 2006); and Galápagos Doves (*Zenaida galapagoensis*; Padilla et al., 2004) have identified endo- and ectoparasites and serologic reactivity to a variety of infectious agents (Parker et al., 2006). This report summarizes causes of mortality and briefly describes common parasites or lesions seen in avian necropsy cases submitted to the CDRS on Santa Cruz Island (00.74258°S and 090.30353°W) from 2002–2004.

Between July 2002 and July 2004, 190 fresh, frozen, or formalin-fixed avian carcasses, comprising 27 different species

TABLE 1. Necropsy submissions to the Charles Darwin Research Station, 2002–2004.

Species	Order	No. of birds
Yellow Warbler ( <i>Dendroica petechia</i> )	Passeriformes	68
Medium Ground Finch ( <i>Geospiza fortis</i> )	Passeriformes	17
Smooth-billed Ani ( <i>Crotophaga ani</i> )	Cuculiformes	13
Small Ground Finch ( <i>Geospiza fuliginosa</i> )	Passeriformes	12
Galápagos Mockingbird ( <i>Nesomimus parvulus</i> )	Passeriformes	10
Dark-billed Cuckoo ( <i>Coccyzus melacoryphus</i> )	Cuculiformes	8
Ground Finch ( <i>Geospiza</i> sp.) <sup>a</sup>	Passeriformes	6
Galápagos Flycatcher ( <i>Myiarchus magnirostris</i> )	Passeriformes	6
Brown Pelican ( <i>Pelecanus occidentalis urinator</i> )	Pelecaniformes	6
Yellow-crowned Night Heron ( <i>Nycticorax violaceus</i> )	Ciconiiformes	5
Barn Owl ( <i>Tyto alba punctatissima</i> )	Strigiformes	5
Galápagos Dove ( <i>Zenaida galapagoensis</i> )	Columbiformes	5
Small Tree Finch ( <i>Camarhynchus parvulus</i> )	Passeriformes	4
Blue-footed Booby ( <i>Sula nebouxii excisa</i> )	Pelecaniformes	4
Cattle Egret ( <i>Bulbulcus ibis</i> )	Ciconiiformes	3
Rock Dove–pigeon ( <i>Columba livia</i> )	Columbiformes	3
Great Blue Heron ( <i>Ardea herodias</i> )	Ciconiiformes	2
Cactus Finch ( <i>Geospiza scandens</i> )	Passeriformes	2
Paint-billed Crake ( <i>Neocrex erythrops</i> )	Gruiformes	2
Common Noddy ( <i>Anous stolidus galapagensis</i> )	Charadriiformes	1
Large Ground Finch ( <i>Geospiza magnirostris</i> )	Passeriformes	1
Lava Gull ( <i>Larus fuliginosus</i> )	Charadriiformes	1
Espanola Mockingbird ( <i>Nesomimus macdonaldi</i> )	Passeriformes	1
Red-billed Tropicbird ( <i>Phaethon aethereus</i> )	Pelecaniformes	1
Vegetarian Finch ( <i>Platyspiza crassirostris</i> )	Passeriformes	1
Common Moorhen ( <i>Porphyrio chloropus</i> )	Gruiformes	1
Audubon Shearwater ( <i>Puffinus lherminieri subalaris</i> )	Procellariiformes	1
Nazca Booby ( <i>Sula granti</i> )	Pelecaniformes	1

<sup>a</sup> not identified to species level, mainly due to trauma (crushed or missing beaks).

(Table 1), were submitted to the Charles Darwin Research Station (CDRS), Santa Cruz, Galápagos from the following islands: Santa Cruz ( $n=171$ ), San Cristobal ( $n=5$ ), Fernandina ( $n=3$ ), Española ( $n=3$ ), Floreana ( $n=1$ ), Isabela ( $n=3$ ), and Santa Fe ( $n=1$ ). Island of origin was unknown for three submitted cases. One hundred seventy-eight of these submissions were evaluated by gross necropsy or histology. Additionally, moribund birds were submitted to the CDRS; nearly all of these birds died or were euthanized within 48 hr following submission. Cases were submitted by CDRS researchers, Galápagos National Park personnel, local residents, or visiting scientists. Of the 171 birds from Santa Cruz, 106 were found on periodic roadkill surveys of the main road between the northern (Itabaca Canal) and

southern (Puerto Ayora) coasts of Santa Cruz Island (Betancourt, et al., 2003; Jiménez-Uzcátegui and Betancourt, 2005) and submitted for necropsy.

Moribund birds were anesthetized with 10 mg/kg of the tiletamine/zolazepam combination (Telazol®, Fort Dodge Laboratories Inc., Fort Dodge, Iowa, USA) intramuscularly and subsequently euthanized with 80 mg/kg of pentobarbital sodium and phenytoin sodium (Beuthanasia-D Special Euthanasia Solution, Schering-Plough Animal Health Corp., Union, New Jersey, USA) intravenously (brachial or jugular vein), and gross necropsies were performed. Representative samples of brain, spinal cord, sciatic nerve, liver, lung, spleen, kidney, reproductive tract, eye, trachea, tongue, esophagus, air sac, tongue, skin, heart, skeletal muscle, pro-

ventriculus, duodenum, small intestines, large intestines, cecum, bursa of Fabricius, pancreas, adrenal glands, and uropygial gland were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin (H&E). Because some cases were not available for histopathology due to trauma or autolysis, only 149 of the 190 cases were evaluated by histology. Special stains were employed, where warranted, based on evaluation of H&E sections. Stains included Prussian blue for detection of iron; Gram stain, Kinyoun's acid fast, or Giemsa stains to detect microorganisms; periodic acid-Schiff stain was used to detect fungal, protozoal, or microsporidial organisms. On most of the fresh postmortem specimens, Diff-Quick® (Fisher Scientific, Pittsburgh, Pennsylvania, USA) stained impression smears of various organs (liver, spleen, and lung) were performed. Although frozen tissues or swabs of various organs were sampled from many of the dead birds submitted, viral or bacterial isolation on these samples was not performed because of permit restrictions on tissue transport or freezer failure.

Causes of mortality are shown in Table 2 and incidental findings are shown in Table 3. Incidental findings were classified as ancillary parasites or histologic lesions present that were not the immediate, nor the ultimate, cause of death. Trauma was the immediate cause of death in 79% ( $n=141$ ) of all examined wild bird submissions. Excluding all 106 roadkill survey birds, trauma (vehicular, human aggression, other) was still the most frequent diagnosis (49%;  $n=35$ ) of submitted cases. Additional trauma-related deaths included animals that were electively euthanized due to unlikely recovery from injury or that died from infectious sequela to trauma ( $n=5$ ). In total, 82.0% (146/178) of deaths were caused by trauma or trauma-related sequela. Most traumatic deaths were due to vehicular collisions in Santa Cruz within the town of

Puerto Ayora and on the main road extending across the island from Puerto Ayora to the Itabaca canal. This road, approximately 40 km in length, is the main conduit for visitors and residents who arrive at the airport on Baltra Island (Betancourt et al., 2003; Jiménez-Uzcátegui and Betancourt, 2005). Similarly, Savidge et al. (1992) describe trauma (vehicular and predation) as the most common cause of death (46%) of Guam avifauna. The proportion of birds dying from vehicular trauma likely overestimates the overall number of trauma deaths relative to other causes of mortality, due to biased sampling; dead birds are highly conspicuous on the Santa Cruz–Baltra road, and this is the major road in the entire archipelago. Island-level population impacts of road kill on Galápagos avifauna are not known. However, the Santa Cruz–Baltra road is a definite local population sink for birds. Although speed bumps and warning signs were installed along the road, roadkill continues to be high. Increased police vigilance, enforcement of speeding regulations, and high speeding fines are management strategies that may be effective in reducing avian mortality.

Mortality was also attributed to *Philornis* myiasis ( $n=6$ ) and gout ( $n=2$ ), and single case mortalities were observed as follows: schistosomiasis (Common Moorhen, *Gallinula chloropus*); electrocution (Brown Pelican, *Pelecanus occidentalis*); avian pox (Galápagos Mockingbird, *Nesomimus parvulus*); foreign body ingestion (Brown Pelican); gastroesophagitis (Brown Pelican); necrotizing hepatitis and splenitis (Galápagos Mockingbird); and panniculitis (Ground Finch; *Geospiza* sp.). Cause of death was undetermined–open in nine (5.0%) cases.

Avian pox was diagnosed by histologic identification of characteristic epithelial eosinophilic intracytoplasmic inclusion bodies and by intralesional parakeratotic and orthokeratotic hyperkeratosis. The only fatal poxvirus infection was in a juvenile Galápagos Mockingbird that had

TABLE 2. Causes of mortality in wild bird necropsy cases evaluated at the Charles Darwin Research Station ( $n = 178$ ).

Cause of Death	Total	Charadriiformes	Ciconiiformes	Columbiformes	Cuculiformes	Gruiformes	Passeriformes	Pelecaniformes	Procellariiformes	Strigiformes
Trauma	141	1	5	3	14	1	111	1		5
Undetermined	9		1		5	1	2			
Elective euthanasia	7			3	2		1			1
<i>Philornis</i>	6						6			
Trauma-related	5	1	2	1				1		
Gout	2							1		
Electrocution	1									
Foreign body	1									
Gastroenteritis	1									
Inanition	1									
Necrotizing hepatitis/splenitis	1									
Panniculitis	1									
Poxvirus	1									
Schistosomiasis	1					1				
Total cases evaluated	178	2	8	7	21	3	124	7	1	5

numerous cutaneous pox nodules on the beak, face, and carpus, and diphtheritic lesions in the oral cavity and pharynx. Additional poxvirus infections were interpreted as incidental findings in passerines (Table 3); these were cutaneous lesions with no evidence of impacts on ability to feed or body condition. Avian pox was also diagnosed by polymerase chain reaction, from multifocal foot lesions in an electively euthanized juvenile Audobon's Shearwater (*Puffinus herminieri*), using primers designed from highly conserved sequences of the intergenic region (CAX) between the genes CAX and thymidine kinase (TK) of the canary pox virus (forward primer: 5'-AGATATAGTA-GAATTTAGTG; reverse primer: 5'-TTCTGCAAGATTTAATATC) (Thiel et al., 2005).

Avian pox is a significant disease of wild birds (Kirmse, 1967), and evidence of avian poxvirus has been seen in endemic birds of Hawaii (Tripathy et al., 2000; Van Riper et al., 2002), the Canary Islands (Smits et al., 2005), and the Galápagos Islands (Vargas, 1987; Thiel et al., 2005). Severe lesions similar to those observed in the juvenile mockingbird in this study have been observed in juvenile mockingbirds on Santa Cruz (Vargas, 1987) and Isabela (unpubl. data). Kleindorfer and Dudaniec (2006) observed a 33% increase in suspected (visual diagnosis) pox-like lesions between 2000 and 2004 in Santa Cruz ground finches and higher apparent lesion prevalence in lowland, as compared to highland, habitats from Isabela, Floreana, and Santa Cruz Islands. The origin of poxvirus infection in Galápagos-endemic birds is unknown, but recent genetic studies suggest that it is most closely related to canarypox viruses (Thiel et al., 2005). *Philornis downsi* larval infestation (larvae identified and removed from subcutaneous and nasal tissues by B. Fessler and S. Kleindorfer) was present in six Galápagos finch (*Geospiza* sp.) chicks (Table 2), three of which had histologic evidence of septicemia (mixed gram-neg-

TABLE 3. Incidental findings in wild bird necropsy cases submitted to the Charles Darwin Research Station, Galápagos, Ecuador.

Diagnostic category	No. of cases (%, n=178)	Species (no. of cases)
Avian pox (nonfatal cutaneous lesions)	7 (3.9%)	Passeriformes: Yellow Warbler (4), Small Ground Finch (1) Vegetarian Finch (1) Procellariiformes: Audubon's Shearwater (1)
Endoparasites		
Renal trematodes ( <i>Renicola</i> spp.)	7 (3.9%)	Pelecaniformes: Brown Pelican (5) Blue-footed Booby (1) Cuculiformes: Dark-billed Cuckoo (1)
Intestinal trematodes	6 (3.4%)	Passeriformes: Galápagos Mockingbird (3) Ciconiiformes: Yellow-crowned Night Heron (1) Pelecaniformes: Brown Pelican (2)
Proventricular nematodes	17 (9.6%)	
<i>Contraecaecum</i> sp.	4 (2.2%)	Ciconiiformes: Yellow-crowned Night Heron (1), Cattle Egret (1) Pelecaniformes: Brown Pelican (1) Gruiformes: Paint-billed Crake (1)
<i>Dispharynx</i> spp. and unidentified	13 (7.3%)	Passeriformes: Yellow Warbler (9) Cuculiformes: Dark-billed Cuckoo (2), Smooth-billed Ani (2)
Enteric coccidia	16 (9.0%)	Passeriformes: Yellow Warbler (12), Galápagos Mockingbird (2), Small Ground Finch (1) Pelecaniformes: Brown Pelican (1)
Protozoal cysts, myocardium	1 (0.56%)	Passeriformes: Galápagos Mockingbird (1)
Ectoparasites		
Cutaneous lice-mites	11 (6.2%)	Passeriformes: Yellow Warbler (1), Vegetarian Finch (1) Cuculiformes: Smooth-billed Ani (4) Pelecaniformes: Blue-footed Booby (2), Brown Pelican (1) Strigiformes: Barn Owl (2)
Oral lice	3 (1.7%)	Pelecaniformes: Brown Pelican (3)
Subcutaneous/air sac mites	2 (1.1%)	Pelecaniformes: Brown Pelican (1) Passeriformes: Medium Ground Finch (1)
Non-specific inflammation		
Lymphoplasmacytic proventriculitis (no nematodes observed)	9 (5.1%)	Passeriformes: Yellow Warbler (5), Galápagos Mockingbird (2), Española Mockingbird (1) Ciconiiformes: Yellow-crowned Night Heron (1)
Cloacal bursitis	3 (1.7%)	Cuculiformes: Smooth-billed Ani (2) Pelecaniformes: Red-billed Tropicbird (1)
Bacterial airsacculitis, moderate	2 (1.1%)	Pelecaniformes: Blue-footed Booby (1) Columbiformes: Galápagos Dove (1)
Pneumonia	4 (2.2%)	Columbiformes: Galápagos Dove (1) Ciconiiformes: Great Blue Heron (1), Yellow-crowned Night Heron (1) Pelecaniformes: Red-billed Tropicbird (1)
Other lesions		
Hemosiderosis	30 (16.9%)	Passeriformes: Galápagos Mockingbird (9), Yellow Warbler (2), Medium Ground Finch (1), Española Mockingbird (1) Cuculiformes: Dark-billed Cuckoo (4), Smooth-billed Ani (2) Columbiformes: Rock Dove (3), Galápagos Dove (1) Ciconiiformes: Great Blue Heron (2), Yellow-crowned Night Heron (1) Pelecaniformes: Brown Pelican (3), Blue-footed Booby (1)

ative and gram-positive bacteria within vessels and parenchyma of numerous tissues) and severe cellulitis and myositis attributed as sequelae to *P. downsi* infestation. *Philornis downsi* larval dipterid infections, reported on many islands in the Galápagos, can cause severe anemia and death in Galápagos Finch nestlings (Dudaniec et al. 2006; Wiedenfeld et al., 2007). Second-stage larvae of *P. downsi* infect the nasal cavity and other subcutaneous tissues of chicks, and late second- and third-stage larvae live in nest material and are ectoparasitic obligate blood feeders on chicks (Arendt, 1985; Fessl and Tebbich, 2002; Fessl et al., 2006). In Puerto Rico, Pearly-eyed Thrashers (*Margarops fuscatus*) experienced 75% mortality attributed to *Philornis* spp. parasitism (Arendt, 1985). In previous studies, 97% of endemic finch nests were parasitized by *P. downsi*, 27% of the nests experienced brood loss due to *P. downsi* parasitism, and 19% of nests examined had complete brood loss (Fessl and Tebbich, 2002). Similar to Eastern Bluebird nestlings (Spalding et al. 2002), infection with *Philornis* sp. larvae in this study caused inflammation associated with subcutaneous parasitic tract lesions.

A Common Moorhen died with a multisystemic trematode infection, consistent with schistosomosis, which caused a severe heterophilic enterocolitis. It is important to further investigate avian schistosomal infections, and identify intermediate hosts, because schistosomal infections are a potentially significant cause of waterbird morbidity and mortality. Targeted disease surveillance is also warranted in Dark-billed Cuckoos (*Coccyzus melacoryphus*); 44% (4/9) of the undetermined deaths were cuckoos (Table 2–5 *Cuculiformes* undetermined deaths: four Dark-billed Cuckoos and one Smooth-billed Ani [*Crotophaga ani*]), and there are anecdotal reports of cuckoo population decline on San Cristobal Island. Although *Dispharynx spiralis* was proposed as a cause of Galápagos Cuckoo mortality

(Vargas and Bensted-Smith, 2000), there did not appear to be severe lesions associated with *Dispharynx*-like proventricular nematodes in cuckoos this study (Table 3).

Additional necropsy findings included endo- and ectoparasitism, liver hemosiderosis, and inflammatory lesions of various organs (Table 3). Although renal trematodes have been associated with nephrosis (Forrester and Spalding, 2003), they caused localized renal inflammation and were considered incidental in this study. Similarly, proventricular nematode infections, consistent with *Contracaecum* spp. in water birds, were associated with moderate lymphoplasmacytic proventriculitis. A mild to moderate lymphoplasmacytic proventriculitis was also present in Yellow Warblers, cuckoos, and anis that had proventricular nematodes (Table 3). Other endoparasites included enteric coccidia in Yellow Warblers and a Ground Finch and myocardial protozoal cysts in an Española Mockingbird; these were not associated with inflammation.

Hemosiderin deposition, present in the liver and spleen of 30% of birds examined (Table 3), was not associated with inflammation. In Galápagos Mockingbirds, Yellow Warbler (*Dendroica petechia*), and finches, hemosiderin deposition was primarily within the hepatocellular cytoplasm, whereas in anis, cuckoos, Rock Doves (*Columba livia*), and Galápagos Doves, hemosiderin was apparent in both hepatocytes and Kupffer cells. Hemosiderosis, relatively common in avian frugivores and insectivores, may be caused by dietary iron overload, repeated starvation and refeeding, oil exposure, increased iron body storage for physiologic reasons (egg production, migration), lead poisoning, and systemic bacterial infection (Ward et al., 1988, Munson and Lowenstine, 1999). Kupffer cell hemosiderin deposition can be associated with avian malaria, but there was no evidence of hemoparasites in submitted cases.

Although no large-scale die-offs were

detected between 2002 and 2004, routine necropsy examination is necessary to measure impacts of pathogens on Galápagos avifauna, to detect emerging infectious diseases, and to reveal anthropogenic causes of mortality such as pesticides. Obviously, trauma is over-represented in necropsy cases. Further active surveillance for sick and dead wild birds, and for potential disease vectors (mosquitoes, ticks) on human-inhabited and uninhabited islands of the Galápagos, is necessary in order to further identify infectious and noninfectious factors that may affect avifaunal population dynamics or threaten species survival. Formalin-fixed and frozen tissues are stored at the Galapagos Genetic Epidemiology and Pathology Laboratory for further diagnostic investigation or for comparison with future cases, if required. In conclusion, continued surveillance for sick or dead birds by scientific investigators, park employees, local residents, and tour guides is important to better characterize and evaluate disease of Galápagos avifauna.

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