

MORTALITY PATTERNS IN ENDANGERED HAWAIIAN GEESE (NENE; *BRANTA SANDVICENSIS*)

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ABSTRACT: Understanding causes of death can aid management and recovery of endangered bird populations. Toward those ends, we systematically examined 300 carcasses of endangered Hawaiian Geese (Nene; *Branta sandvicensis*) from Hawaii, Maui, Molokai, and Kauai between 1992 and 2013. The most common cause of death was emaciation, followed by trauma (vehicular strikes and predation), and infectious/inflammatory diseases of which toxoplasmosis (infection with *Toxoplasma gondii*) predominated. Toxicoses were less common and were dominated by lead poisoning or botulism. For captive birds, inflammatory conditions predominated, whereas emaciation, trauma, and inflammation were common in free-ranging birds. Mortality patterns were similar for males and females. Trauma predominated for adults, whereas emaciation was more common for goslings. Causes of death varied among islands, with trauma dominating on Molokai, emaciation and inflammation on Kauai, emaciation on Hawaii, and inflammation and trauma on Maui. Understanding habitat or genetic-related factors that predispose Nene (particularly goslings) to emaciation might reduce the impact of this finding. In addition, trauma and infection with *T. gondii* are human-related problems that may be attenuated if effectively managed (e.g., road signs, enforcement of speed limits, feral cat [*Felis catus*] control). Such management actions might serve to enhance recovery of this endangered species.

Key words: *Branta sandvicensis*, Hawaiian Goose, mortality, Nene, pathology.

INTRODUCTION

The Hawaiian Islands have the highest per capita number of endangered birds in the US (Dobson et al. 1997). Of these endangered birds, one of the most charismatic and visible is the Hawaiian Goose (*Branta sandvicensis*), known as the Nene. The Nene is the largest extant native terrestrial bird in the Hawaiian Islands and the official state bird. Before the 20th century, numbers of this bird were estimated statewide to be as high as 25,000 or as few as 250 (Paxinos et al. 2002), but those numbers declined to less than 50 individuals by the 1940s (Baldwin 1945). Hunting, habitat loss, and introduced predators have historically contributed to declines of Nene, particularly in coastal lowlands of the islands where habitat alterations have been most pronounced (US Fish and Wildlife Service 2004). In the early 1950s, captive breeding efforts were started in attempts to recover the species and these attempts ended in 2011. Numbers currently fluctuate at approxi-

mately 2,000 birds statewide. Recently, populations have increased somewhat, but the current stocks are relegated to the islands of Hawaii, Kauai, Maui, and Molokai. Although genetic diversity of Nene is lowest on Kauai (Rave 1995), populations on that island are thriving, probably because of the absence of mongoose (*Herpestes auro-punctatus*), an introduced predator, and because of the greater availability of lowland habitat where Nene reproductive success is greater (US Fish and Wildlife Service 2004).

Nene are generalist herbivores feeding on a variety of native and introduced vegetation. Nesting occurs on the ground in protected brush in lowlands and highlands. Highland nesting is largely because human intervention results in lowland habitat loss and Nene introduction occurs predominantly in upland habitats (US Fish and Wildlife Service 2004). During summer, grass seed and herbs dominate the diet; grass, leaves, and berries are favored in winter (Black et al. 1994). Nene will

range across and rarely among islands. For example, on the island of Hawaii, Nene spend the nonbreeding season (May–August) in high-elevation shrubs and the breeding and molting seasons in mid-elevation areas (Hess et al. 2012). Although hunting was partly responsible for historical declines of Nene, current threats include habitat loss, introduced predators, poor nutrition, human interactions, inbreeding, and possibly disease (US Fish and Wildlife Service 2004).

A goal of the US Fish and Wildlife Recovery plan (US Fish and Wildlife Service 2004) is to increase the Nene population statewide to self-sustaining populations on Hawaii, Maui, Lanai, Molokai, Kahoolawe, and Kauai. This goal is being instigated by a combination of habitat management, captive propagation (ceased in 2011), and establishment of new populations (US Fish and Wildlife Service 2004). Captive propagation and establishment of new populations have the potential to introduce or exacerbate disease (Cunningham 1996), so it is important to understand potential causes of death in recovering stocks and identify mortality factors that could be exacerbated by recovery actions. The critically endangered Laysan duck (*Anas laysanensis*) is an example for which wildlife health surveys contributed to endangered species recovery. This duck was successfully reintroduced to the island of Midway from the island of Laysan (Reynolds and Klavitter 2006). However, the helminth parasite *Echinuria uncinata* was partly responsible for catastrophic mortalities of Laysan ducks on Laysan Island, their native range (Work et al. 2004). To avoid translocating the parasite to Midway, ducks selected for translocation were treated with anthelmintics (Work et al. 2010).

Understanding causes of death in endangered birds in tropical ecosystems can be challenging because these animals are inherently uncommon in the wild; samples sizes are small; and decomposition is rapid, making it difficult to retrieve specimens suitable for laboratory diagnostics.

Thus, detecting mortality patterns in rare animals requires examination of specimens in the longer term. Here, we present necropsy findings of endangered wild and captive Nene collected over 22 yr.

MATERIALS AND METHODS

Nene carcasses were submitted from throughout the Hawaiian Islands to the US Geological Survey National Wildlife Health Center Honolulu Field Station (Honolulu, Hawaii, USA) either chilled or frozen. Post-mortem condition was subjectively judged as excellent, good, fair, poor, or decomposed, based on general appearance, odor, presence of maggots, and integrity of feathers and skin. Birds were weighed to the nearest gram and underwent a systematic external and internal exam. Age was determined based on banding history, plumage (Hunter 1995), or evidence of prominent bursa, and sex was determined based on visualization of gonads. As appropriate, and depending on the stage of decomposition of the carcass, representative tissues were fixed in 10% neutral-buffered formalin, trimmed, embedded in paraffin, sectioned at 5 μm , and stained with hematoxylin and eosin for microscopic examination. Grocott's methenamine silver or Gram stains were used to visualize fungal hyphae or bacteria, respectively (Prophet et al. 1992).

Depending on gross and microscopic findings, additional laboratory tests were done to confirm particular suspected etiologies. For suspected bacterial infections (based on gross evidence of inflammation or histologic presence of bacteria associated with tissue necrosis), organs or heart blood was plated on blood and MacConkey's agars and incubated at 37 C for 2–3 d; bacteria were identified using standard metabolic profiles (Klingler et al. 1992). Helminths were identified based on morphologic characteristics (McDonald 1969). For birds dying in good body condition with no evident gross lesions, we confirmed botulism type C by assaying heart blood using the mouse cross-protection test (Hatheway 1979); for those birds with a history suggestive of exposure, cholinesterase inhibition assays were used as evidence of organophosphate or carbamate exposure by comparison with normal values for wild fowl (Hill 1988, 1992; Franson and Smith 1999). Cholinesterase inhibition in the absence of reactivation was considered evidence for organophosphate exposure, but return of cholinesterase activity to normal levels after overnight incubation of the sample was consistent with carbamate

exposure (Franson and Smith 1999). For birds suspected of lead poisoning (emaciation, fecal pasting, with or without esophagus or proventriculus dilated with ingesta suggestive of paralysis), we tested livers for lead by using atomic absorption spectrometry (Franson and Smith 1999). Elevated (2–6 µg/g wet weight) or toxic (>10 µg/g wet weight) liver lead levels were defined as described by Franson and Pain (2011). In cases suggestive of virus infection (histologic evidence of nonsuppurative inflammation and necrosis with or without syncytia, or gross evidence of multifocal hemorrhage), virus isolation was attempted by inoculating tissue homogenates into embryonated chicken eggs (Senne 2008) or duck embryo fibroblasts.

Birds with histologic evidence of necrosis associated with tachyzoites or protozoal cysts, were tested for *Toxoplasma gondii* by immunohistochemistry (IHC) as described by Work et al. (2002), with minor modifications. In brief, embedded tissues were deparaffinized in xylene and rehydrated in ethanol series. Antigens were unmasked using EnVision FLEX target retrieval solution, pH 6 (Dako, Carpinteria, California, USA) at 97 C for 30 min. Slides were cooled for 20 min, rinsed in EnVision FLEX wash buffer (Dako), blocked for 5 min with Envision FLEX block solution (Dako), washed briefly with buffer, and then blocked with serum-free protein block (Dako) for 5 min. Sections were incubated 30 min with polyclonal rabbit anti-*T. gondii* tachyzoite antibody (PA1-38789, Thermo, Rockford, Illinois, USA) at 1:50 in Dako antibody diluent. Sections were washed, incubated for 30 min with undiluted ImmPRESS HRP Universal Antibody (MP-7500, Vector Laboratories, Inc., Burlingame, California, USA), washed, and visualized with diaminobenzidine (Dako) for 5 min. Sections were then counterstained with Hematoxylin QS solution (H-3404, Vector) for 30 s, dehydrated with ethanol and xylene, and coverslipped with Cytoseal 60 (Richard-Allan Scientific, Kalamazoo, Michigan, USA). Positive controls for *T. gondii* IHC included Nene lungs previously confirmed to be *T. gondii* positive (Work et al. 2002), and negative controls were lungs from Nene that died from trauma.

Causes of death were classified into six categories, including emaciation, trauma, inflammation, toxic, miscellaneous, or unknown, based on the most severe gross and microscopic lesions encountered. Trauma included cases with evidence of internal hemorrhage, broken bones, bruising, and no gross or microscopic evidence of other significant disease processes. Causes of trauma were

determined based on the history that accompanied the specimen. Emaciation included cases with gross evidence of atrophy of breast muscles and internal organs or with microscopic evidence of liver, heart, or kidney atrophy, and no evidence of trauma or other significant disease processes. Inflammation included cases with evidence of various types of suppurative or nonsuppurative cellular infiltrates or necrosis with or without infectious agents. Toxicoses included cases with no evidence of inflammatory or traumatic changes with characteristic gross lesions of lead poisoning (Franson and Pain 2011) accompanied by toxic liver lead concentrations, depressed brain cholinesterase with or without reactivation, or heart blood positive for botulism type C. Miscellaneous included all other known causes of death. Unknown included all other cases where lesions explaining cause of death were not evident. For analyses, birds were classified as captive birds (>1 wk in captivity before death) or free-ranging birds. Age classes included goslings, immatures, or adults (Hunter 1995).

The number of nonindependent variables (age, sex, captive status, diagnostic category, and island of collection) examined yielded 288 possible combinations (3×2×2×6×4). The low sample size relative to potential explanatory variables precluded meaningful statistical analysis. Accordingly, data are summarized as percentages and examined over time and by island.

RESULTS

Of 300 birds examined, the most common cause of death was emaciation (24%), followed by trauma and inflammation (23% each), toxicosis (4%), and miscellaneous (2%), with the remainder unknown. Goslings dominated (45%), followed by adults (38%), immatures (15%), and unknowns (Table 1). Eighty percent of birds examined were free-ranging birds, and females slightly outnumbered males. Most birds originated from the island of Maui (38%), followed by Kauai (30%), Hawaii (22%), and Molokai (10%). There was a marked increase in numbers of birds submitted after 2005 (Fig. 1).

Birds dying from emaciation presented with varying degrees of pectoral muscle atrophy and loss of body fat. Most (96%) cases of emaciation were uncomplicated;

TABLE 1. Number of Hawaiian Geese or Nene (*Branta sandvicensis*) per diagnostic category partitioned by age, sex, or island for 300 birds necropsied between 1992 and 2013.

	Emaciation	Trauma	Inflammation	Toxicosis	Miscellaneous	Unknown	Total
Age							
Gosling	48	16	31			39	134
Adult	13	43	27	9	4	17	113
Immature	9	11	11	2	1	11	45
Unknown	1					7	8
Captivity status							
Free ranging	59	62	41	11	2	64	239
Captive	12	8	28		3	10	61
Sex							
Female	23	29	31	5	3	24	115
Male	16	31	26	6	2	15	96
Unknown	32	10	12			35	89
Island							
Maui	13	35	34	2	2	28	114
Kauai	21	13	20	9	2	24	89
Hawaii	32	12	9		1	14	68
Molokai	5	10	6			8	29
Total diagnoses	71	70	69	11	5	74	300

however, two birds had attendant mild infection with intestinal coccidia, and another bird had mild inflammation of the liver (see Supplementary Material Table S1). Emaciation dominated in goslings, free-ranging birds, females, and birds originating from Hawaii (Table 1).

Vehicular impact and predation were the most common causes of trauma. A variety of miscellaneous trauma findings included pulmonary hemorrhage, aspiration, blunt trauma, crushing, foreign body ingestion, electrocution, oviduct rupture, entrapment, gunshot, perforation, yolk sac rupture, forced submergence, intraspecific aggression, and unspecified. Rare cases of trauma were complicated by avian pox, emaciation, or botulism type C (see Supplementary Material Table S1). Trauma dominated in adults, free-ranging birds, males, and birds originating from Maui (Table 1).

The leading cause of inflammatory conditions was toxoplasmosis followed by omphalitis (see Supplementary Material Table S1). Of 129 different isolates of bacteria comprising 41 different species of bacteria, *Escherichia coli* dominated (29%), followed by *Enterococci* and *Pseu-*

domonas spp. (9% each), with 38 species comprising the remainder. Of eight fungal isolates, *Aspergillus fumigatus* comprised the majority (87%), with one case of *Candida albicans*. No viruses were isolated from any specimens. Of 33 cases where organisms were associated with lesions, protozoa dominated (39%), followed by fungi (27%), bacteria (24%), and helminths (10%). Inflammatory conditions usually affected multiple organs (42%) (see Supplementary Material Table S1) and were more common in adults and goslings, free-ranging birds, and females, and most often seen in birds from Maui (Table 1).

The majority of toxicoses were lead poisoning (45%), followed by botulism (36%) and suspect organophosphate poisoning (19%). Birds with lead poisoning manifested varying combinations of emaciation, fecal pasting, and accumulations of forage in the esophagus and proventriculus. Liver lead concentrations in birds with lead poisoning were in the range 13.4–20.6 μg lead/g wet weight. Remnants of lead were not observed grossly within the gizzard, nor were characteristic intranuclear inclusions (Locke et al. 1967) seen in the kidneys. Birds with suspect organophosphate

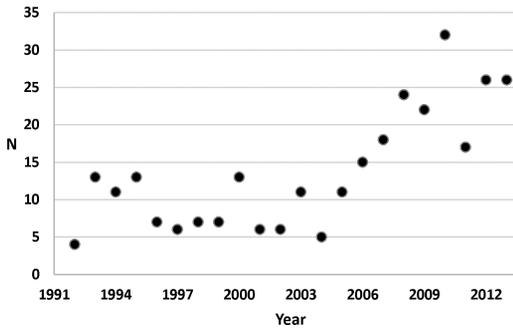


FIGURE 1. Number of Hawaiian Geese or Nene (*Branta sandvicensis*) carcasses (*N*) submitted to the US Geological Survey, National Wildlife Health Center, Honolulu Field Station (Honolulu, Hawaii, USA) for necropsy, by year, 1991–2012.

poisoning had no gross evidence of pesticide-laden bait within the gastrointestinal tract. Toxicoses were exclusively seen in free-ranging adult birds, about equally represented among males and females, and dominated in birds from Kauai (Table 1).

Miscellaneous conditions included two egg-bound adults and neoplasias in the heart, liver, or skeletal muscle of adult birds. Because of the rarity of neoplastic lesions, efforts were not made to confirm their specific identity. Unknown comprised 25% of birds, with most (80%) of the birds in this category graded as in fair or poor postmortem condition. The remainder had no pathologic or laboratory findings that could explain cause of death.

DISCUSSION

Data on health of Nene are limited to isolated case reports or parasite surveys. The only existing mortality surveys of Nene in Hawaii have focused on grossly evident causes such as trauma (Hoshide et al. 1990; Bergman et al. 2009). In a comprehensive summary of parasites and diseases in Hawaiian birds (van Riper and van Riper 1984), Nene were documented having cecal worms, aspergillosis, unidentified coccidia, and gizzard worms (*Amidostomum* sp.). A parasite survey in

Nene from Maui (Bailey and Black 1995) documented eggs of *Syngamus* sp., *Ascaridia* sp., *Heterakis* sp., *Amidostomum anseris*, *Trichostrongylus tenuis*, *Echinuria uncinata*, and *Capillaria* sp. shed in 22% or 28% of captive or wild birds, respectively. Avian pox virus was isolated from one captive Nene from the island of Hawaii (Kim and Tripathy 2006).

This study expands upon earlier reports by focusing on mortality factors by using gross and microscopic pathology along with confirmatory laboratory tests. However, our study reveals that, broadly, the major causes of death for Nene include emaciation, trauma, and inflammatory conditions. Emaciation was more common in free-ranging birds and goslings, and it was notably prevalent on the island of Hawaii, and to a lesser extent, Kauai. The high prevalence of emaciation in birds from Hawaii seems to mirror a trend that hatching and fledgling success for Nene on Hawaii are relatively low, 35% and 30%, respectively, and starvation in goslings is common (Rave et al. 2005). In contrast, emaciation was relatively uncommon on Maui, perhaps because habitats there are more conducive to raising goslings. Nene can use exotic grass and human-modified landscapes or native shrublands during the breeding and molting seasons, and proximity to water is important during molt (Leopold and Hess 2013). Perhaps access to these types of forages is not adequate to support Nene in some areas of the island of Hawaii. Genetic inbreeding could be another reason why Nene fail to thrive; this condition has been documented in Nene from the island of Hawaii (Rave et al. 1998). More data on foraging behavior of goslings, habitat suitability, and effects of parental care of gosling survival might shed further light on why this age class is prone to starvation.

Trauma, the second most common finding, affected mostly adult or free-ranging Nene, comprised mainly vehicular impact and predation, and it was most

common on Maui. These findings confirm historical patterns observed for causes of death in Nene. For example, vehicle collision was the most common cause of death in adult Nene on Hawaii (Rave et al. 2005), dog-induced trauma was documented by US Department of Agriculture Wildlife Services in Hawaii (Bergman et al. 2009), and dog predation was considered historically a major cause of death in Nene from Kauai (US Fish and Wildlife Service 2004). As Nene populations continue recovering, and possibly migrating to lowland habitats, it is likely that trauma associated with human presence (vehicles, dogs) will continue to play a major role in mortality of this species. Devising ways of having Nene coexist with humans while minimizing traumatic episodes to sustain recovery of the species would be a worthy goal of future recovery efforts.

Inflammatory diseases were almost equal in frequency to trauma as a finding for Nene in the Hawaiian Islands and were dominated by *T. gondii*, which comprised 16% of inflammatory diagnoses. This parasite was documented in Nene by Work et al. (2002) and is a major cause of death in Hawaiian crows (*Corvus hawaiiensis*; Work et al. 2000). Of the 11 toxoplasmosis cases, most were from free-ranging birds, with cases originating from Kauai, and fewer from Maui and Hawaii. Nene on Kauai mostly frequent lowlands, whereas Hawaiian crows frequent midelevation forests. Thus, feral cats, the definitive host of *T. gondii*, pose a disease threat to two species of native Hawaiian birds with vastly different life histories and habitats. The parasite is endemic in feral cats on the island of Hawaii where approximately 30% of animals are antibody positive (Danner et al. 2007); so, this parasite will probably continue playing a role in mortality of Nene. Bacterial diseases (mainly from enteric bacteria) played a secondary role and affected mainly immature Nene in captivity. Avian malaria accounted for only one death in a captive Nene, whereas avian pox was

seen in an emaciated bird but was judged to be a secondary finding. In contrast, these two pathogens are major causes of morbidity and mortality in native Hawaiian honeycreepers (Atkinson et al. 2005; Atkinson and Samuel 2010). The rarity of malaria in Nene also fits with findings of Bailey and Black (1995) who were unable to detect blood parasites in Nene.

Of the toxicoses, lead poisoning was the most common, and the source of lead was undetermined. Because waterfowl hunting is not allowed in Hawaii, lead shot ingestion (Kendall et al. 1996) from wetlands is unlikely. In contrast, Hawaii allows hunting of upland game birds, so Nene could ingest spent shot from those areas. Ingestion of fishing sinkers is another possible source of lead in birds (Pokras and Chafel 1992); however, unlike saltwater, fresh water fishing opportunities in Hawaii are also extremely limited, so fishing sinkers are also an improbable source. The Hawaiian Islands have a high concentration of existing and defunct military installations, so ingestion of bullets or lead paint (Sileo and Fefer 1987) could be a source of lead. More focused studies are needed to sort out sources of lead exposure in Nene. Unlike other waterfowl in the Hawaiian Islands, such as critically endangered Laysan ducks on Midway (Work et al. 2010), botulism poses a minor threat to Nene, perhaps because they tend to forage on grasses rather than aquatic invertebrates.

We report the first systematic investigation of causes of death in Nene. Passive surveillance strategies such as those used here have limitations, particularly when interpreting temporal trends when efforts to detect specimens are poorly documented or diagnostic effort is inconsistent (Russel and Franson 2014). In our case, animals were consistently examined by the same group by using similar techniques throughout the study, which should decrease variability. Results showed that Nene continue to be challenged by limitations in fledgling success, principally due to emaciation, and that human-in-

duced sources of trauma are significant for wild Nene. However, infectious disease and other inflammatory conditions are also important contributors to mortality. These findings should serve as a valuable baseline to guide recovery. Specifically, although it might be difficult to reduce impacts of emaciation, the relative impact of human-induced trauma and toxoplasmosis could be decreased with management. Doing so might enhance recovery of Nene in their native range.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at <http://doi:10.7589/2014-11-256>.

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