A Health Survey for Hawaiian Duck (*Anas wyvilliana*) at Hanalei National Wildlife Refuge, Kaua‘i

Final Report
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INTRODUCTION

The Hawaiian duck or Koloa maoli (*Anas wyvilliana*; hereafter Koloa) is endemic to Hawaii and is one of three extant waterfowl species occurring on the Hawaiian Islands (Olson and James 1982, Engilis et al. 2002). Relatively common during the early 1900s, the Koloa has since experienced a significant statewide population decline because of factors including wetland loss, over harvest by hunters, and introduced predators. More recently, the genetic integrity of the species has been threatened by hybridization with feral mallards (*Anas platyrhynchos*; Engilis et al. 2002). Consequently, Koloa was listed as a federally endangered species in 1967 and has the highest recovery priority for the four wetland birds that are listed and occur on the main Hawaiian Islands (USFWS 2011). Kauaʻi is the only island that supports a viable population of pure Koloa and consequently Hanalei and Huleia National Wildlife Refuges (NWRs) are the most important sites for this species in Hawaii.

The range of Koloa overlaps with humans and domestic birds (e.g. feral chickens, Muscovy duck [*Cairina moschata domestica*]). They are also exposed to migratory birds including waterfowl from both Asia and North America. These associations and contacts allow for free movement of pathogens between groups. This risk is magnified by the concentration of animals on small warm water wetland areas with ideal conditions for the survival and even multiplication of certain pathogens (Wobeser 1997). Additionally, the domestic mallard not only represents a threat from hybridization (Engilis 2002), but is also a potential reservoir for several pathogens of ducks and related waterfowl (Wobeser 1997). As well as being a public and animal health concern, some diseases (e.g., Avian Influenza, Newcastle Disease) could have significant economic impact for the state of

Hawaii by interfering with the free movement of people and animals between islands and the mainland.

Our study examined a sample of Koloa maoli living in and around Hanalei NWRs. In this study, we assessed the physical condition of the animals and collected samples to screen for known important pathogens (viruses, bacteria, parasites) and environmental contaminants. Our work addressed the stated objectives of the Avian Health and Disease Program by conducting surveillance of Koloa maoli on Kauaʻi to establish health baselines and identify emerging avian health and disease risks. This information will help develop and guide effective management actions if indicated. Related to the specific Region 1 RFP, our work provides support to management of waterbirds on Kauaʻi related to health and disease impact investigations (item 1) and collaborations (item 7). This study addressed four of the seven topics identified within the Avian Health and Disease National Strategic Plan including: collection of baseline data on avian species to assess health and disease concerns; evaluation of critical avian populations (endangered species); investigation of infectious diseases (viruses, bacteria, fungal infections), and investigation of non-infectious diseases (parasites, biotoxins, contaminants). While our survey focused on Koloa maoli, the results provide insight into parasite, disease, and contaminant issues related to the endangered Hawaiian goose (*Branta sandvicensis*), and three other species of endangered waterbird (Hawaiian coot [*Fulica alai*], Hawaiian common gallinule [*Gallinula galeata sandvicensis*], and Hawaiian stilt [*Himantopus mexicanus knudseni*]) that occur with Koloa maoli at Hanalei and Hule'ia NWR.

Objectives

1. Examine the physical condition of a sample of Koloa maoli living in and around Hanalei and Huleʻia NWRs.
2. Collect samples to screen for known important pathogens (viruses, bacteria, parasites) and environmental contaminants.

METHODS & MATERIALS

Study Area

Trapping and tagging of Koloa occurred at Hanalei NWR on the north shore of Kauaʻi County, Hawaii (21° 12.052' N, 159° 28.352' W; Fig. 1). Elevation on the island ranges from sea level to 1598 m on Mount Waiʻaleʻale, and principle wetland habitat types on the island include riparian wetlands, montane bogs, low elevation seasonal wetlands, and agricultural wetlands (Schwartz and Schwartz 1953, Swedberg 1967, Ramage and Schroeder 1999). Rainfall on Kauaʻi varies significantly with altitude and latitude, and between Hanalei NWR and Mt. Waiʻaleʻale (1,010 cm/yr), precipitation increases by over 50 cm/km (NCDC 2012). The Hanalei River headwaters form on Mount Waiʻaleʻale and flow 25.2 km north to Hanalei Bay. The lower 5.6 km section of the Hanalei River flows through the 372 ha Hanalei NWR where water is diverted to taro (kalo, *Colocasia esculenta*; 57.0 ha) loʻi and managed wetlands (40 ha). The remainder of the refuge consists of lowland forest and shrubland (130 ha) and riparian habitat (148 ha). Precipitation at Hanalei NWR varies between a relatively dry season (10.9 – 16.4 cm/mo from May to October) and a wet season (17.3 – 23.1 cm/mo from November to April); mean annual rainfall at Hanalei NWR is 208.8 cm/yr (NCDC 2012). Hanalei NWR and the surrounding taro agriculture of the north shore of Kauaʻi is believed to be the most important region for Koloa on the Hawaiian islands (Banko 1987, USFWS 2011).

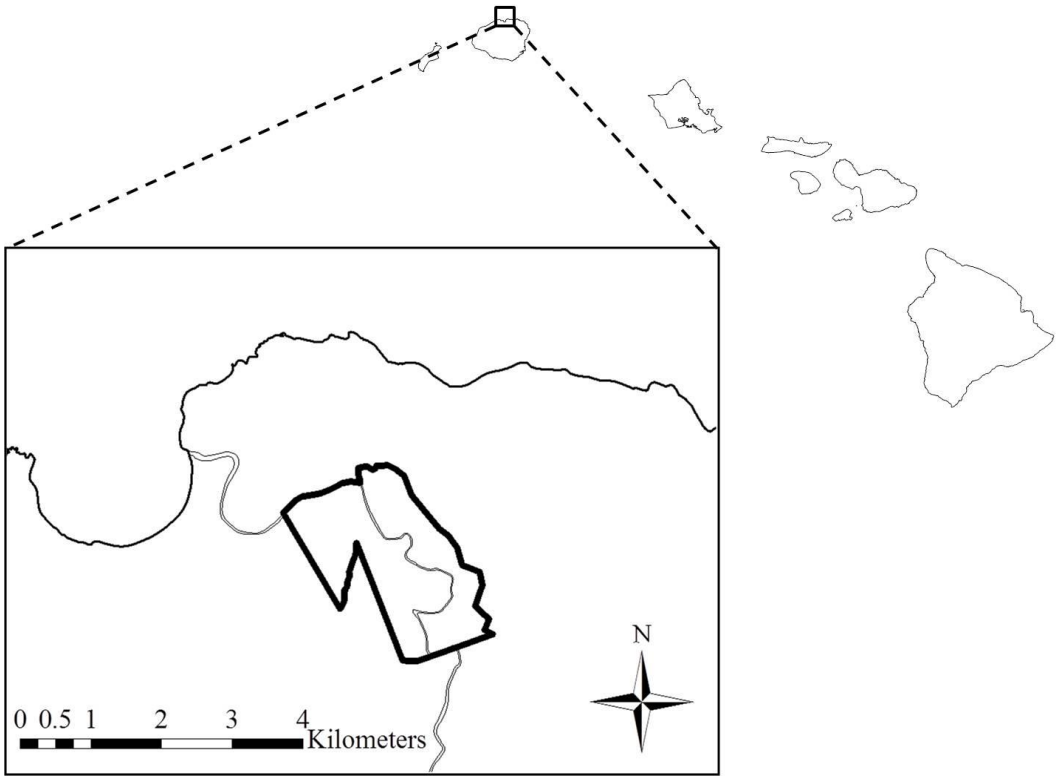

Figure 1. Map of the main Hawaiian Islands showing the location of Hanalei National Wildlife Refuge (refuge boundary outlined in bold above) on the north shore of Kaua‘i (source: Malachowski 2012).

Bird Capture

We captured Koloa using baited swim-in traps (Hunt and Dahlka 1953) that were customized to minimize chance of harm to Koloa and non-target species (Malachowski 2013). Birds were carefully removed from traps by hand or using a long-handled dip net. The net mesh size was approximately 1.3 cm to prevent entanglement of legs, bills, and wings of ducks in the netting. All non-target birds were immediately released. All captured Koloa were placed in poultry crates covered with thin, dark cloth to provide for security and a visual barrier to reduce stress. Crates were placed in a sheltered location to protect birds from the sun or rain as they waited to be processed.

Examination and Sample Collection

We determined sex and age (duckling, juvenile, adult) of each captured bird using plumage and bill characteristics (Engilis et al., unpublished data) and cloaca examination. Since birds in formative and first alternate plumage (i.e., first year birds) were not always discernible from birds in definitive basic and alternate plumage, they were grouped with adults. We measured body mass (nearest 5g), culmen length (mm), culmen width (mm), and wing arc (mm). We removed wing arc measurements from the sample for birds with damaged or heavily worn primaries. We calculated body condition indices, an indirect measure of physiological condition, as body mass divided by culmen length (Harder and Kirkpatrick 1996). Also, we scored the condition of the pectoral muscle as 0 (strongly emaciated; muscle is markedly concave), 1 (emaciated; muscle is mild to moderately concave), 2 (moderate condition; muscle is flat or mildly convex), or 3 (good condition; muscle is convex; Phillips et al. 2010). Some birds were re-captured and re-measured

multiple times during trapping seasons; however, we used only one record per bird to calculate morphometric summary statistics. Also, ducklings were removed from the sample.

We performed additional health assessments on a sample of 60 Koloa (n_{male} = 59, n_{female} = 1). We examined birds for clinical signs of disease. In addition, for each of these birds, we collected 3 mls of blood from the right jugular vein using a heparinized three ml syringe attached to a 25-gauge needle. After collection, two drops of blood were placed in a sterile vial for PCR analysis for hemoparasites. Blood smears were also made on two slides, dried and stained with Wrights-Giemsa to evaluate for the presence of hemoparasites (i.e. *Hemoproteus, Leukocytozoan, Plasmodium spp*.) to compare with the information obtained from PCR analysis. One ml of blood was placed in a two ml cryotube for later mineral (elemental) analysis. In addition to whole blood, feathers were collected from the flanks, placed in plastic bags, and submitted to a commercial facility (Utah State Veterinary Diagnostic Lab, Logan, Utah, 84341) to determine the concentration of the following elements: silver (Ag), aluminum (Al), arsenic (As), boron (B), barium (Ba), beryllium (Be), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), antimony (Sb), selenium (Se), silicon (Si), tin (Sn), strontium (Sr), thallium (Ti), vanadium (V), and zinc (Zn). The remaining two mls of blood was placed in a two ml serum separator tube, centrifuged at 3,000 rpm for five mins, the plasma decanted into a two ml cryotube and frozen. The plasma samples were then submitted to the National Wildlife Health Center (Madison, Wisconsin) for antibody titer testing for avian

influenza and paramyxoviruses (Newcastle Disease). A sterile culturette was inserted into the cloaca and then submitted for culture for *Salmonella* and *Campylobacter spp*. at a commercial laboratory (Veterinary Diagnostic Lab, Oregon State University Veterinary School) using standard techniques. Two swabs from the oral cavity and cloaca were collected and stored in sterile cryotubes for later analysis at a commercial molecular diagnostic facility (the Zoological Molecular Laboratory, University of Florida) for the presence of herpes, adeno, and paramyxoviruses.

RESULTS

Between November 23 and December 14, 2012 and November 29 and December 19, 2013, we captured 377 individual Hawaiian ducks, including 307 males (81.4%) and 70 females (18.6%). We also recorded 358 recaptures. Males were larger and had greater body condition scores than females of similar age classes (Table 1). Also, first year and adult birds were larger and had better body condition than juveniles for each sex.

Three (5%; $n = 60$) of the cloacal swabs submitted for bacterial culture were positive for *Campylobacter spp*., including one identified as *C. jejuni*. None of the samples ($n = 60$) were positive for Salmonella.

The blood PCR analysis revealed eight samples (14%; $n = 59$) positive for Plasmodium and related hemoparasites. Review of the blood smears revealed no hemoparasites. The PCR analysis of the oral swabs revealed the presence of a novel herpes in four ducks (7%; $n = 58$). Three distinct adenoviruses (5%; $n = 60$) were identified from the cloacal swabs. No paramyxoviruses were detected with PCR in either the oral or cloacal swabs. There was no serological evidence of exposure to avian

influenza viruses, but 9% of ducks showed potential exposure to a paramyxovirus similar to Newcastle Disease Virus.

All elements except silver (in feathers) were detected in the blood and feathers of one or more birds at concentrations greater than the minimum measurable limits (Table 2).

Table 1. Mean morphometric measurements and body condition scores for Hawaiian Duck (n = 376) captured at Hanalei National Wildlife Refuge, Kaua‘i in November – December 2012 and 2013. Values are given as mean ± SE along with the sample size.

	Male		Female	
Measurement	Adult and 1st year	Juvenile	Adult and 1st year	Juvenile
Culmen length (mm)	47.26 ± 0.13 (n = 280)	47.84 ± 0.49 (n = 25)	44.16 ± 0.27 (n = 59)	44.62 ± 1.00 (n = 11)
Culmen width (mm)	18.83 ± 0.04 (n = 278)	18.44 ± 0.14 (n = 25)	17.87 ± 0.08 (n = 59)	17.66 ± 0.19 (n = 11)
Wing arc (mm)	240.33 ± 0.45 (n = 275)	237.42 ± 1.49 (n = 24)	227.83 ± 0.92 (n = 58)	226.23 ± 2.24 (n = 11)
Mass (g)	722.82 ± 3.76 (n = 279)	652.24 ± 9.28 (n = 25)	657.53 ± 7.63 (n = 59)	571.18 ± 11.45 (n = 11)
Body condition index	15.31 ± 0.07 (n = 278)	13.65 ± 0.18 (n = 25)	14.9 ± 0.17 (n = 59)	12.86 ± 0.37 (n = 11)
Condition of pectoral muscle	2.01 ± 0.03 (n = 278)	1.50 ± 0.08 (n = 25)	1.8 ± 0.05 (n = 59)	1.59 ± 0.15 (n = 11)

Table 2. Feather and whole blood micro- and macro-element concentrations (ppm) for Hawaiian Ducks (n = 60) captured at Hanalei National Wildlife Refuge, Kauaʻi in December 2012. Values are given as mean ± SD (range).

Element	Feathers	Blood
Ag	--	0.0003 ± 0.0008 (0-0.004)
Al	69.3 ± 56.5 (20-386.8)	0.126 ± 0.061 (0.056-0.438)
As	0.180 ± 0.065 (0.059-0.355)	0.022 ± 0.015 (0-0.062)
B	0.448 ± 0.348 (0.073-1.724)	0.066 ± 0.072 (0.015-0.492)
Ba	0.714 ± 0.489 (0.231-3.648)	0.008 ± 0.007 (0.002-0.035)
Be	0.009 ± 0.012 (0-0.052)	0.004 ± 0.008 (0-0.04)
Ca	409 ± 80 (270-608)	61 ± 6 (50.7-79)
Cd	0.019 ± 0.016 (0.002-0.073)	0.004 ± 0.007 (0-0.039)
Co	0.666 ± 0.428 (0.113-1.895)	0.019 ± 0.01 (0.006-0.053)
Cr	1.12 ± 0.62 (0.45-4.64)	0.575 ± 0.149 (0.393-0.872)
Cu	13.1 ± 1.7 (9.5-16.5)	0.322 ± 0.107 (0.205-0.963)
Fe	294 ± 190 (73-881)	489 ± 46 (395-650)
Hg	0.405 ± 0.275 (0.120-1.410)	0.025 ± 0.019 (0.007-0.09)
K	176 ± 131 (52-696)	2057 ± 183 (1678-2507)
Li	0.025 ± 0.016 (0.004-0.068)	0.011 ± 0.011 (0-0.04)
Mg	156 ± 33 (101-233)	73.0 ± 12.8 (30.3-98)
Mn	13.8 ± 8.3 (2.2-36.1)	0.026 ± 0.009 (0.014-0.052)
Mo	0.126 ± 0.128 (0.032-0.658)	0.029 ± 0.018 (0.011-0.096)
Na	499 ± 236 (192-1672)	1717 ± 179 (1324-2155)
Ni	2.87 ± 2.31 (0.46-9.52)	0.011 ± 0.008 (0.002-0.045)
P	159 ± 93 (77-492)	1485 ± 110 (1212-1696)
Pb	0.346 ± 1.139 (0.034-8.751)	0.038 ± 0.059 (0.002-0.309)
Sb	0.021 ± 0.014 (0.004-0.069)	0.004 ± 0.008 (0-0.039)
Se	1.33 ± 0.59 (0.53-3.38)	0.930 ± 0.485 (0.390-3.123)
Si	124 ± 33 (79-230)	26.9 ± 2.9 (21.9-35.9)
Sn	0.044 ± 0.045 (0.001-0.182)	0.008 ± 0.012 (0-0.052)
Sr	1.44 ± 0.56 (0.51-2.81)	0.033 ± 0.013 (0.016-0.079)
Tl	0.008 ± 0.012 (0-0.048)	0.003 ± 0.006 (0-0.029)
V	0.594 ± 0.419 (0.142-2.559)	0.03 ± 0.012 (0.014-0.062)
Zn	128 ± 14 (100-161)	4.72 ± 0.47 (3.87-6.13)

DISCUSSION

The major health concern for Koloa in the Hanalei Wildlife Refuge is botulism. Since this is a well-recognized issue, the present study focused on other potential disease problems for both waterfowl and humans living in and around the refuge. Cloacal cultures for potential bacterial pathogens revealed a low prevalence of *Campylobacter* spp. (5%) and no *Salmonella* isolates. It is possible the delay between collection and culture due to overnight shipping may have affected the sensitivity and the ability to detect these organisms. This apparent low sensitivity would have been added to if these organisms were present in low numbers. The *Campylobacter* spp. (including one isolate of *C. jejuni*) are a potential threat to both animal and human health (Thomas et al. 2007) and warrant further investigation. The use of molecular testing techniques (quantitative PCR) could be a more sensitive way to assess for the presence of this and other bacteria in Koloa, as well as other waterfowl (i.e. Hawaiian goose). Chickens are commonly identified carriers of *Campylobacter* and other potentially pathogenic bacteria. Screening of the feral chickens present on refuge for potential pathogens may help determine their potential for harboring these organisms. The presence of *Pasteurella multocida* was also not assessed in this study and is a major known pathogen of waterfowl.

The PCR analysis of the oral and cloacal swabs revealed the presence of novel herpes and adenoviruses in four (7 %) and three (5 %) ducks, respectively. The significance of these viruses in these animals is unknown. Serological analysis of the plasma samples showed high percentages of the birds had been exposed to avian influenza and Newcastle like paramyxoviruses.

As indicated previously, botulism is the major disease issue identified on the refuge, with several recent outbreaks associated with Koloa deaths. This survey for potential pathogens revealed no other major concerns in the population of ducks examined. However, we did not search for all possible pathogens, but selected to search for those with disease potential as documented in previous reports on waterfowl. There is a concern that debilitation and stress-related immunosuppression could result in the proliferation and dispersal of other pathogens. The presence of *Pasteurella multocida* in either the environment or ducks was also not examined in this survey, but there is one record of a gallinule at Hanalei infected with Pasturella (NWHC Case #21957).

The absence of *Salmonella spp*. from the cloacal swabs may have been due to the less than ideal time between sampling and plating. Alternatively, it more likely reflects a low to negligible shedding amongst these ducks. PCR or an alternative collection technique might yield positive results. The isolation of a *Campylobacter* species, including *C. jejuni*, from three birds is moderately significant. *Campylobacter* have been known to cause disease in waterfowl and poultry, as well as humans (Thomas et al. 2007). Feral chickens are ubiquitous on Kauaʻi and common on the refuge. Chickens have been implicated as potential reservoirs and may be the source of the infections in these birds. Since a portion of the refuge is used for the production of the human food source taro, further studies to better identify the prevalence and biology of this zoonotic organism are indicated. Other waterfowl, including the endangered Hawaiian coot, Hawaiian common gallinule, and Hawaiian goose, also inhabit the wildlife refuge and may either act as a reservoir for *Campylobacter* or could become infected.

The significance of the novel adeno and herpes viruses identified from the cloacal/fecal samples is unknown. It is not surprising these birds should harbor a diverse range of viruses and bacteria. No deaths of Koloa have previously been associated with a potential viral disease. We ran the primers for barcoding on the 8 positive samples and the two strong positives were the only ones to amplify. This is pretty good evidence that those two birds are infected with relictum, but we will sequence the products to get final verification. The remaining 6 birds were probably positive for relictum, but we can't verify at this point in time.

It is interesting that two identifiably novel viruses were identified in Koloa. Although not necessarily pathogenic, these viruses could be used to identify contact between Koloa and migratory waterfowl. Many species of waterfowl, from both Alaska and mainland USA, winter in Hawaii, but more importantly some birds from Asia may also pass through. This opens up the opportunity for the spread of pathogens from these areas and the introduction into naïve populations.

The reason for the high prevalence of antibody titers to Newcastle virus is unknown. The titers do, however, indicate exposure to the same or a similar paramyxovirus. The pathogenicity of these paramyxoviruses varies within and between species. It would be of value to understand if this virus is reservoired within the ducks, one of the other waterfowl species, or potentially in the feral chickens. There is no evidence of overt disease at this time associated with Newcastle virus or a similar virus.

Analysis of metal concentrations in feathers is a common methodology for surveying exposure to elements in birds. It is commonly reported that such concentrations reflect a bird's body burden for different metals, but correlations showing this are either

lacking, or weak (Lodenius and Solonen 2013). However, such data can help track changes in exposure to metal with time (Solonen and Kuusela 1985), thus, our data do provide a baseline for future such surveys of Koloa. Concentrations of elements attributable to contaminants were generally low in Koloa.

Overall, no major potential pathogens were identified in this population of ducks. Botulism continues to be the major disease issue for Koloa in the Hanalei NWR. There is some concern that stress and immunosuppression due to botulism may be added to by other pathogens. The significance of the novel herpes and adenoviruses are undetermined. *Campylobacter* is present on the refuge at an apparently low prevalence. Its significance for human health warrants further exploration of its source and the relationship of the feral poultry and the potential for pathogen movement to the native waterfowl and other birds.

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