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Authors: Heard, Darryl J., Mulcahy, Daniel M., Iverson, Samuel A., Rizzolo, Daniel J., Greiner, Ellis C., et al.

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## A Blood Survey of Elements, Viral Antibodies, and Hemoparasites in Wintering Harlequin Ducks (*Histrionicus Histrionicus*) and Barrow's Goldeneyes (*Bucephala Islandica*)

Darryl J. Heard,<sup>1,6</sup> Daniel M. Mulcahy,<sup>2</sup> Samuel A. Iverson,<sup>3</sup> Daniel J. Rizzolo,<sup>2</sup> Ellis C. Greiner,<sup>1</sup> Jeff Hall,<sup>4</sup> Hon Ip,<sup>5</sup> and Daniel Esler<sup>3</sup> <sup>1</sup> College of Veterinary Medicine, University of Florida, Gainesville, Florida 32610, USA; <sup>2</sup> US Geological Survey, 1011 East Tudor Rd, Anchorage, Alaska 99503, USA; <sup>3</sup> Simon Fraser University, Centre for Wildlife Ecology, 5421 Robertson Rd, RR1, Delta, British Columbia V4K 3N2, Canada; <sup>4</sup> Utah State Veterinary Diagnostic Laboratory, Logan, Utah 84322, USA; <sup>5</sup> US Geological Survey, National Wildlife Health Center, 6006 Schroeder Rd, Madison, Wisconsin 53711, USA; <sup>6</sup> Corresponding author (email: HeardD@mail.vetmed.ufl.edu)

**ABSTRACT:** Twenty-eight Harlequin Ducks (*Histrionicus histrionicus*) and 26 Barrow's Goldeneyes (*Bucephala islandica*) were captured in Prince William Sound, Alaska, between 1 and 15 March 2005. Blood was collected for quantification of element concentrations, prevalence of antibodies to several viruses, and hemoparasite prevalence and identification. Although we found selenium concentrations that have been associated with selenosis in some birds ( $\geq 2.0$  ppm ww), our findings contribute to a growing literature describing relatively high selenium in apparently healthy birds in marine environments. Avian influenza virus antibodies were detected in the plasma of 28% of the ducks. No antibodies against adenovirus, reovirus, or paramyxovirus 1 were detected. Several hemoparasite species were identified in 7% of ducks. Our findings are similar to those in other free-living marine waterfowl and do not indicate unusual concerns for the health of these species in this area in late winter.

**Key words:** Avian influenza virus, Barrow's Goldeneye, *Bucephala islandica*, Harlequin Duck, *Histrionicus histrionicus*, *Leucocytozoon simondi*, reovirus, selenium.

Although described for some waterfowl species (Wobeser, 1997), basic information about the prevalence and population effects of contaminants and pathogens is unknown for most ducks (Kear, 2005). Screening for elemental and organochlorine contaminants has been conducted in several Arctic waterfowl (Henny et al., 1995; Grand et al., 1998; Franson et al., 1999, 2002, 2004; Braune et al., 2006) to determine causes for declining populations (Goudie et al., 1994). Lead appears

to be a significant cause of mortality and influence on Spectacled Eider (*Somateria fischerifischeri*) populations (Grand et al. 1998). Elevated selenium concentrations have been identified in several Arctic waterfowl, but the health significance of these findings is unknown (Franson et al., 1999, 2002, 2004; Wayland et al., 2001). Sea ducks in Prince William Sound (PWS), Alaska, have been shown to be chronically exposed to residual oil from the Exxon Valdez spill (Trust et al., 2000). Continued exposure was found to be affecting population dynamics of Harlequin Ducks (*Histrionicus histrionicus*, hereafter HARD) as many as 9 yr after the spill (Esler et al., 2002). In addition to contaminants, several viruses have been associated with duck die-offs in the Arctic including adenovirus in Long-tailed Ducks (*Clangula hyemalis*) in northern Alaska, and adenovirus and reovirus in Common Eiders (*Somateria mollissima*) in Finland (Hollmén et al., 2002, 2003a, b).

We examined blood from HARD and Barrow's Goldeneyes (*Bucephala islandica*, hereafter BAGO) wintering in coastal Alaska to quantify concentrations of elements and the prevalence of viral antibodies and hemoparasites. This information is particularly important in our study area, PWS, which was subject to acute and chronic effects of the Exxon Valdez oil spill (Peterson et al., 2003) that may be additive to other causes of disease.

Twenty-eight (male  $n=20$ , female  $n=8$ ) HARD and 26 (male  $n=20$ , female  $n=6$ ) BAGO were captured at Knight Island and Montague Island in PWS ( $60^{\circ}20'N$ ,  $147^{\circ}30'W$ ), Alaska, between 1 and 15 March 2005. All the animals were examined and then weighed using an electronic scale. Ducks were captured primarily for other studies, and blood samples were collected according to approved protocols (Simon Fraser University IACUC No. 743B-05).

Ducks were captured using floating mist nets and decoys placed over feeding areas (Kaiser et al., 1995). After entanglement, birds were immediately removed from the net and transferred to commercial animal carriers. Ducks were then transported to a chartered research vessel, where they were anesthetized with isoflurane in oxygen for liver biopsy (Trust et al., 2000). All blood samples were collected either after surgery or immediately prior to release following at least 1 hr recovery. All ducks were released within 8 hr of capture. Three milliliters of blood were collected from the right jugular vein using a heparinized (sodium heparin, 1,000 USP units  $ml^{-1}$ , Baxter Healthcare Corp., Deerfield, Illinois, USA) 3 ml syringe, and 25 g needle. Approximately 0.50 to 0.75 ml was placed in a 1.2 ml cryogenic vial (Corning Inc., Corning, New York, USA) for whole blood mineral analysis. The remainder was divided equally between two 1.3 ml serum separator tubes (Sarstedt Inc., Newton, North Carolina, USA), centrifuged (2 ml centrifuge, Cole-Parmer Instrument Co., Vernon Hills, Illinois, USA) at 3,000 rpm for 5 min, and the plasma was then decanted into a 1.2 ml cryogenic vial. The whole blood, plasma, and blood cells remaining in the separator tubes were stored in a cooler with crushed ice for the duration of the capture period. Samples were then frozen at  $-62^{\circ}C$  ( $-80^{\circ}F$ ) for  $\leq 1$  month until analysis. There was insufficient whole blood or plasma for some analyses; therefore sample size was  $<54$  in some instances.

Fifty-three (HARD  $n=27$ , BAGO  $n=26$ ) whole blood samples were submitted to the Utah Veterinary Diagnostic Laboratory (Logan, Utah, USA) for measurement 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), selenium (Se), silicon (Si), tin (Sn), strontium (Sr), titanium (Ti), vanadium (V), and zinc (Zn) (Table 1). Samples were digested (1:1) with trace mineral-grade nitric acid (Fisher Scientific, Pittsburgh, Pennsylvania, USA) in sealed 10 ml Oak Ridge Teflon digestion tubes (Nalge Nunc International, Rochester, New York, USA) for 1 hr at  $90^{\circ}C$ . One milliliter of the digest was then added to 9.0 ml of 18.3 Mohm water in a 15 ml polypropylene trace-metal-free tube (Elkay, Mansfield, Massachusetts, USA). This provided a 5% nitric acid matrix for the analysis, which was matrix matched for all standard curve and quality control samples. Mineral content analysis was performed using an ELAN 6000 inductively coupled plasma mass spectrometer (Perkin Elmer, Shelton, Connecticut, USA). For all elements except selenium, five-point standard curves from 0.01 to 0.50 mg/l were used to quantify the minerals. Selenium analysis utilized a four-point standard addition curve to prevent analytical interference. Sequential 1:10 dilutions were made, using 5% nitric acid, for minerals exceeding the standard curve. Standard curves and quality control samples were analyzed every five samples. The NIST standards also were analyzed to verify accuracy of the analytical results. All concentrations were given as ppm wet weight.

Plasma samples from 46 ducks (HARD  $n=23$ , BAGO  $n=23$ ) were tested for antibodies against paramyxovirus 1 (Newcastle Disease Virus, NDV), adenovirus,

TABLE 1. Whole blood micro- and macro-element concentrations in ppm ww. Values are given for mean  $\pm$ SD (range) for Harlequin Ducks ( $n=27$ ) and Barrow's Goldeneye ( $n=26$ ) captured in Prince William Sound, Alaska, in late winter.

Element	Minimum detectable concentration	Barrow's Goldeneyes	Harlequin Ducks
Al	0.001	0.07 $\pm$ 0.03 (0.04–0.20)	0.07 $\pm$ 0.04 (0.04–0.23)
As	0.001	0.24 $\pm$ 0.06 (0.12–0.33)	0.59 $\pm$ 0.28 (0.16–1.19)
B	0.001	0.38 $\pm$ 0.23 (0.10–1.02)	0.27 $\pm$ 0.11 (0.10–0.63)
Ca	0.01	47 $\pm$ 5 (36–56)	48 $\pm$ 4 (38–56)
Co	0.001	0.007 $\pm$ 0.004 (0.003–0.020)	0.003 $\pm$ 0.002 (0.001–0.007)
Cr	0.001	0.25 $\pm$ 0.02 (0.19–0.30)	0.25 $\pm$ 0.02 (0.22–0.28)
Cu	0.001	0.38 $\pm$ 0.04 (0.29–0.48)	0.46 $\pm$ 0.04 (0.40–0.54)
Fe	0.001	563 $\pm$ 63 (369–676)	606 $\pm$ 44 (525–692)
Hg	0.0001	0.98 $\pm$ 0.37 (0.42–1.71)	0.82 $\pm$ 0.32 (0.27–1.46)
K	0.01	1,943 $\pm$ 142 (1,611–2,236)	2,302 $\pm$ 128 (2,023–2,623)
Li	0.001	0.013 $\pm$ 0.007 (0.003–0.029)	0.010 $\pm$ 0.003 (0.004–0.016)
Mg	0.01	130 $\pm$ 57 (53–261)	153 $\pm$ 73 (74–365)
Mn	0.001	0.025 $\pm$ 0.004 (0.018–0.031)	0.027 $\pm$ 0.006 (0.018–0.040)
Mo	0.001	0.030 $\pm$ 0.008 (0.019–0.050)	0.038 $\pm$ 0.011 (0.024–0.063)
Na	0.01	1,595 $\pm$ 140 (1,343–2,024)	1,640 $\pm$ 122 (1,355–1,895)
Ni	0.001	0.006 $\pm$ 0.001 (0.005–0.008)	0.007 $\pm$ 0.002 (0.005–0.015)
P	0.001	1,748 $\pm$ 290 (1,121–2,195)	1,900 $\pm$ 234 (1,226–2,347)
Pb	0.001	0.021 $\pm$ 0.011 (0.005–0.046)	0.026 $\pm$ 0.014 (0.010–0.070)
Se	0.001	9.8 $\pm$ 3.2 (3.1–17.0)	5.5 $\pm$ 3.0 (2.0–11.1)
Si	0.001	32.7 $\pm$ 2.9 (25.8–37.9)	30.7 $\pm$ 3.2 (26.3–39.9)
Sr	0.001	0.04 $\pm$ 0.01 (0.02–0.09)	0.06 $\pm$ 0.02 (0.04–0.09)
V	0.001	0.06 $\pm$ 0.01 (0.03–0.09)	0.05 $\pm$ 0.01 (0.02–0.08)
Zn	0.001	4.14 $\pm$ 0.34 (3.35–4.69)	5.01 $\pm$ 0.34 (4.34–5.53)

and reovirus at the US Geological Survey, National Wildlife Health Center (NWHC; Madison, Wisconsin, USA). Samples were submitted to the Wisconsin Veterinary Diagnostic Laboratory (Barron, Wisconsin, USA) for avian influenza serology using an agar gel immunodiffusion test (Thayer and Beard, 1998). An antibody titer of  $\geq 1/40$  was considered positive for all other assays.

Samples were tested for NDV antibody with a hemagglutination inhibition test using an isolate from a coot (NWHC case no. 406-77-383, Washington, 1977) (Payment and Trudel, 1993). Plasma samples were assayed for adenovirus antibody with a microneutralization test (Thayer and Beard, 1998) using a strain of adenovirus (NWHC case no. 4672-9605) isolated from Long-tailed Ducks in Alaska (Hollmén et al., 2003a). Reovirus antibody titers were assayed using the same microneutralization test (Thayer and Beard,

1998), except an Eider Duck isolate (NWHC case no. 19062-01, Massachusetts, 2005) was used instead of the adenovirus.

Approximately 5  $\mu$ l of blood from 54 (HARD  $n=28$ , BAGO  $n=26$ ) ducks were used immediately after collection to prepare thin smears on glass microscope slides. The slides were air dried at room temperature, immersed in absolute methanol for 10 min, and again air dried. They were then stored in a box slide holder at room temperature for 1–3 wk before staining with an automated stainer using the Wright-Giemsa technique. Stained smears were covered with 0.16-mm-thick glass coverslips using a commercial mounting reagent (Eukitt, Calibrated Instruments Inc., Hawthorne, New York, USA) to allow examination at high (500 $\times$ ) light microscopic magnification under oil immersion for parasite identification. Each slide was examined at 300 $\times$ , and

then at 500 $\times$  for at least 20 min. Parasites were identified based on morphology and size (Valkiūnas, 2004).

The mean $\pm$ 1SD body weights (g) for BAGO males ( $n=20$ ) and females ( $n=6$ ) were 1,102 $\pm$ 66 and 731 $\pm$ 87, respectively. The body weights for HARD males ( $n=20$ ) and females ( $n=8$ ) were 628 $\pm$ 27 and 582 $\pm$ 32, respectively. All elements except Ag, Be, and Ti were detected in the blood of one or more ducks at concentrations above minimum measurable limits (Table 1). Antibodies to avian influenza virus at titers  $\geq 1/40$  were identified in the plasma of 13 (28%) of the duck 46 samples examined (HARD=10 of 24 [42%]; BAGO=3 of 22 [14%]). No antibodies against adenovirus, reovirus, or paramyxovirus 1 were detected in any of the submitted samples. Several hemoparasite species were identified in the erythrocytes in 7% (4/54) of examined blood smears. The macrogametocytes of *Leucocytozoon simondi* were identified in five erythrocytes in a blood smear from one HARD (4%). The macrogametocytes of *Haemoproteus nettionis* and *H. greineri* were identified in blood smears from two (8%) and one (4%) BAGO, respectively.

All of the ducks were healthy based on attitude, physical examination, body weight, and survival following inhalation anesthesia and liver biopsy. This is not surprising, since an ill bird is unlikely to survive the physiologic and energetic stressors of winter in PWS (Esler et al., 2000). Several findings in this survey, however, warrant further discussion including high blood selenium concentrations, a high prevalence of antibodies against avian influenza, and the presence of hemoparasites.

The whole blood selenium concentrations in these HARD (mean $\pm$ SD 5.5 $\pm$ 3.0, range 2.0–11.1 ppm wet weight) and BAGO (9.8 $\pm$ 3.2, 3.1–17.0) were similar to those measured in birds (Heinz, 1996) and other vertebrates (Puls, 1994) with clinical selenosis. High blood selenium concentrations also have been de-

scribed in otherwise healthy Alaskan waterfowl including Emperor Geese (*Chen canagica*), Long-tailed Ducks, Spectacled Eider, King Eider (*Somateria spectabilis*), and Common Eider (Franson et al., 1999, 2002; Stout et al., 2002; Wayland et al., 2003; Franson et al., 2004; Wilson et al., 2004). Similarly, high tissue concentrations have been described in ducks from Canada (Henny et al., 1995; Wayland et al., 2001, 2003; Braune and Malone, 2006) and Russia (Stout et al., 2002). Samples have usually been collected from nesting birds when they are most easily caught. Blood concentrations declined through the breeding period in Emperor Geese (Franson et al., 1999) and Spectacled Eiders (Wilson et al., 2004), suggesting exposure occurs on marine wintering grounds.

Although well described in waterfowl (Ohlendorf, 2002), selenosis has not been reported in either HARD (Robertson and Goudie, 1999) or BAGO (Eadie et al., 2000). The most frequently reported effects are teratogenesis, embryonic death, and poor neonatal growth. These detrimental reproductive effects are associated with high egg tissue selenium concentrations, where  $\geq 10$   $\mu$ g/g is considered the detrimental threshold (Skorupa, 1998). Interestingly, there has been no association of these findings with elevated blood or tissue concentrations in Arctic waterfowl. Chronic selenosis is characterized by low body weight and pectoral muscle wasting (Albers et al., 1996). This was not observed in these HARD and BAGO; body weights were within published reference ranges for these species (Robertson and Goudie, 1999; Eadie et al., 2000). More subtle signs not evaluated in this study include immunosuppression (Wayland et al., 2003) and hepatic injury (Franson et al., 2002).

The form of selenium in the HARD and BAGO blood was not determined. Ingestion of selenomethionine is the main exposure route in birds and other animals (Fordyce, 2005). Although concentrations in marine water are insufficient to induce



toxicosis, it is accumulated and magnified by marine organisms (Fordyce, 2005). Mollusks (especially blue mussels, *Mytilus edulis*, and periwinkles, *Littorina* sp.) make up a major proportion of the diet of BAGO during winter (Eadie et al., 2000). The main diet of HARD in winter is intertidal and subtidal invertebrates, predominantly crustaceans (Robertson and Goudie, 1999). Bivalves are very effective bioaccumulators because they assimilate most of the selenium they ingest and only slowly release them (Rainbow, 1996).

The source of selenium exposure in these ducks may be from natural sources, such as volcanism (Fordyce, 2005). Because the BAGO and HARD appeared healthy despite high blood selenium concentrations, it is likely that these species and other Arctic waterfowl have developed mechanisms to cope with dietary exposure to selenium. This hypothesis is supported by a Dutch study of Oystercatchers (*Haematopus ostralegus*) that demonstrated no affect on reproduction despite high blood selenium concentrations in adult birds (Goede, 1993). Recent studies show aquatic vertebrates may detoxify either mercury or selenium by forming biologically inert complexes such as mercuric selenide (HgSe) (Ikemoto et al., 2004). It is possible the BAGO and HARD use this mechanism to prevent selenosis and the deposition of selenium into egg tissue. Understanding of selenium detoxification mechanisms would benefit from studies with concurrent analyses of tissue (e.g., liver) and whole blood concentrations, as well as the evaluation of egg tissue selenium content.

Identification and monitoring of avian influenza strains in these and other ducks in Alaska are indicated because of the potential for contact with ducks from the western Pacific (Winker et al., 2007). Avian influenza is important because some strains (e.g., highly pathogenic H5N1) cross species boundaries and infect other animals, including waterfowl, commercial

poultry flocks, and humans (Olsen et al., 2006). Also, influenza could affect populations of HARD and BAGO through mortality of individuals, which could be exacerbated in the presence of other stressors. The high prevalence of antibodies to avian influenza in this study is not surprising, as wild ducks are known to be a primary reservoir (Olsen et al., 2006). The avian influenza strain(s) carried by ducks in this study were not identified because of insufficient plasma for comparative serologic testing (Wobeser, 1997).

A recent avian influenza survey of Alaskan waterfowl based on PCR or viral culture of fecal samples showed a very low prevalence of birds shedding virus (Winker et al., 2007) and concluded that transmission of avian influenza was limited in Alaskan environments. The results of the present study, however, show high exposure in HARD and BAGO. This difference reflects the persistence of antibodies indicating past exposure, while fecal viral shedding during active infection is likely to occur for a short period of time (Olsen et al., 2006). Further, Winker et al. (2007) sampled birds from only western Alaska and not PWS.

The diversity of serologic testing in these ducks was limited by available plasma volumes. Selection of pathogen antibodies to assay was based on published reports of viral diseases in arctic marine ducks (Wobeser, 1997; Hollmén et al., 2002, 2003a, b). The absence of detectable antibodies against paramyxovirus 1, reovirus, and adenovirus is most likely due to absence of exposure.

The low prevalence of hemoparasites in the blood of the sampled ducks is similar to those described in late winter for other avian species (Valkiūnas, 2004). Many hemoparasites are sequestered in tissue during winter. This is due, in part, to the absence of immuno-incompetent birds (i.e., ducklings) and intermediate hosts that might transmit new infections (Valkiūnas, 2004). Although there is much argument as to whether *Hemoproteus*

species are pathogenic, *Leucocytozoon simondi* is a well-described cause of morbidity and mortality in geese and ducks (Valkiūnas, 2004). It is interesting to note *H. greineri* has been found in anatids across the expanse of the Nearctic (Valkiūnas, 2004). The most recent anatid blood parasite survey, however, did not find it in the prairie provinces of Canada (Bennett et al., 1982). Assessment of the health effects of hemoparasites is preferably performed during nesting, when birds are exposed to the intermediate hosts and circulating macrogametocyte levels are highest.

In conclusion, our sample of the population of sea ducks from PWS appeared healthy at the time of capture. This finding, however, is not unexpected because ill birds would not be expected to survive late winter in PWS. A complete health evaluation of these duck populations, therefore, would require sampling throughout the year. Further investigation is recommended to evaluate what effect, if any, elevated selenium concentrations have on marine bird populations in Arctic and subarctic areas, and to better understand mechanisms for selenium tolerance in marine birds. In addition, research is indicated to determine and monitor the strains of avian influenza in these duck populations.

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