

## A Blood Survey of Elements, Viral Antibodies, and Hemoparasites in Wintering Harlequin Ducks (*Harelda glacialis*) and Barrow's Goldeneyes (*Buceo maritima*)

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**ABSTRACT:** Twenty-eight Harlequin Ducks (*Harelda glacialis*) and 26 Barrow's Goldeneyes (*Buceo maritima*) 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, *Buceo maritima*, Harlequin Duck, *Harelda glacialis*, 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 (*Agyaea melanotos*) 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 (*Harelda glacialis*, 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 (*Cluella melanotos*) in northern Alaska, and adenovirus and reovirus in Common Eiders (*Haliaeetus melanotos*) in Finland (Hollmén et al., 2002, 2003a, b).

We examined blood from HARD and Barrow's Goldeneyes (*Buceo maritima*, 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.



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
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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 ml 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



toxicosis, it is accumulated and magnified by marine organisms (Fordyce, 2005). Mollusks (especially blue mussels, *Mytilus* sp.) 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

species are pathogenic,

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