A Lesser Scaup (*Aythya affinis*) Naturally Infected with Eurasian 2.3.4.4 Highly Pathogenic H5N1 Avian Influenza Virus – Movement Ecology and Host Factors

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**Summary**

Despite the recognized role of wild waterfowl in the potential dispersal and transmission of highly pathogenic avian influenza (HPAI) virus, little is known about how infection affects these birds. This lack of information limits our ability to estimate viral spread in the event of an HPAI outbreak, thereby limiting our abilities to estimate and communicate risk. Here we present telemetry data from a wild Lesser Scaup (*Aythya affinis*), captured during a separate ecology study in the Chesapeake Bay, Maryland. This bird tested positive for infection with clade 2.3.4.4 HPAI virus of the A/goose/Guangdong/1/1996 (Gs/GD) H5N1 lineage (results received post-release) during the 2021-22 ongoing outbreaks in North America. While the infected bird was somewhat lighter than other adult males surgically implanted with transmitters (790g, =868g, n=11), it showed no clinical signs of infection at capture, during surgery, nor upon release. The bird died 3d later, pathology undetermined as the specimen was not able to be recovered. Analysis of movement data within the 3d window showed that the infected individual’s maximum and average hourly movements (3894.3m, 428.8m respectively) were noticeably lower than noninfected conspecifics tagged and released the same day (=21594.5m, =1097.9m, respectively; n=4). We identified four instances where the infected bird had direct contact (fixes located within 25m and 15 min) with another marked bird during this time. Collectively, these data suggest that the HPAI positive bird observed in this study may have been shedding virus for some period prior to death, with opportunities for direct bird to bird or environmental transmission. Although limited by low sample size and proximity to the time of tagging, we hope that these data will provide useful information as managers continue to respond to this ongoing outbreak event.

Keywords: avian influenza virus, disease, HPAI, H5N1, Lesser Scaup, telemetry, movement

**Introduction**

Wild waterfowl have long been recognized as the reservoirs of low pathogenic influenza A viruses (LPAI; Olsen et al., 2006), which often circulate asymptomatically (Kuiken, 2013). Wild waterfowl are also a source for viral introduction of avian influenza viruses (AIVs) to domestic poultry, both within the United States (Li et al., 2018) and globally (Lycett et al., 2016). The 2.3.4.4 clade of the A/goose/Guangdong/1/1996 (Gs/GD) H5N1 lineage of highly pathogenic influenza A virus (HPAI) was first recognized in 2010 and has spread across the globe resulting in losses to some wild bird populations and major economic losses to commercial poultry producers (Lycett et al., 2019). The continued circulation of the 2.3.4.4 GsGD lineage of HPAI has led to recent calls for increased vigilance and preparation given the potential for such viruses to result in a pandemic (Horwood, 2021; Ramey et al., 2022), and the recent spread to North America (Bevins et al., 2022).

Historically, HPAI viruses were not known to be maintained in wild avian species or disseminated through migration, but this has changed since the emergence of the GsGD HPAI lineage (Ramey et al., 2022). Recent research has established the connection between wild waterfowl migration and HPAI viral spread (Tian et al., 2015; Yoo et al., 2022). Wild birds have been implicated in the transmission of H5 HPAI virus from Asia to the Pacific flyway of North America in 2014-15 (Lee et al., 2015), and are hypothesized to be the source of recent (2021) transmission of H5N1 from Europe to the North American Atlantic Flyway (Caliendo et al., 2022).

Despite the recognized role of wild waterfowl in the dispersal and transmission of HPAI, relatively little is known about how infection affects these animals in the wild. Laboratory-based challenge studies using naïve birds have demonstrated a large degree of variability among species related to pathogenicity and the extent of viral shedding (Hénaux and Samuel, 2011). While beneficial, such laboratory-based studies cannot predict how infected birds will move and interact with their natural habitat, with compounding biotic stressors. The only currently available data on how HPAI infection affects wild bird movement ecology comes from a white-faced whistling duck (*Dendrocygna viduata*) marked in Nigeria that completed a full migration despite infection with H5N2 (Gaidet et al., 2008), and five spot-billed ducks in South Korea infected with H5N6 (Yoo et al., 2022) that had local and migratory movements. However, it should be noted that researchers have reported LPAI infection can result in reduced feeding rates, delayed migration, and prolonged stopovers (van Gils et al., 2007), though results differ between studies (i.e., Latorre-Margalef et al., 2009).

Here we present telemetry data from a wild Lesser Scaup (*Aythya affinis*), captured and marked for a separate ongoing ecology study and opportunistically sampled for AIV, that tested positive for Eurasian clade 2.3.4.4 GsGD lineage Highly Pathogenic H5N1 during the 2021-2022 outbreak that is rapidly unfolding in North America. Our objective is to characterize the movement of the infected individual relative to uninfected conspecifics marked on the same day, and to relate this information to existing HPAI challenge studies in this species. We believe that such information, despite some limitations in our dataset, will benefit efforts to understand and model HPAI transmission in wild waterfowl, and contribute to information that may help in surveillance and response efforts.

**Materials and Methods**

Capture, Sampling, and Marking

We captured Lesser Scaup using a closed barrier trap (Haramis et al., 1982) baited with corn from Dec 2021 - Jan 2022. Capture occurred on the Chesapeake Bay at the Eastern Neck National Wildlife Refuge, Rock Hall, Maryland (39° 1' 4", -76° 13' 19"). Upon capture, birds were placed in large holding containers and stored out of direct sunlight until they could be processed. Birds were individually banded with USGS Bird Banding Laboratory metal bands and data on age, sex, weight and body condition on a scale of 1 to 5 were obtained (Olsen et al., 1996). Birds were then either selected (>700 g, preference for adult birds) for surgical implantation with a tracking transmitter or released. Transmitters were 30-g Global Positioning System-Global System for Mobile Communications (GPS-GSM) transmitters (OrniTrak-I30 3 G; Ornitela, UAB, Vilnius, Lithuania) programmed to take points every hour with data uploads to the mobile network occurring daily. Selected individuals were transported to the USGS Eastern Ecological Science Center veterinary hospital in Laurel, Maryland for surgical implantation of transmitters. Surgical implants were necessary as opposed to traditional backpack harnesses due to diving feeding behavior by this species (Olsen et al., 1992).

Upon arrival to the laboratory, a pre-surgery medical examination was performed, and following anesthesia and intubation, transmitters were implanted in the celomic cavity (Olsen et al., 1992; Korschgen et al., 1996). Prior to surgery, transmitters were covered with nylon mesh to aid in attachment during surgery and ensure proper placement of the transmitter and antenna, then sterilized with ethylene oxide. Following completion of all procedures, birds were monitored for up to 3h to ensure successful recovery from surgery. Once recovered, they were transported back to the site of initial capture and released.

While in the hospital, one cloacal and one oropharyngeal swab (Hanson et al., 2003) were collected from each bird using sterile polyester-tipped applicators and placed as paired samples (by bird) in vials containing 2 mL of chilled Brain Heart Infusion media (Becton Dickinson and Co., Sparks, MD) supplemented with penicillin G (1,000 units/ml), streptomycin (1 mg/ml), kanamycin (0.5 mg/ml), gentamicin (0.25 mg/ml), and amphotericin B (0.025 mg/ml) (Sigma Chemical Company, St. Louis, MO). Swabs were stored on ice for <12h during sample collection and transport to a holding facility where they were then stored at -70 °C until being shipped on dry ice to the laboratory where they were held at -80 °C until processing. During surgery, < 3 ml of whole blood was collected from each bird to be used for avian influenza antibody testing (results from such testing are not yet available and thus lab methods not reported here).

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received (USGS EESC ACUC 2014-02P and 2021-16P; University of Delaware IACUC 1385-2022-A; Federal banding permit #6570). The U.S. National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

Laboratory Analysis

We extracted viral RNA from all swab samples using the MagMAX-96 AI/ND Viral RNA Isolation Kit (Ambion/Applied Biosystems, Foster City, CA) (Spackman et al., 2002) and screened using real-time reverse transcriptase-PCR (rRT-PCR) that targeted the influenza A virus (IAV) matrix (M) gene (Spackman et al., 2002) at the University of Georgia Southeastern Cooperative Wildlife Disease Study. Any sample that did not yield a cycle threshold (Ct) value ≤45 was considered negative for IAV. IAV positive samples were screened for 2.3.4.4 HP H5 via rRT-PCR; suspect positives from this assay were sent to the National Veterinary Services Laboratory (NVSL), Ames, Iowa for HPAI testing. IAV laboratory testing was completed approximately 8 days after capture and release, and NVSL HPAI final results were received approximately 3 weeks later, hence the infection status was not known at time of release.

Hereafter, HPAI positive detection is noted as HPAI+; lack of HPAI positive detection, or HPAI negative, is noted as HPAI-. These terms throughout the manuscript refer to testing results from samples collected on the day of capture and marking for each transmitter-marked Lesser Scaup; no repeat samples were taken during the study, as birds were not recaptured following marking and release.

Movement Data Processing and Analysis

We downloaded all telemetry data from the online system and filtered to include only fixes believed to be from live birds based upon manual review of movements and internal temperature readings. We further filtered to include only fixes occurring from 23 Jan 2022 through 26 Jan 2022, the days for which data is available for bird 214474 before death (HPAI+ individual; see results). Following all filtering we divided movement data into three groups: (1) bird 214474 (HPAI+), (2) the four individuals marked and released concurrent to 214774 (individuals 214470-214473; HPAI-), and (3) all other individuals that were HPAI- and were captured, marked, and released on a different day than 214470 through 214474. To understand movement rates of marked birds we calculated the distance moved between consecutive hourly fixes (± 5 min) for the infected individual and others marked on the same day, and found the minimum, maximum, and average distance moved for each individual. We only calculated these metrics for birds marked on the same day as the infected bird (HPAI+, 214474) to ensure that rates were comparable between individuals given potential initial impacts from tagging (Geen et al., 2019) and any possible interaction with weather. We calculated each of the three movement metrics for the HPAI+ individual using two sets of fixes: (1) the full set of data from release to last live point, and (2) from release until the bird constricted movement to a reduced area (<0.5 km in diameter; Figures 1-2) preceding death.

Finally, we manually identified all instances in which any other marked bird had a “direct contact” with the HPAI+ bird. Direct contact was considered any fix within 25 m and 15 min. These buffers were used since individual transmitters were turned on at different times, depending on time of surgery implantation, hence although all transmitters are set to the same fix cycle, the fixes are not taken at the same time. Finally, to determine if there were any apparent trends in temperature as the HPAI+ bird fought infection, we visually compared internal body temperature as recorded by the transmitter for 214474 and the four individuals marked on the same day. All data visualization was performed in ArcMap 10.6.1 (Environmental Systems Research Institute, Inc., Redlands, CA) and R 4.0.2 (R Core Team, 2021). All data presented in this manuscript can be found in the USGS ScienceBase repository (Prosser et al., 2022).

**Results**

During this study we collected paired telemetry and avian influenza virus data for 25 Lesser Scaup (11 males, 14 females); only one individual, an after-second-year male (bird 214474) tested positive (HPAI+) for clade 2.3.4.4 Highly Pathogenic H5N1 (NVSL accession Number 22-003098). This bird showed no apparent signs of infection at the time of capture and behaved normally throughout the surgical procedure and post-surgical release. Preliminary lab analysis of suspected H5 results were received 8 days post-sampling and release. HPAI confirmation was received from USDA NVSL 28 days post-sampling and release. Although the bird appeared healthy and comparable to the other four birds marked the same day and it had normal body condition (BCI score 4 out of 5), its mass was low compared to the other males tagged in the study (790g vs mean 868g ± 68g SD for n=11 males).

For the first day following marking and release (23-24 Jan 2022), the HPAI+ individual behaved comparably to other individuals tagged on the same day, with movements around the Southeastern portion of Eastern Neck National Wildlife Refuge (Figure 1). However, when other individuals began to leave the area moving across the Chesapeake Bay, the HPAI+ bird remained near the refuge until confining movements to a marshy area and subsequently dying (Figure 2). Death was determined when internal body temperature, as indicated by the transmitter, fell from 42 to 35 °C over the course of an hour, then followed near-ambient temperatures. When movement rates were compared to conspecifics marked and released concurrently (HPAI-), we found the average and maximum hourly movements were meaningfully lower for the HPAI+ bird (Table 1). For instance, the HPAI+ bird had ~ 41% lower average hourly movements than the collective average of the HPAI- cohort when examining points prior to movement restriction proceeding death. Interestingly, all five birds marked and released this day (HPAI+ and HPAI-) showed a slight increase in body temperature (not unusual following surgical procedures), as recorded by the transmitter post-release (Figure 3), but neither the HPAI+ nor the HPAI- birds sustained temperature readings consistent with those of infected individuals in laboratory-based challenge studies (Stephens et al., 2019).

After examining point locations of all individuals marked in this study, we identified four instances with three separate individuals that met our definition of direct contact (fixes recorded within 25m and 15 min) between the HPAI+ bird and HPAI- birds. These four contacts all occurred on 24 Jan 2022 and were with individuals tagged between 6 Jan and 13 Jan 2022. No instances of direct contact were observed with birds marked and released concurrently with the HPAI+ individual. Manual examination of individuals noted as having direct contact with bird 214474 as well as those transported and tagged concurrently did not result in any identification of abnormal movements following contact within the time period examined (time from release to death of HPAI+ bird 214474, approximately 3 days).

**Discussion**

The data described in this manuscript represent the first telemetry information documenting the movements of HPAI positive waterfowl in North America. While the sample size is limited to one individual and covers only a brief window between release and death, these data provide important insights into how infected birds may utilize their landscape and interface with conspecifics, other taxa, and the environment. One of the most notable findings from these data is that while the HPAI+ Lesser Scaup exhibited reduced maximum and average movements relative to uninfected conspecifics, its maximum movement was nearly four kilometers with declines in movement occurring closer to death. Because we do not know how long this bird was infected prior to capture, this limited movement may not be representative of potential movement behavior during the entire course of infection; however, these movements would suggest that for at least some window of time post-infection, this individual may have served as a competent host for HPAI and could have transported the virus to other waterbodies. The potential role of this individual in transmission of HPAI is furthered by the direct contact between the infected bird and uninfected individuals seen in the telemetry data. While the subsequent movements of these exposed individuals does not indicate any abnormal behaviors that would indicate transmission, this could be the result of wild birds having some level of immunity due to previous infections with other strains (Costa et al., 2011; Berhane et al., 2014). Still, such exposure events do provide an opportunity for transmission even if migratory capacity of wild birds is inhibited by infection or eventual mortality (Blagodatski et al., 2021). In addition, the three days that this infected individual remained alive following release may have contributed to indirect environmental transmission to other birds utilizing these habitats. Transmission of AIV can occur via a fecal-oral route through fecal contaminated water and can remain infective in the environment across seasons (Brown et al., 2007; Ramey et al., 2020).

The collection of telemetry data from a naturally infected Lesser Scaup was opportune given that this species has also been used in HPAI laboratory challenge studies (A/Northern

Pintail/WA/40964/2014 H5N2, A/Gyrfalcon/WA/41088/2014 H5N8, A/turkey/IN/1403-1/2016 H7N8, A/chicken/TN/17-007147-2/2017 H7N9), presenting us the opportunity to compare the outcomes observed in both controlled environments and a natural setting. For instance, the birds monitored throughout HPAI challenge studies showed no clinical signs of infection (Spackman et al., 2017; Stephens et al., 2019), which compares well with our wild caught bird that was also asymptomatic at the time of examination. In addition, while our wild caught bird weighed less than most males captured in this study, a trend previously reported with LPAI infected waterfowl (Latorre-Margalef et al., 2009), there is still disagreement concerning if low weight or reduced body condition is a result of infection or if such individuals are more susceptible to AIV infection (Flint and Franson, 2009). While Lesser Scaup continued to gain weight while infected with H7 HPAI during a laboratory challenge study (Stephens et al., 2019), those birds had reduced energy requirements compared to birds in natural settings. Additionally, there are likely variations in clinical response associated with different GsGd lineage viruses, thus additional challenge studies on wild species with this current strain of Eurasian HPAI Clade 2.3.4.4 would be valuable.

Differences in environmental conditions may explain elements of our study that disagree with previous challenge studies. For instance, our HPAI+ bird did not display the same elevated body temperature observed in infected birds during challenge studies (Stephens et al., 2019). However, diving ducks are known to fluctuate internal temperature based on time of year (Bevan and Butler, 1992) which correlates with ambient temperature. Thus, the controlled environment of the challenge study may have resulted in different baseline body temperatures than would have been found at our study location during the winter season. Similarly, while challenge studies report no Lesser Scaup mortalities following infection with HPAI, our HPAI+ wild bird died within a few days of marking. Unfortunately, efforts to recover the infected individual post-mortality were unsuccessful, preventing necropsy and leaving us to acknowledge the possibility that mortality may have been driven by causes outside of HPAI. One possible explanation for this discrepancy in mortalities between our wild bird and those in challenge studies is that the energetic demands of survival or confounding factors in the wild were too high to overcome infection for this individual while laboratory conditions were suitable for survival and even growth (Stephens et al., 2019). We also cannot rule out a potential role of delayed marking effects or predation on mortality and movements. At least one other marked bird in the study was likely predated by a bald eagle (*Haliaeetus leucocephalus*), as its transmitter was relocated in/near a tree with an eagle nest while an additional two are believed to have been predated but with less clear evidence on predator identification. Interestingly, several bald eagles have recently been recovered dead and tested positive for H5 HPAI (USDA, 2022) within the flyway, though it is unclear if they contracted the virus through environmental transmission, predating infected live birds, or scavenging infected carcasses.

It is not well understood to what extent this strain of virus affects Lesser Scaup or other waterfowl. The HPAI+ bird in this study was asymptomatic when sampled but died 3d later. Ongoing national active surveillance efforts that target dabbling ducks (Anatinae) generally show asymptomatic infection (USDA, 2022), and passive surveillance mortality events are mainly being observed in raptors (Accipitres) and geese (Anserinae); however, one large-scale mortality event in Lesser Scaup in Florida is being investigated (1000 dead birds, with 8 samples each testing positive for HPAI; (USGS, 2022)). Large scale mortality events have not been observed in this region (Chesapeake Bay, Maryland and surrounding states) based on surveys from the ecology study where this Lesser Scaup was marked, weekly waterfowl surveys at the Eastern Neck National Wildlife Refuge (NWR) where the bird was marked (USFWS, 2022a), as well as weekly / biweekly surveys in prime waterbird habitats in the region (other NWRs (USFWS, 2022b), Hart-Miller Island (MD DNR, 2022; MES, 2022a), and Poplar Island Restoration site (MES, 2022b)). We know from active surveillance data that many infected wild birds appear to be asymptomatic (USDA, 2022). However, only movement studies can show if otherwise asymptomatic birds’ movements are affected, informing how infection may impact capacity to spread virus to other birds, the environment, and new areas. Additional survey data, movement ecology studies, avian influenza surveillance, and lab challenge studies in Lesser Scaup and other waterfowl species will be helpful in improving our understanding of wild bird HPAI transmission. The results from this study show HPAI asymptomatic infection of a wild diving duck in addition to reduced movements compared to non-infected birds marked on the same day, indicating potential for virus spread. Although these data suffer from admitted limitations, we hope that they will provide useful information as managers continue to respond to this ongoing outbreak event, and that additional studies will continue to build our understanding of the changing ecology of wild birds and HPAI infection.

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**Conflict of Interest Statement**

Authors declare no conflict of interests.

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**Tables**

Table 1. The hourly movements of a cohort of Lesser Scaup (*Aythya affinis*) captured and released together on Eastern Neck National Wildlife Refuge, Rock Hall, Maryland, USA. Movements reflect distance, in meters, between consecutive hourly points from post release (1/23/2022 21:58 EST) through the end of the day on 1/26/2022 (the day 214474 died).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Transmitter | HPAI+ | Number of Hourly Points | Min (m) |  | Max (m) |
| 214470 | No | 73 | 1.8 | 757.8 | 11562.1 |
| 214471 | No | 73 | 0.8 | 909.4 | 21672.5 |
| 214472 | No | 73 | 1.4 | 1414.9 | 31407.1 |
| 214473 | No | 73 | 2.2 | 1309.6 | 21736.2 |
| 214474a | Yes | 57 | 4.3 | 428.8 | 3894.3 |
| 214474b | Yes | 34 | 9.2 | 645.0 | 3894.3 |

a Contains all points following release until death

b Contains all points prior to the bird constraining movements to a small area prior to death.  214474b average hourly movements is ~41% lower than  of hourly movements for 214470, 214471, 214472, and 214473 during this time period.

**Figures**

Figure 1. The annotated movements of a Lesser Scaup (*Aythya affinis*) 214474 that tested positive for current infection with Eurasian lineage clade 2.3.4.4 highly pathogenic avian influenza H5N1 (left) relative to the four other uninfected individuals (right) from release through end of day, 26 January 2022 (the day the infected bird died).

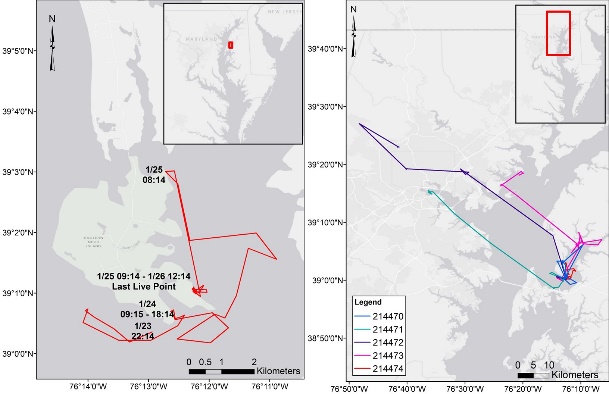


Figure 2. The spatially constricted movements of a lesser scaup (*Aythya affinis*; PTT 214474) that tested positive for current infection with HPAI EA 2.3.4.4 H5N1 prior to death.

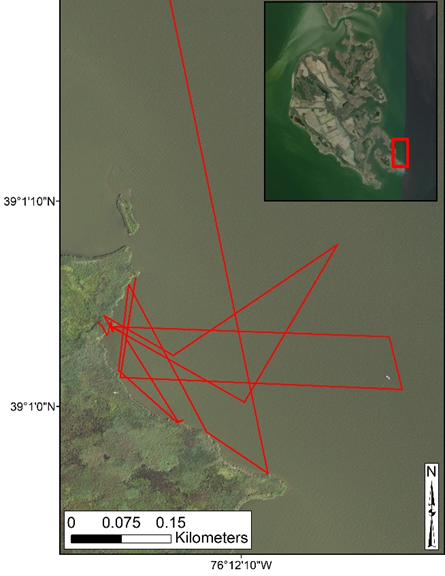


Figure 3. Internal body temperature of Lesser Scaup (*Aythya affinis*) from release through end of day January 26, 2022 when the HPAI+ bird died. While bird 214474 tested positive for current infection with Eurasian lineage clade 2.3.4.4 highly pathogenic avian influenza H5N1, all other birds were negative when sampled on January 23, 2022 (the day of capture, marking, and release). The horizontal line indicates the approximate temperature of infected birds observed during challenge studies (Stephens et al., 2019).

Chart, scatter chart

Description automatically generated