Identifying Wellfleet Bay virus and its contribution to a Nantucket Island bird mortality event

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Introduction

- An unusual mortality event occurred on January 31, 2025, on Nantucket Island, approximately 30 miles off the coast of Cape Cod in MA, USA.
  - Hundreds of Common Eiders and Surf Scoter mortalities were discovered in addition to smaller numbers of Herring Gulls, Great Black-Backed Gulls, Long-tailed Ducks, and Common Loons
- The primary differential was H5N1 Highly Pathogenic Avian Influenza (HPAI)
  - Only 3 birds tested positive for HPAI, but this was not a viable explanation for this mass mortality

Question/Hypothesis

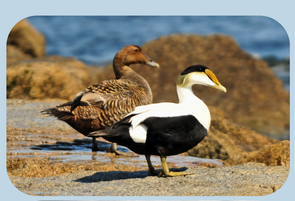
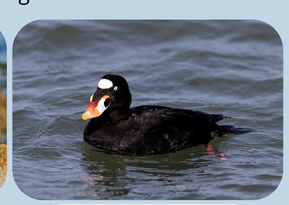
Research Question: *With negative HPAI results, what is the cause of this unusual mortality event?*

Hypothesis: Wellfleet Bay virus is the cause of this multi-host unusual mortality event.

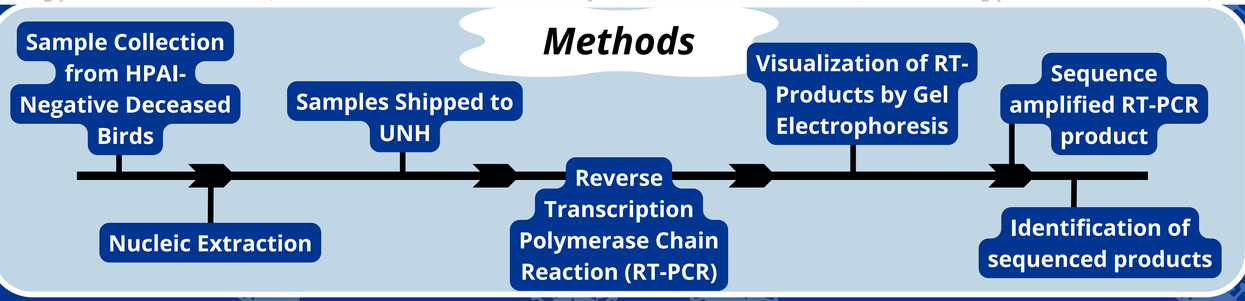
Available Metadata

All animals were collected 1/30 = 1/31 2025

- 56 Common eiders (top left image)[9]
- 13 Surf scoters (top right image)[4]
- 7 White-winged scoters (bottom left image)[7]
- 6 Black scoters (bottom right image)[5]
- 4 Long-tailed ducks
- 3 Herring gulls
- 1 Greater Black-backed gull

Methods



Two-Step RT-PCR

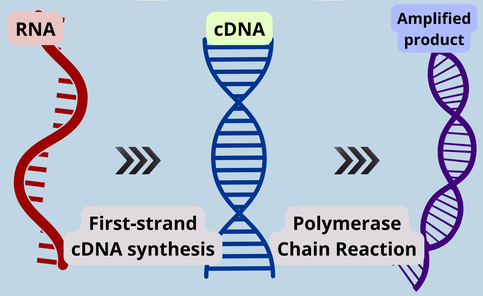


Figure 1: Two-Step RT-PCR separates the conversion of RNA to cDNA and the PCR process (ThermoFisher, 2026). The first step in the two-step RT-PCR utilizes a reverse transcriptase to convert the target RNA into a complementary DNA strand (ThermoFisher, 2026). Then the cDNA is amplified, and that product is added to the PCR master mix for the last amplification step. For this study, the primers used to target the WFBv are the forward primer ACGATTGGAAGAAAAGGAAAGAC and reverse primer CGATGGAAGAGAGAACCC.
The separation of the two steps allows for condition optimization for each step, and flexibility with reverse transcription priming (ThermoFisher, 2026).

Gallery

Depicted here from left to right: the Long-tailed duck (left)[13], greater black-backed gull (lower middle)[8], and the herring gull (right)[10].

Results

	1	2	3	4	5	6	7	8	9	10	11	12
A	25NE004001	25NE004009	25NE004017	25NE004225	25NE004333	25NE004411	25NE004499	25NE004577	25NE004655	23WP003328	23WP003336	25WP00004
B	25NE004002	25NE004010	25NE004018	25NE004226	25NE004334	25NE004422	25NE004500	25NE004588	25NE004666	23WP003329	23WP00337	PCR_neg
C	25NE004003	25NE004011	25NE004019	25NE00427	25NE004335	25NE004433	25NE004511	25NE004599	25NE004677	23WP003330	23WP00338	Pos_ctrl
D	25NE004004	25NE004012	25NE004020	25NE004228	25NE004336	25NE004444	25NE004522	25NE004600	25NE00468	23WP003331	23WP003339	No_RT_ctrl
E	25NE004005	25NE004013	25NE004211	25NE004229	25NE004337	25NE004455	25NE004533	25NE004611	25NE00469	23WP003332	23WP00340	
F	25NE004006	25NE004014	25NE004220	25NE004300	25NE004338	25NE004466	25NE004544	25NE004622	23WP00325	23WP003333	25WP000001	
G	25NE004007	25NE004015	25NE004231	25NE004311	25NE004399	25NE004477	25NE004555	25NE004633	23WP00326	23WP003334	25WP000002	
H	25NE004008	25NE004016	25NE004402	25NE004322	25NE004400	25NE004488	25NE004566	25NE004644	23WP00327	23WP003335	25WP000003	

Figure 2: Sample ID. Samples 25NE00427 (extracted from the brain of a great black-backed gull), 25NE00468 (extracted from the brain of a herring gull), and 25NE00469 (extracted from the brain of a common eider) tested positive for HPAI, highlighted in yellow. The negative control (in red) has no sample in the well. The positive control (in green) has the positive WFB virus control in the well. Then, as another control (in blue) no reverse transcriptase was added to the well.

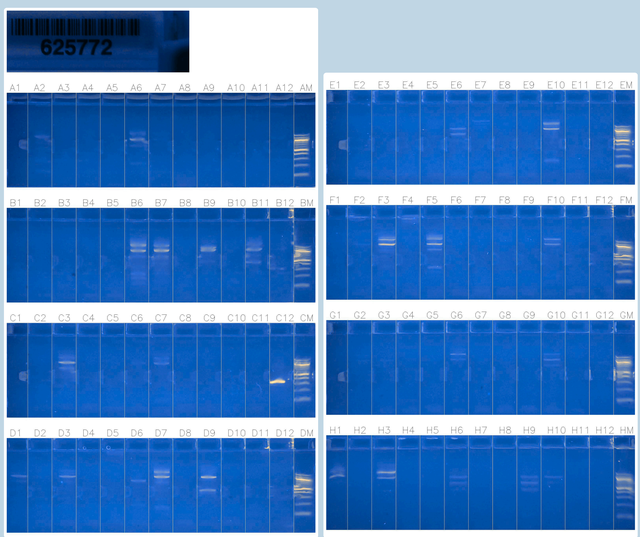


Figure 3: PCR Product Gel Electrophoresis. The amplified RT-PCR product was diluted 1:10 before plating the samples in the 2% agarose gel. The amplified RT-PCR product, non-diluted, was then sent for sequencing to verify the bands shown on the gel.

Conclusion

- Wellfleet Bay virus (WFB) first identified in 2006[1]
  - Most often infecting common eiders (*Somateria mollissima*) along the MA coast
  - Family *Orthomyxoviridae*, genus *Quaranjavirus*[2]
  - Negative-sense ssRNA genome; enveloped
  - Causes hepatic, pancreatic, and splenic necrosis[2]
- Other potential causes of brief, significant, multi-species, bi-familial (*Anatidae, Laridae*), unusual mortality events include:
  - Avian cholera (*Pasteurella multocida*) is another avian infectious disease that has caused recurrent outbreaks, threatening populations of endangered seabirds on Amsterdam Island[4]
  - Harmful algal blooms are growing as a threat to marine birds and other wildlife, caused by recent strong marine heatwaves[3]

- The 2025 mortality event was not consistent with WFBv infection. Successful amplification of the positive control confirms a successful assay, however no tested birds were found to have the virus.
- Next steps include testing for additional possible causes of mortality including toxicity and alternate pathogens.

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