

Wellfleet Bay Virus Workshop

Tufts University

June 11, 2014



WFBV Presentations

June 11, 2014

Agenda

8:30 Welcome/Opening Remarks

8:45 Introduction - An Overview of the Mortality Events, Timing and General Ecology of Common Eiders on Cape Cod Randall M. Mickley, Chris Dwyer, Julie Ellis, Jennifer R. Ballard, Samantha E. J. Gibbs

9:15 Pathology of Disease Caused by Wellfleet Bay Virus, an Emerging Pathogen of Common Eiders (*Somateria mollissima*) Valerie Shearn-Bochsler, Anne Ballmann, Jeffrey Hall, Chris Dwyer, and Hon Ip

9:45 Biological, Genetic, Antigenic, and Phylogenetic Analysis of Wellfleet Bay Virus

Andrew B. Allison, Jennifer R. Ballard, Robert B. Tesh, Randall M. Mickley, Hon S. Ip, Valerie Shearn-Bochsler, Edward C. Holmes, and Chris Dwyer

10:15 Experimental Inoculation of the Common Eider with Wellfleet Bay Virus

Jennifer R. Ballard, Samantha E. J. Gibbs, Chris Dwyer, John Fischer, Valerie Shearn-Bochsler

10:45 Geographic Patterns of Wellfleet Bay Virus Seroprevalence in the Common Eider (*Somateria mollissima*) Jennifer R. Ballard, Samantha E. J. Gibbs, Chris Dwyer, Randall Mickley, Catherine Soos, H. Grant Gilchrist, N. Jane Harms, Jeffrey Hall, Jean-Francois Giroux, Stéphane Lair, Randy Milton, Glen Parsons, Brad Allen, John Fischer, and Daniel G. Mead

11:15 Early Lunch (to beat the crowd)

12:30 Narrowing the Focus: Annual Movement Patterns of Satellite-Marked Common Eiders Breeding in Boston Harbor Lucas Savoy, Chris Dwyer, Randall M. Mickley, Samantha E. J. Gibbs, Glenn Olsen, H Heusmann, Susannah Corona, Jorge Ayub

1:00 An Investigation into the Potential Source Population of Common Eiders Found Dead at Jeremy Point via DNA Analysis and Comparison to Major Breeding Areas Sarah Sonsthagen, Chris Dwyer, Randall M. Mickley, Samantha E. J. Gibbs, Jean-Francois Giroux, Brad Allen, Randy Milton

1:15 A Comparison of Mercury Concentrations in Common Eiders from Boston Harbor and Other New England Locations Lucas Savoy, Randall M. Mickley, Josh Beuth, Dustin Meattay, Kevin Regan

1:30 Deuterium Dilution: A Non-lethal Method for Measuring Body Composition of Common Eiders Joshua M. Beuth, Scott R. McWilliams, Peter W. C. Paton, Jason E. Osenkowski

2:00 Wellfleet Bay Virus and Sea Duck Conservation: More Questions than Answers

Samantha E. J. Gibbs, Chris Dwyer, Jennifer R. Ballard, Andrew B. Allison, Randall M. Mickley

2:30 Panel Discussion – Ideas, Priorities and Next Steps.

3:00 Conclusion/Closing Remarks/Acknowledgements

Introduction - An Overview of the Mortality Events, Timing and General Ecology of Common Eiders on Cape Cod Randall M. Mickley, Chris Dwyer, Julie Ellis, Jennifer R. Ballard, Samantha E. J. Gibbs

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In Massachusetts, multi-agency collaborative wildlife disease investigation involving common eiders (*Somateria mollissima*) has been underway for several years. Die-offs of these sea ducks have been occurring annually on Cape Cod beaches since at least 2006, and have affected thousands of birds. The mortality events are taking place approximately 20 miles from an important common eider overwintering area in Nantucket sound. The morbidity and mortalities have been occurring on a publicly accessed National Seashore, creating concern among its visitors.

In 2010, the multi-agency investigation discovered a new virus, tentatively named Wellfleet Bay Virus, after the location where it was first detected. Funding from the US Fish and Wildlife Service's Avian Health and Disease Program has thus far enabled the collection of several thousand biological samples, hundreds of necropsies, viral transmission studies, genetics studies, and a satellite telemetry study of the affected common eider population. More than eighteen agencies/institutions have taken part in aiding this investigation to understand the potential impacts of this virus and implications for common eider management decisions. This on-going wildlife disease investigation is a model for interagency collaboration.



Pathology of Disease Caused by Wellfleet Bay Virus, an Emerging Pathogen of Common Eiders (*Somateria mollissima*) Valerie Shearn-Bochsler, Anne Ballmann, Jeffrey Hall, Chris Dwyer, and Hon Ip

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A previously unrecognized virus, now named Wellfleet Bay Virus (WFBV), was first isolated from a Common Eider (*Somateria mollissima*) from Cape Cod, Massachusetts in 2006 from carcasses submitted for necropsy to the USGS National Wildlife Health Center (NWHC) during a mortality event. Based on genetic analysis of five different RNA segments, the virus was determined to be a novel orthomyxovirus (family *Orthomyxoviridae*, genus *Quarjavirus*).

Since 2006, the NWHC has continued to investigate the disease caused by WFBV in both free-flying eiders during subsequent die-offs, and through laboratory inoculation trials in juvenile eiders. Pathologic findings in free-flying eiders infected with WFBV were variable, but consistently included hepatic necrosis and lymphoid depletion. In 2010, healthy eider ducklings were inoculated with WFBV in a controlled laboratory setting. Twenty-five percent of inoculated eiders became severely ill and had to be euthanized. Inoculated eiders developed serum antibodies by Day 4 following inoculation. At necropsy, lesions consistent with those seen in free-flying eiders were present in inoculated birds that became ill; control birds were unaffected. Virus was re-isolated from multiple tissues from affected birds, completing the fulfillment of Koch's postulates for WFBV in Common Eiders.



Biological, Genetic, Antigenic, and Phylogenetic Analysis of Wellfleet Bay Virus

Andrew B. Allison, Jennifer R. Ballard, Robert B. Tesh, Randall M. Mickley, Hon S. Ip, Valerie Shearn-Bochsler, Edward C. Holmes and Chris Dwyer

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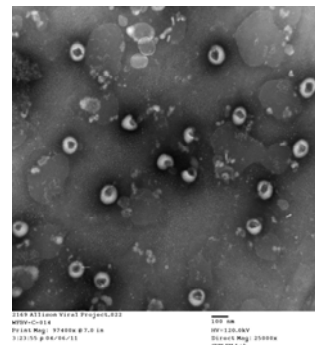
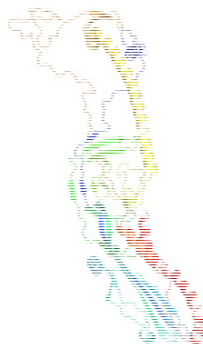
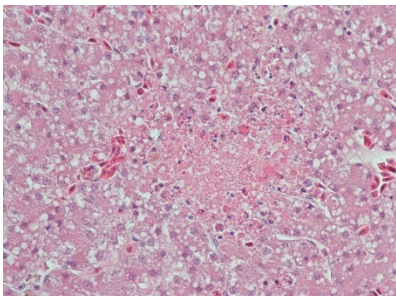
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Since 1998, cyclic mortality events in common eiders (*Somateria mollissima*) have been documented along the coast of Cape Cod, Massachusetts, USA. Although longitudinal disease investigations have uncovered potential contributing factors responsible for these outbreaks, detecting a primary etiological agent has proven enigmatic. Here, we identify a novel orthomyxovirus, tentatively named Wellfleet Bay virus (WFBV), as a potential causative agent of these outbreaks. Genomic analysis of WFBV revealed that it is most closely related to members of the *Quaranjavirus* genus within the family *Orthomyxoviridae*. Similar to other members of the genus, WFBV encodes an alphabaculovirus gp64-like glycoprotein, tentatively suggesting it is tick-transmitted in nature. However, in addition to the six RNA segments encoding the prototypical structural genes identified in other quaranjaviruses, a previously unknown RNA segment (segment 7) was discovered in WFBV that is predicted to encode a novel protein of ~14.3 kDa. Although WFBV shows low to moderate levels of sequence similarity to Quarantil virus and Johnston Atoll virus, the original members of the *Quaranjavirus* genus, additional antigenic, genetic, and phylogenetic analyses demonstrated that it is closely related to the recently identified Cygnet River virus (CyRV) from South Australia, suggesting that WFBV and CyRV may be geographic variants of the same virus.



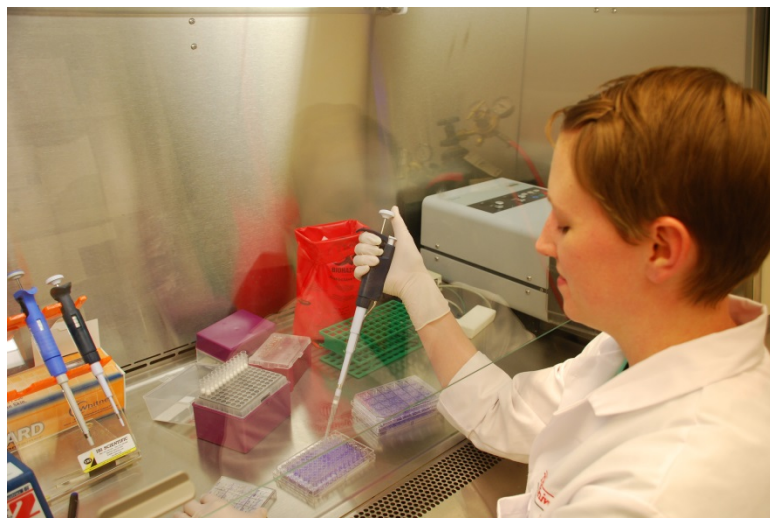
Experimental Inoculation of the Common Eider with Wellfleet Bay Virus Jennifer R. Ballard, Samantha E.J. Gibbs, Chris Dwyer, John Fischer, Valerie Shearn-Bochsler

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Wellfleet Bay virus (WFBV) is an Orthomyxovirus isolated from common eiders (*Somateria mollissima*) collected during recurrent epornitics in Massachusetts. Other viruses in the Quaranjavirus genus are tickborne, but routes of transmission have not been identified for WFBV. In the summer of 2012, three groups of eider ducklings were inoculated with WFBV by intradermal, oral, or tracheal routes. Two to three sham-inoculated birds were cohoused with each group, and a sham-inoculated control group was maintained. All birds were observed for clinical signs. Blood samples were collected at multiple time points; serologic testing was conducted using a microneutralization assay. Oropharyngeal and cloacal swabs were collected throughout the study, and birds from each group were euthanized at multiple times points. At necropsy, tissues were collected for virologic testing and histologic examination. Swabs and tissue samples were tested by virus isolation and confirmatory reverse transcription PCR (rt-PCR). Histologic examination was conducted by routine methods, and immunohistochemical staining (IHC) was applied to affected tissues. Minimal clinical disease was observed in the majority of inoculated birds. Seroconversion occurred in all inoculated groups but not in control nor in contact control birds. Histologic lesions included splenic and hepatic necrosis. Isolates of WFBV were obtained from oropharyngeal swabs, cloacal swabs, and multiple tissues collected before seven dpi. Samples collected 8-30 dpi were negative by virus isolation. Confirmatory rt-PCR and IHC results are pending. These results indicate that WFBV can be transmitted experimentally by multiple routes, although direct transmission to co-housed birds did not occur. Successful transmission via intradermal inoculation and the lack of direct transmission support but do not confirm that WFBV may be arthropod-borne. Minimal clinical disease suggests that field strains of WFBV may be more virulent than the experimental strain or additional factors may be associated with the development of more severe disease.



Geographic Patterns of Wellfleet Bay Virus Seroprevalence in the Common Eider

Jennifer R. Ballard, Samantha E.J. Gibbs, Chris Dwyer, Randall M. Mickley, Catherine Soos, H. Grant Gilchrist, N. Jane Harms, Jeffrey Hall, Jean-Francois Giroux, Stéphane Lair, Randy Milton, Glen Parsons, Brad Allen, John Fischer, and Daniel G. Mead

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From 1998 to the present, mortality events have been reported on a nearly annual basis in American common eiders (*Somateria mollissima dresseri*) on Cape Cod, Massachusetts. Early investigations of these events revealed consistent lesions in affected birds, indicating a shared etiology, and a novel Orthomyxovirus, tentatively named Wellfleet Bay virus (WFBV), has been isolated from multiple birds collected during these events. Concern that WFBV could adversely affect common eider population stability prompted a serologic survey to determine the prevalence, geographic distribution, seasonality, and demographic patterns of WFBV infection in multiple common eider populations. Antibodies against WFBV were detected using a microneutralization assay. Seroprevalences of WFBV in the American common eiders sampled were: Nova Scotia 3.5% (3/85), Maine 3.2% (33/341), Massachusetts 31.7% (46/145), Rhode Island 3.6% (5/137), and Quebec 2.7% (5/195). No antibodies against WFBV were found in two populations of the adjacent subspecies, the northern common eider (*S.m. borealis*), sampled in Nunavut, Canada and Iceland (0/96 and 0/34, respectively). Opportunistic sampling of sympatric species has identified WFBV antibodies in two herring gulls (*Larus argentatus*), two ring-billed gulls (*Larus delawarensis*), and one white-winged scoter (*Melanitta fusca*) in Massachusetts. Statistical analysis of seroprevalence by location, season, and demographic characteristics is underway. Preliminary results indicate that Massachusetts represents an epicenter of WFBV transmission, and that WFBV exposure likely is limited to the American subspecies of common eider and its sympatric species. Further investigation of this area and its role in WFBV epidemiology is warranted.

Narrowing the Focus of the Wellfleet bay Virus Investigation: Annual Movement Patterns of Satellite-marked Common Eiders Breeding in Boston harbor, MA Lucas Savoy, Chris Dwyer, Randall M. Mickley, Samantha E.J. Gibbs, Glenn H. Olsen, H Heusmann, Susannah Corona and Jorge Ayub

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Between 1998 and 2011, 11 recognized mortality events occurred in Common Eiders (*Somateria mollissima*) along the coast of Cape Cod, Massachusetts, USA. The numbers of eiders involved in these outbreaks ranged from 30 to 2,800 individuals, with estimated total losses exceeding 6,000 birds. In 2010, a novel orthomyxovirus tentatively named Wellfleet Bay Virus (WFBV) was isolated from the tissues of four of these birds.

In 2012, biologists along the North American Atlantic coast visited several Common Eider nesting colonies in order to collect blood samples to screen for the presence of the WFBV. Colonies from Nova Scotia, Canada, and Maine and Massachusetts, USA were sampled. Also, wintering eiders from Rhode Island were screened for the virus. Overall, 6% of eiders from Nova Scotia, 0% from Maine, and 4% from Rhode Island tested positive. In Massachusetts, 41% tested positive and one nesting colony in Boston Harbor contained 96% of eiders testing positive for WFBV.

In the spring of 2013, we initiated an annual movement study aimed at following the trail of the eiders breeding in Boston Harbor. We implanted 12 Common Eiders with satellite transmitters in the spring of 2013 and an additional 19 in 2014. The transmitters have the ability to provide location data for 2.5 years. Ten of the eiders from 2013 provided movement data. Molting locations varied, including areas of Maine, Massachusetts, and Boston Harbor. One of the marked birds died in November 2013, at the same location and time period as the eider WFBV die-off that season. Eiders wintered in two locations; Boston Harbor area and Nantucket Sound area. Data collected from this study provides a better understanding of the annual movements of eiders from a high virus outbreak area and their potential interactions with other Atlantic populations of eiders.



An Investigation into the Potential Source Populations of Common Eiders Affected by Wellfleet Bay Virus through DNA Comparison with Eider Breeding Populations in the Eastern U.S. and Canada

Sarah Sonsthagen, [Chris Dwyer](#), Randall M. Mickley, Samantha E. J. Gibbs, Jean-Francois Giroux, Brad Allen and Randy Milton

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Recent mortality events of common eiders (*Somateria mollissima*) associated with the Wellfleet Bay virus (WFBV) on Cape Cod, MA, USA have led to questions regarding the geographic origin and potential population impacts (if any) of this disease on common eiders. Eiders that were previously banded in Maine, Nova Scotia and Quebec have been found dead on Cape Cod, although the limited number of recoveries continues to be insufficient for identifying the source population(s) of eiders affected. Given that the number of breeding common eiders associated with nearby Boston Harbor Islands has remained stable at approximately 300-500 pairs and mortality events are approximately half that number annually, is unlikely that (but currently unknown), eiders involved in the mortality events on Cape Cod are from the local population. Common eiders are unique among sea ducks as they exhibit fine scale spatial genetic structure at both mitochondrial and nuclear markers. Thus, it may be possible to assign birds collected during the annual mortality events to breeding colonies based on their genetic signature. This study is designed to develop a multi-locus data matrix containing reference breeding colonies sampled throughout Maine, Nova Scotia and the Gulf of St. Lawrence. Under a scenario of genetic structure among breeding colonies, we are working toward probabilistically assigning common eiders involved in annual mortality events to breeding areas using the data matrix to examine the spatial distribution and proportion of local and migratory birds involved in dieoff events on Cape Cod.



A Comparison of Mercury Concentrations in Common Eiders from Boston Harbor and Other New England Locations

Lucas Savoy, Randall M. Mickley, Josh Beuth, Dustin Meattley, Kevin Regan

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Mercury is a persistent contaminant, and readily available to most fish and wildlife through atmospheric deposition and localized industrial point sources. Levels of methylmercury, the organic and highly toxic form of mercury, is bio-magnified through marine and freshwater food chains and at certain levels can be harmful to wildlife. Determining mercury concentrations in wildlife by analysis of whole blood is important in understanding contaminant accumulation through consumption of local food sources. Mercury accumulated over time in long lived species, such as the Common Eider (*Somateria mollissima*), can be an added physiological stressor and impact the overall health of an individual bird or population. We collected blood samples from 20 eiders captured near Calf Island (Boston Harbor, Massachusetts) in 2013 to measure the concentration of Hg in their blood. We analyzed the samples for total mercury concentrations, and compared them to Common Eiders sampled at sites in Maine, northern Massachusetts, and Rhode Island. Samples from a total of 144 eiders were used for comparison. Common Eiders sampled from Boston Harbor in 2013 (n=20) contained significantly higher Hg levels than eiders sampled from Maine (n=56) and Rhode Island (n=41). Boston Harbor eiders contained significantly lower levels of Hg than eiders collected in northern Massachusetts (n=27) (Plum Island).



Deuterium Dilution: A non-lethal method for measuring body composition of Common Eiders

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Thousands of sea ducks winter along the nearshore and offshore waters of southern New England and these waters have been recently considered for offshore wind energy development. Anthropogenic disturbances introduced into habitat used by wintering eider as well as diseases have the potential to negatively impact their body condition. Biologists presently lack an accurate, non-lethal method for measuring body composition of sea ducks that allows them to assess body condition. Our goal was to validate the deuterium dilution method for measuring body composition American common eiders (*Somateria mollissima dresseri*). We captured 19 eiders during winter in southern New England, injected each eider with $1,066 \pm 4.5$ mg of 99.9% deuterium oxide, allowed them to rest for 90 minutes and then collected approximately 200 μ l of blood from each eider. We euthanized eiders after collecting blood, recorded structural measures, and performed a carcass analysis. We measured deuterium concentration in blood water using an isotope ratio mass spectrometer, from which we estimated total body water. Deuterium over-estimated body water by $13.30 \pm 0.73\%$ ($R^2 = 0.931$). We derived linear regression models using combinations of 5 variables to estimate wet lean and fat mass. Using a jack knife validation, we determined that the top two models predicted with 2.04 and 2.02 % relative error, respectively, and the top ranked fat mass model predicts with 20.24% relative error. Using these models, we estimated the body composition of 86 captured and released eider. We determined that both male and female eiders significantly increased fat mass in late winter compared to early winter, whereas wet lean mass of males remained constant throughout the winter, while wet lean mass of females was significantly less than males during late winter and decreased compared to early winter. Deuterium dilution is a method that enables field biologists to obtain relatively accurate measurements of common eider body composition without needing to kill the bird to perform extensive and expensive total carcass analyses.



Wellfleet Bay Virus and Sea Duck Conservation: More Questions than Answers Samantha E. J. Gibbs, Chris Dwyer, Jennifer R. Ballard, Andrew B. Allison, Randall M. Mickley

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Recent investigations to determine the ecology and epidemiology of Wellfleet Bay virus (WFBV) have resulted in several important findings: a) a description of the novel virus, b) clinical signs and pathology associated with natural and experimental WFBV infection, c) geographic range of the virus in common eiders (*Somateria mollissima*) based on antibody prevalence, and d) seasonal movements and body condition of common eiders in the affected population. This work has given us a greater understanding of WFBV and common eiders, but has also generated many more questions than answers. Still unknown are: the transmission route(s) of the virus, other host species, possible vectors, the influence of dual infections (could this be a disease complex, role of the eider immune response), the connection between the Boston Harbor Islands and Wellfleet Bay, relationship to Cygnet River virus, what lies behind the timing and tendency of the outbreaks to affect one sex at a time, whether WFBV is signaling negative changes in sea duck health and habitats, and the threshold at which common eiders will be impacted by the virus at a population scale. We will continue our investigations, as the answers to these questions will be critical in helping us to determine and manage for the impacts of WFBV on common eiders and other avian species.

