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PREVALENCE AND DISTRIBUTION OF WELLFLEET BAY VIRUS EXPOSURE IN THE COMMON EIDER (*SOMATERIA MOLLISSIMA*)

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ABSTRACT: Between 1998 and 2014, recurrent mortality events were reported in the Dresser's subspecies of the Common Eider (*Somateria mollissima dresseri*) on Cape Cod, Massachusetts, US near Wellfleet Harbor. The early die-offs were attributed to parasitism and emaciation, but beginning in 2006 a suite of distinct lesions was observed concomitant with the isolation of a previously unknown RNA virus. This novel pathogen was identified as an orthomyxovirus in the genus *Quaranjavirus* and was named Wellfleet Bay virus (WFBV). To assess evidence of exposure to this virus in Common Eiders, we conducted a longitudinal study of the prevalence of WFBV antibodies at multiple locations from 2004–14; we collected 2,258 serum samples from six locations and analyzed each using a microneutralization assay. Results corroborate the emergence of WFBV in 2006 based on the first detection of antibodies in that year. Significantly higher prevalence was detected in Common Eiders sampled in Massachusetts compared to those in Maine, Nova Scotia, and Québec. For birds breeding and wintering in Massachusetss, viral exposure varied by age, sex, and season of sampling, and prevalence by season and sex were highly interrelated with greater numbers of antibody-positive males in the autumn and females in the spring. No evidence of viral exposure was detected in the Northern subspecies (*Somateria mollissima borealis*). Among the locations sampled, Massachusetts appears to be the epicenter of Common Eider exposure to WFBV. Further research is warranted to understand the factors controlling the epidemiology of WFBV in Massachussetts, including those that may be limiting geographic expansion of this virus.

Key words: Common Eider, serology, seroprevalence, Wellfleet Bay virus.

INTRODUCTION

Between 1998 and 2014, recurrent mortality events were reported in the Dresser's subspecies of the Common Eider (*Somateria mollissima dresseri*; hereafter Dresser's ssp.) on Cape Cod, Massachusetts, US near Wellfleet Harbor (41°54′15″N, 70°2′33″W). These events occurred in the spring (April–May) and autumn (October–December) and involved

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from <10 to an estimated 2,400 birds per event (National Wildlife Health Center [NWHC] 2015; J.R.B. unpubl data). The early die-offs were attributed to parasitism and emaciation (NWHC 2015). However, beginning in 2006, a suite of consistent lesions was observed in the dead eiders including hepatocellular, pancreatic, and splenic necrosis, concomitant with the isolation of a previously unknown RNA virus (NWHC 2006; V. Shearn-Bochsler unpubl. data). Preliminary genomic characterization identified the virus as a novel orthomyxovirus in the genus *Quaranjavirus*; it was subsequently named Wellfleet Bay virus (WFBV; Allison et al. 2015).

Field investigations of the recurrent mortality events included carcass counts, band recoveries, serum sampling of moribund individuals, necropsy of suitable specimens, and tissue collection for isotope and genetic analysis. Leg bands collected from the carcasses traced individual birds to nesting colonies in Maine, Nova Scotia, Québec, and Labrador. These band recoveries represented all of the known banding programs at the time of the mortality events and most of the major nesting colonies of the Dresser's ssp. These findings raised concern among migratory bird managers that WFBV could have adverse, population-level effects in this subspecies.

Globally, there are six subspecies of Common Eider, three of which were included in this study (Clements et al. 2015). The Dresser's ssp. breeds from Labrador to Massachusetts and generally winters south of the Gulf of St. Lawerence. The Northern subspecies (*Somateria mollissima borealis*; hereafter Northern ssp.) breeds in northeastern Arctic Canada, Greenland, and Iceland and winters in the maritime provinces of Canada, Southern Greenland, and Iceland. Although overlap between these two subspecies occurs, there is reasonable temporal separation through most of their ranges. The third subspecies noted herein is the Hudson Bay subspecies (*Somateria mollissima sedentaria*: hereafter Hudson Bay ssp.), which breeds and winters in James and Hudson Bays and overlaps the Northern ssp. in some areas. Although some intermixing of subspecies occurs, morphologic differences exist that aid in classification (Mendall 1986; Waltho and Coulson 2015).

Two experimental inoculation trials have examined the pathologic, immunologic, and clinical effects of WFBV in Common Eiders (J.R.B. unpubl. data; V. Shearn-Bochsler unpubl. data). These studies examined, among other things, seroconversion of naïve eiders following exposure to WFBV. Seroconversion was detected by 5 d postinoculation and could be detected through day 30. Studies examining long-term antibody persistence have not been performed. Based on these findings, detection of WFBV antibodies from free-ranging Common Eiders should be a useful indicator of viral exposure, though temporal interpretation is limited.

Here we report the findings of a longitudinal study on the prevalence of WFBV antibodies in multiple subpopulations of Common Eiders. We directed particular attention to the geographic and demographic distribution of antibody-positive birds. Our objectives were to: 1) estimate the timing of WFBV emergence in the Dresser's ssp. through analysis of banked serum samples; 2) document the geographic range over which exposure to WFBV can be detected in the Dresser's ssp.; 3) examine the prevalence of WFBV exposure within the Dresser's ssp. by location, season, sex, and age; and 4) look for evidence of WFBV exposure in the Northern ssp. Our results will aid in assessing the potential for adverse, population-level effects associated with WFBV in Common Eiders and in directing future research into WFBV epidemiology.

MATERIALS AND METHODS

Sample acquisition

Serum samples were acquired from: 1) banked serum from previous studies in Maine, Nunavut, and Québec (2004–11); 2) targeted sampling of apparently healthy, sick, and recently dead birds collected in Massachusetts by multiple methods (2011–14); and 3) samples from apparently healthy Common Eiders collected during normal banding activities or in conjunction with on-going

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research in Iceland, Maine, Nova Scotia, Nunavut, Québec, and Rhode Island (2011–14).

Samples were collected in Iceland from apparently healthy, female Common Eiders during spring nesting; the study site and handling procedures were described by Dusek et al. (2014). Historic samples from Maine were collected from apparently healthy, molting eiders captured in the autumn near Metinic Island (43°52′51″N, 69°7′26″W). More-recent samples from Maine were collected from hunter-harvested eiders in the autumn and from nesting females on seven islands along the state's south-central coastline. Sick and dead eiders collected on Cape Cod, Massachusetts were gathered by hand or captured using dip nets during spring and autumn mortality events; samples also were submitted by local wildlife rehabilitation clinics. Apparently healthy eiders from Massachusetts were captured using dip nets and floating mist nets in areas of Cape Cod (41°53′37″N, 70°4′12″W) and Boston Harbor (42°20′35″N, 70°53′47″W) in the spring and autumn, and additional samples were collected from hunter-harvested birds in the autumn. Samples from Nova Scotia were collected from nesting eiders on 13 islands along the eastern and southern coasts of the province. Samples from Nunavut were collected from nesting eiders at a study site described by Buttler et al. (2011). Historic and recent samples were collected from nesting eiders in Québec at study sites in the St. Lawrence estuary (Joint Working Group 2004; J.-F.G. unpubl. data). Samples were collected from apparently healthy eiders in Rhode Island captured with floating mist nets in the autumn and winter (Beuth 2013).

Blood sampling techniques and data collection varied according to the institution overseeing the fieldwork and the individual collector. Generally, samples were collected using sterile, 25–22-ga hypodermic needles and 3-mL plastic syringes. Routine venipuncture was conducted from the jugular, basilic, or medial metatarsal veins. Postmortem samples were collected from the jugular vein or heart. Samples were centrifuged within 24 h of collection. Serum was pipetted or decanted into clean vials and stored at –20 C. Protocols for bird handling and sample collection were reviewed by the institutional animal care and use committee or equivalent body of the institution responsible for each field study. Sampling conducted solely for this project was performed using techniques approved by the University of Georgia Institutional Animal Care and Use Committee (A2011 02-011-Y3-A3). For each sample, sex, age class, band number, and the date and location of sampling were requested but were not always available.

Microneutralization assays

Two microneutralization assays were used to detect WFBV antibodies, and each sample was tested by one or both techniques. The first assay screened each serum sample at a 1:8 dilution using Vero MARU [Middle America Research Unit] cells as the biological indicator (National Veterinary Services Laboratories 1981). The challenge virus was a cell-line adapted, fourth passage of WFBV originally isolated from the liver of a sick Common Eider collected in 2010. The challenge virus was diluted to $10^{3.0}$ tissue culture infective doses ($TCID_{50}$)/25 μL (back titration mean $10^{3.2}$ $TCID_{50}$/25 μL; range $10^{2.2}$–$10^{3.6}$ $TCID_{50}$/25 μL). The virus and serum were incubated together in a 96-well plate for 1 h at 37 C and 5% CO_2, after which Vero MARU cells in suspension were added to each well. The plates were incubated for 10–11 d and fixed with 10% neutral-buffered formalin with 0.25% crystal violet. Two control wells and two experimental wells were used for each sample. Samples for which both experimental wells demonstrated >50% monolayer destruction were considered negative for WFBV antibodies. Samples for which either control well demonstrated >50% monolayer destruction were considered to contain endogenous, cytotoxic constituents; these were excluded from further analysis. Samples with at least one positive experimental well (<50% monolayer destruction) were tested by the second microneutralization format if sufficient volume was available.

Samples found positive by the first assay, and all samples collected in Massachusetts, were tested using the second microneutralization format to quantify antibody titers and detect birds with titers <8 for increased sensitivity. For this assay, each serum sample was serially diluted from 1:4 to 1:256 and incubated in the same manner described above with $10^{3.0}$ $TCID_{50}$/25 μL of virus (back titration mean $10^{3.0}$ $TCID_{50}$/25 μL; range $10^{2.4}$–$10^{3.7}$ $TCID_{50}$/25 μL). Four replications per sample were conducted if volume was sufficient; rarely, one to two replicates were used. The geometric mean titer (GMT) of all replicates was considered the final result, and samples with a GMT ≥8 were classified as positive. For samples where small volumes only permitted use of the first assay, the GMT was calculated from that assay and interpreted as above.

All tests were interpreted by the same individual and blinding was not attempted. However, these assays consistently yielded strong positive or negative results, with minimal risk of subjective interpretation.

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Statistical analysis

Statistical analysis was conducted with STATA®13.1 (StataCorp, College Station, Texas, USA). Subpopulations were considered to be portions of each subspecies separated geographically with a low likelihood of within-season exchange of birds, recognizing that intermixing of these groups undoubtedly occurs between seasons (Beuth 2013). Based on these criteria, results from Rhode Island and Massachusetts were combined for analysis and, because the latter is of primary interest, this group is hereafter referred to as the "Massachusetts subpopulation." The dates of sample collection were assigned to either breeding (April–July) or nonbreeding (August–March) seasons, and age classes were designated as juvenile (hatch-year and second-year birds) and adult (after-second-year) (Waltho and Coulson 2015). Subspecies identity was assumed on the basis of location and season. All samples from Nunavut and Iceland were classified as the Northern ssp., although the Hudson Bay ssp. may have occurred as an unreported minority at Nunavut. All samples from Maine, Massachusetts, Nova Scotia, and Québec were classified as Dresser's ssp. Due to the aforementioned morphologic differences, it is unlikely that significant intermixing of subspecies would have been overlooked.

The prevalence of WFBV exposure was calculated for each sampling location with 95% confidence intervals (CI). The relationship between WFBV exposure and subspecies designation was assessed using chi-square (χ^2) analysis for independence. A second comparison was made between location and antibody prevalence within the Dresser's ssp. only. This comparison was run twice, first using data for the entire subspecies and again using only data from nesting females. Within the Dresser's ssp., the relationships between antibody prevalence and the remaining categorical predictor variables (sex, age, season of sampling) were examined using χ^2 analysis. Finally, antibody prevalence and the categorical predictor variables were compared within the Massachusetts subpopulation. No between-year comparisons were undertaken, as variability in sampling by region would prohibit interpretation.

We used backward selection to construct a multivariate logistic regression model to examine the relationship between demographic characteristics and WFBV exposure in the Massachusetts subpopulation. Initially, the model included all three independent covariates (sex, age, and season) and all possible two-way interactions. The interactions for sex/age and age/season were removed because several of the possible combinations were not represented in the data, and those interactions could not be assessed. The final model retained all covariates with a P-value <0.05 and those that resulted in a $>10\%$ change in the coefficient value of another covariate when removed. The model was assessed for goodness of fit using Pearson's χ^2 statistic.

RESULTS

In total 2,258 serum samples were acquired from 2,233 birds, and 2,211 results were generated. Samples for which results could not be generated were clotted, cytotoxic, or of insufficient volume. Table 1 presents the number of positive results over the number of results generated for each year and location. No WFBV antibodies were detected in 2004 or 2005. The first WFBV exposure we detected was in three eiders sampled in the St. Lawrence Estuary of Québec in 2006. Based on this apparent year of emergence, it is questionable whether birds sampled prior to 2006 would have had any opportunity for exposure to WFBV, and the results generated for these years could artificially dilute the apparent antibody prevalence for some locations. Thus, the 215 samples collected in 2004 and 2005 were excluded from analysis.

The prevalence of WFBV antibodies at our sampling locations during or after 2006 were: Iceland 0% (0/52; 95% CI $\leq$5.6%), Maine 3.2% (11/346; 95% CI 1.8–5.7%), Massachusetts 16.3% (63/387; 95% CI 12.9–20.3%), Nova Scotia 3.3% (6/180; 95% CI 1.5–7.2%), Nunavut 0% (0/530; 95% CI $\leq$0.06%), and Québec 1.4% (7/501; 95% CI 0.7–2.9%). Antibody prevalence was significantly different ($P<0.001$) between the Northern ssp. (0/582; 0%) and Dresser's ssp. (87/1,414; 6.2%). Because the total prevalence for the Northern ssp. was 0%, no further statistical analyses were performed on these data.

Within the Dresser's ssp., antibody prevalence did not vary significantly by sampling location when comparing the (combined breeding and nonbreeding season) subpopulations in Québec, Nova Scotia, and Maine (P=0.150). Prevalence and location ceased to be independent when Massachusetts was included in the comparison ($P<0.001$), indicating that the higher prevalence at this

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TABLE 1. Prevalence of antibody to Wellfleet Bay virus in the Common Eider (*Somateria mollissima*) by location and year. Results reflect combined breeding and nonbreeding season samples, and intermixing of birds by location is likely within subspecies. Dashes indicate that no samples were available for that combination of year and location.

	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Subtotal
Massachusetts/ Rhode Island[a]	—	—	—	—	—	—	—	8/86	27/126	16/71	12/104	63/387 (16.3%)
Maine[a]	0/39	—	—	—	—	—	—	0/10	11/336	—	—	11/385 (2.9%)
Québec[a]	0/20	0/156	3/197	—	—	—	—	—	4/304	—	—	7/677 (1.0%)
Nova Scotia[a]	—	—	—	—	—	—	—	—	3/85	3/95	—	6/180 (3.3%)
Nunavut[b]	—	—	—	0/151	0/107	0/82	0/31	0/96	0/63	—	—	0/530 (0%)
Iceland[c]	—	—	—	—	—	—	—	—	0/34	0/18	—	0/52 (0%)
Subtotal	0/59 (0%)	0/156 (0%)	3/197 (1.5%)	0/151 (0%)	0/107 (0%)	0/82 (0%)	0/31 (0%)	8/192 (4.2%)	45/948 (4.7%)	19/184 (10.3%)	12/104 (11.5%)	Total: 87/2,211

[a] Samples for *Somateria mollissima dresseri*.
[b] Samples for *Somateria mollissima borealis* with possible *Somateria mollissima sedentaria* (all analyzed as *S. m. borealis*).
[c] Samples for *S. m. borealis*.

TABLE 2. Demographic characteristics and total prevalence of antibody to Wellfleet Bay virus within the Dresser's subspecies of the Common Eider (*Somateria mollissima dresseri*) after 2005 in the USA and Eastern Canada.

	No. positive/results (%)	P[a]
Male	20/229 (8.7)	0.061
Female	65/1,180 (5.5)	—
Juvenile	2/63 (3)	0.312
Adult	81/1,283 (6.3)	—
Breeding	70/1,112 (6.3)	0.67
Nonbreeding	17/302 (5.6)	—

[a] P-value based on Pearson's chi-square test. Dashes indicate a paired comparison to the preceding variable.

location was statistically significant. The majority of sampling in Maine, Nova Scotia, and Québec targeted nesting females, with only a small number of males sampled (n=44). Consequently, a second analysis of prevalence by location was conducted using only results from nesting females to determine if the observed pattern persisted. The prevalences of WFBV antibodies in nesting female Dresser's ssp. during and after 2006 by location were: Maine 3.7% (11/299; 95% CI 2.0–6.5%), Massachusetts 48% (39/82; 95% CI 37.0–58.4%), Nova Scotia 3.4% (6/179; 95% CI 1.5–7.3%), and Québec 1.4% (7/495; 95% CI 0.7–2.9%). Maine, Nova Scotia, and Québec had similar prevalences (P=0.096), but prevalence was not independent of sampling location when the Massachusetts subpopulation was included in the analysis (P<0.001), corroborating the previous finding.

The relationships of antibody prevalence to sex, age, and season were assessed within the Dresser's ssp. (Table 2). In short, these analyses indicate that prevalence over the entire subspecies was independent of sex, age, and season.

Due to its high antibody prevalence, the Massachusetts subpopulation was evaluated further (Table 3). Prevalence for this subpopulation was significantly higher in the breeding season (P<0.001) and in adult birds (P=0.001). Overall, there was a higher prevalence in females than in males (females=20.8% n=197; males=10.8% n=185; P=0.008), but this varied

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TABLE 3. Demographic characteristics and prevalence of antibody to Wellfleet Bay virus in the Massachusetts/Rhode Island, USA subpopulation of Dresser's subspecies of Common Eider (*Somateria mollissima dresseri*) after 2005.

	No. positive/results (%)	*P*[a]
Male (year-round)	20/185 (10.8)	0.008
Female	41/197 (20.8)	—
Male (breeding)	7/50 (14)	<0.001
Female	39/82 (47.6)	—
Male (nonbreeding)	13/135 (9.6)	0.009
Female	2/115 (1.7)	—
Juvenile	2/58 (3)	0.001
Adult	57/262 (21.8)	—
Breeding	46/132 (34.8)	<0.001
Nonbreeding	17/255 (6.7)	—

[a] *P*-value based on Pearson's chi-square test. Dashes indicate a paired comparison to the preceding variable.

by season. In the breeding season, females had a higher prevalence (females=48% *n*=82; males=14% *n*=50; $P<0.001$) while in the nonbreeding season males had a higher prevalence (females=1.7% *n*=115; males=9.6% *n*=135; $P=0.009$).

The final multivariate logistic regression model for the Massachusetts subpopulation included the explanatory variables sex, age, season, and a sex/season interaction (Table 4). The age covariate was not statistically significant but was retained to control confounding in the season covariate. The model demonstrated good fit based on the Pearson χ^2 statistic ($P=0.87$). During the breeding season, the odds of detecting WFBV exposure in males were 82% lower than in females (odds ratio [OR]=0.18; 95% CI 0.07–0.45), but during the nonbreeding season the odds of detecting WFBV exposure in males were 390% higher than in females (OR=4.9; 95% CI 1.04–22.7), after adjusting for age. In females, the odds of detecting WFBV exposure during the nonbreeding season were 97% lower than during the breeding season (OR=0.03; 95% CI 0.01–0.14), after adjusting for age. In males, the odds of antibody detection during the nonbreeding season were 14% lower than during the breeding season (OR=0.86; 95% CI 0.31–2.4), but this finding was not statistically significant. The odds of detecting WFBV exposure in adult birds were 280% higher than in juvenile birds (OR=3.8; 95% CI 0.84–17.1), after adjusting for sex and season, but this finding was not statistically significant.

We recaptured and sampled 24 birds in multiple years, and the majority of these were from Massachusetts. Fourteen were negative at every sampling event. Results for the remaining 10 birds demonstrated marked

TABLE 4. Multivariate logistic regression model for prediction of Wellfleet Bay virus exposure in Dresser's subspecies of Common Eider (*Somateria mollissima dresseri*) from Massachusetts/Rhode Island, USA.

Variable	Coefficient (SE)	Odds ratio (95% CI)[a]	*P*
Sex			
Female	Referent		
Male	−1.72 (0.47)	NR	<0.001
Age			
Juvenile	Referent		
Adult	1.33 (0.77)	3.8 (0.84, 17.09)	0.083
Season			
Breeding	Referent		
Nonbreeding	−3.46 (0.75)	NR	<0.001
Sex by Season			
Male/nonbreeding	3.30 (0.91)	NR	<0.001
Constant	−1.36 (0.78)	—	0.082

[a] CI = confidence interval; NR = odds ratios not reported because they depend on the level of the interacting variable; see text. Dash indicates that an odds ratio is not calculated for the constant.

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TABLE 5. Interannual variation in serology for Wellfleet Bay virus in recaptured Common Eiders (*Somateria mollissima dresseri*) from Massachusetts/Rhode Island, USA. Titers are included in parentheses; titers ≥8 were considered positive. Dashes indicate that the sampling date does not fall within the specified year.

Bird band number	Sampling date	2012	2013	2014
2047-12111	14 May 12	Positive (32)	—	—
	30 April 13	—	Negative (5.7)	—
2047-12110	14 May 12	Positive (64)	—	—
	30 April 13	—	Positive (8)	—
2047-12101	14 May 12	Positive (53.8)	—	—
	11 June 13	—	Negative (4.8)	—
2047-12102	14 May 12	Positive (19)	—	—
	1 May 14	—	—	Negative (4.8)
2047-12103	14 May 12	Positive (8)	—	—
	30 April 14	—	—	Negative (4)
2047-12107	14 May 12	Positive (16)	—	—
	30 April 14	—	—	Negative (4.8)
2047-12108	14 May 12	Positive (11)	—	—
	12 June 14	—	—	Negative (4)
2047-12120	14 May 12	Positive (16)	—	—
	1 May 14	—	—	Negative (4.8)
2047-65031	1 May 13	—	Negative	—
	1 May 14	—	—	Positive (11.3)
2047-65040	11 June 13	—	Positive (26.9)	—
	12 June 14	—	—	Positive (19)

year-to-year variation in individual titers (Table 5).

DISCUSSION

The first isolation of WFBV from free-ranging eiders and the first detection of WFBV antibodies in this study corroborate an apparent WFBV emergence in 2006 (NWHC 2006). Although the initial serologic detections came from samples collected in Québec, there is no further evidence supporting this location as the origin of the virus. No mortality events associated with WFBV have been reported in Québec, and subsequent sampling found a low antibody prevalence in Québec. Samples were not available from other locations for 2006, and all subsequent information indicates that Massachusetts is likely more important in the epidemiology of WFBV.

For the Dresser's ssp. as a whole, birds sampled in Massachusetts/Rhode Island had significantly higher prevalences than those sampled in Maine, Nova Scotia, or Québec, but overall prevalence was independent of age, sex, and season. The relationship between location and prevalence appears to be robust, but the demographic findings should be considered in the context of sample distribution. Common Eiders spend large amounts of time in flocks on the open ocean, making them difficult to sample. Only during the nesting season do females spend extended periods on land, and it is during this time that they are most-often studied. Thus, the samples available for this study were heavily biased toward breeding females. For the same reason, the subpopulations in Maine, Nova Scotia, and Québec were sampled more frequently in the breeding seasons. Finally, nestling birds were not sampled during the breeding season and, therefore, sampling of juvenile birds predominately occurred in the nonbreeding season. In summary, more-homogenous sampling across subspecies could reveal patterns of exposure not detected here. In an effort to account for these limitations, a

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separate analysis of WFBV antibody prevalence by location was conducted comparing only nesting females. The results corroborate the observed relationship between sampling location and prevalence. This corresponds well with the observation that WFBV-associated mortality has been limited to Massachusetts and indicates that WFBV epidemiology at this location warrants further examination.

Somewhat more-homogenous sampling was conducted in the Massachusetts subpopulation, allowing for better analysis of demographic patterns of exposure. Chi-square analysis of independence and multivariate logistic regression of data from this subpopulation provided complementary results. Higher antibody prevalence was detected in adults than in juvenile birds. The small sample size of juveniles likely affected the significance of this finding, but it is biologically reasonable if age affects opportunity for exposure. The inclusion of age in the logistic regression model controlled confounding in the season covariate, likely due to sampling of juvenile birds predominantly during the nonbreeding season.

Within the Massachusetts population, antibody prevalence by sex and season were variable but closely interrelated. During the breeding season, prevalence was greater in females than in males. The reason for this is unclear, but it may relate to differences in susceptibility or exposure. An obvious sex-based difference in behavior is the time females spend on land during incubation, but other behavioral differences likely exist.

Wellfleet Bay virus is related taxonomically to the known arboviruses Quaranfil virus and Johnston Atoll virus (Presti et al. 2009; Allison et al. 2015). If WFBV is vector-borne, time spent on land could increase exposure risk. A modest variety of ectoparasites has been documented in Common Eiders, including the tick *Ixodes uriae*, but attempts to recover plausible vector species from nesting colonies in Massachusetts have failed (Smith et al. 2006; J.R.B. unpubl. data). Additionally, transmission associated with land-based arthropods fails to explain autumn mortality events when Common Eiders reside offshore. In Massachusetts during the nonbreeding season, WFBV antibodies were detected in a larger proportion of males than of females, but the overall prevalence in the population was lower than during the breeding season. The increased proportion of antibody-positive males may be the result of sampling bias associated with the investigation of male-dominated mortality events, but it also may reflect a true association where increased exposure of males to the virus results in mortality as well as increased antibody prevalence. Massachusetts is one of the southernmost breeding locations for the Common Eider and a major wintering ground for the Dresser's ssp., including birds from more-northerly breeding colonies. Thus, the lower overall prevalence in Massachusetts during the nonbreeding season may be due to a migratory influx from low-prevalence colonies; waning titers in previously exposed birds; the natural addition of naïve, hatch-year birds; or some combination of these.

Serial samples from a small number of recaptured birds demonstrated marked interannual variation in serologic status. To consider these findings in context, it should be noted that an extremely high antibody prevalence (96%; 21/22) and high individual titers were observed in nesting females on Calf Island, Boston Harbor during the spring of 2012 (J.R.B unpubl. data). Subsequent sampling of this population in 2013 and 2014 demonstrated lower prevalences (34%; 10/29 and 26%; 8/31, respectively) and lower individual titers. Reversion to antibody-negative status was observed in multiple individuals. A general pattern of bimodal mortality (autumn and spring) continued to occur throughout this time including autumn 2011, autumn 2012, spring 2013, autumn 2013, and spring 2014, with no mortality reported in the winter or summer. Unfortunately, small sample sizes prevented statistical evaluation among years, but the relationship between population immunity and outbreak frequency/severity warrants further investigation.

Although the prevalence of WFBV antibody was higher in the Massachusetts subpopulation, antibodies were detected in birds from

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nesting colonies in Maine, Nova Scotia, and Québec. Massachusetts and Rhode Island are a common wintering area for birds from these colonies, and mortality events occur regularly in Massachusetts during the nonbreeding season. Therefore, it is possible that Massachusetts is the site of exposure for antibody-positive birds detected in the other nesting colonies. The low prevalence of antibodies in these populations may result from one or more mechanisms: 1) only a small proportion of the nesting birds from Maine, Nova Scotia, and Québec are migrating to Massachusetts in the nonbreeding season; 2) the rate of WFBV transmission in Massachusetts is lower during the nonbreeding season; 3) antibody titers may wane from the time of nonbreeding season exposure to breeding season sample collection; and 4) mortality associated with WFBV may be higher in eiders that migrate farther, resulting in fewer birds with convalescent titers. We cannot rule out the possibility that WFBV transmission is occurring on the northern nesting colonies, but it appears that such transmission, if present, occurs at a much lower frequency than in Massachusetts. One possible source for such exposures could be WFBV-infected eiders from Massachusetts interacting with birds at these locations during postbreeding or molt-migrations; there is preliminary evidence that such migrations occur (L. Savoy unpubl. data).

It appears that coastal Massachusetts is an epicenter for WFBV exposure, but the reason for this is unclear. The virus' source(s) and route(s) of transmission are unknown, as is the mechanism for bimodal mortality. The duration of our study beyond the apparent emergence of WFBV should have been sufficient to detect increases in antibody prevalance indicative of viral range expansion, as is typically observed following the introduction of a new pathogen. Yet, no such increases were detected. Therefore, it seems likely that some unidentified, epidemiologic factor has limited the geographic spread of WFBV and may be controlling the observed patterns of exposure and disease in Massachusetts. Common Eiders in Massachusetts may be experiencing stressors, demonstrating migratory patterns, or sharing habitat with species that differ from other Common Eider populations. Additionally, much of the Massachusetts breeding population is found close to a large urban center (Boston, Massachusetts) and likely experiences substantial anthropogenic influence. This greatly expands the possible sources for virus introduction, pathogen maintenance, and population stress. From these findings, it appears that the Massachusetts eider population is at greatest risk for adverse effects due to WFBV; however, ongoing surveillance indicates that this population has been increasing in recent years (Paton et al. 2005; C.D. unpubl. data). Nonetheless, further investigation of WFBV in this area is warranted to better understand its epidemiology and predict its potential for future range expansion.

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