Factors affecting Western Atlantic red knots (*Calidris canutus rufa*) and their prey during spring migration on Virginia's barrier islands

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ACADEMIC ABSTRACT

Understanding factors that influence a species' distribution and abundance across their annual cycle is needed for range-wide conservation planning. Every year during spring migration, thousands of federally threatened (U.S.A.) and endangered (Canada) migratory Western Atlantic red knots (*Calidris canutus rufa*, 'red knot') use Virginia's barrier islands as stopover habitat to regain the fat required to continue flights to breeding grounds. Because the red knot completes one of the longest avian migrations in the world and relies on variable prey resources at its stopover grounds, the red knot exemplifies the challenges faced by long-distance migrant shorebirds. These challenges may be exacerbated by climate change, as long-distance migrants may be unable to adapt quickly to changing prey ranges and abundances, resulting in spatial and temporal mismatches between predators and prey. More specifically, as climate change causes ocean temperatures near Virginia's barrier islands to rise, organisms that live within the intertidal zone, like blue mussels (*Mytilus edulis*), are experiencing range shifts. Here, we 1) confirmed what prey red knots select in Virginia, 2) addressed the factors that affect red knot site selection, red knot flock size, and prey abundances across Virginia's barrier island intertidal shoreline during 2007 – 2018, and 3) predicted the origin of juvenile blue mussels, a key prey resource for red knots in Virginia.

To determine which prey are most available to red knots in Virginia, we collected sand and peat substrate core samples from Virginia's ocean intertidal zone and counted the number of prey in each sample. We compared these prey availability data to prey DNA data obtained from fecal DNA metabarcoding analyses on red knot feces (*n* = 100) collected on peat and sand substrates between 2017 – 2019. Red knots consumed prey from Orders Veneroida (clams), Mytiloida (mussels), Diptera (flies), and Amphipoda/Calanoida (crustaceans). While crustaceans were the most abundant prey on both sand (70.80% of total prey counted) and peat (74.88%) substrates, red knots selected crustaceans less than expected given their availability. Red knots selected clams and mussels, supporting their status as bivalve specialists in Virginia.

After determining which prey red knots consumed and selected in Virginia, we predicted the number of red knots using Virginia's barrier island stopover during their migratory stopover (May 14 – 27, 2007 – 2018) annually. We used confirmed prey, tide, distance to known roosts, and red knot winter counts from Tierra del Fuego to inform zero-inflated negative binomial mixed-effects regression models of red knot site selection and flock size in Virginia. We also used generalized linear mixed-effects regression models to determine how climatic and geomorphological factors affected prey abundances. Modeled red knot peak counts were highest in 2012 (11,644) and lowest in 2014 (2,792; $\bar{x}$ = 7,055, SD = 2,841); the trend over time was variable but there was no evidence of a linear increase or decrease. Red knots selected foraging locations with more prey, though red knot flock size did not consistently relate to prey abundance. Tide, substrate, and water temperature affected prey availability. While different prey responded to these covariates in variable ways, prey generally were most abundant on peat banks at low tide.

Given the importance of blue mussels in the red knot's diet and distribution in Virginia, if the blue mussel's range continues to contract northward, red knots could be faced with additional fat replenishment challenges. We analyzed the variation in blue mussels from 2010 – 2018 by

collecting core samples on peat banks in Virginia and counting the number of blue mussels in the cores. To approximate the origin of Virginia's juvenile blue mussels and determine how continued ocean temperature warming may further affect the blue mussel's range contraction, we conducted oxygen stable isotope ($\delta^{18}O_c$) analyses on 74 blue mussel shell umbos (the first portion of the shell precipitated) and shell edges (the most recently precipitated shell) to compare and predict where different portions of the shell were formed. We compared blue mussel shell compositions to $\delta^{18}O_c$ calculated in equilibrium with regional ocean water using recorded $\delta^{18}O_w$ data and sea surface temperature data from ocean buoys between New Hampshire and Virginia. Blue mussel abundance/core sample declined over the duration of our study (Spearman's rank correlation coefficient: ρ(rho) = -0.31, $p < 0.001$), with the highest abundance in 2010 ($\bar{x}$ blue mussels/core sample = 537.88, SE = 85.85) and lowest in 2016 ($\bar{x}$ = 34.08 blue mussels/core sample, SE = 6.96). Blue mussel umbos ($\bar{x}$ $\delta^{18}O_c$ = -0.23‰, SE = 0.12) contained more positive $\delta^{18}O_c$ than shell edges ($\bar{x}$ $\delta^{18}O_c$ = -0.53‰, SE = 0.20), suggesting that Virginia's blue mussels originated from ocean populations in more saline and/or colder water than that within Virginia's intertidal zone. Blue mussel umbo $\delta^{18}O_c$ were not different than $\delta^{18}O_c$ calculated in equilibrium with regional ocean water off the Virginia and Delaware coasts, suggesting that Virginia's blue mussels originated in ocean waters between Delaware and Virginia; however, they may have originated in waters as far north as New York in some years, potentially decreasing the risk of blue mussels being completely extirpated from Virginia in the near future.

While red knots currently use spring migratory stopovers across the United States' Atlantic Coast, from Florida to New Jersey, the largest spring concentrations of knots are confined to the Delaware Bay and Virginia's barrier islands. Because these stopover grounds support large proportions of the red knot's migratory population, any changes in the factors that affect red knots at these stopover sites could have lasting implications for red knots. The blue mussel's range contraction and decline over time in Virginia, for example, is concerning from a conservation perspective. Red knots require easily accessible and abundant prey resources to efficiently replenish fat-stores needed for continued migration and breeding. Additionally, because red knots breed within a narrow period, any delays on stopover grounds could prevent red knots from breeding, even if they survive migration.

Our research demonstrates that red knots use prey abundance as a determinant when selecting foraging locations, and that peat banks, while only sporadically available across the barrier islands at mid- to low-tides, contain higher prey abundances than sand. Thus, to continue maximizing the availability of prey in Virginia, measures should continue to be taken to allow natural island migration processes that encourage the presence of both sand and peat substrates. Beach nourishment and stabilization projects are often used on coastal beaches to prevent shoreline erosion; however, such actions prevent the formation of peat banks by blocking island migration processes. A reduction in peat banks could decrease the abundance of prey available to red knots, making weight gain during the critical stopover period more challenging for red knots. Additionally, beach nourishment through sand replenishment buries invertebrate prey, potentially causing mass prey mortality and reducing shorebirds' ability to access deeply buried prey. To prevent the loss of important peat banks on these islands, and to prevent disrupting predator-prey interactions, managers should continue their ongoing focus on allowing natural processes to occur on Virginia's barrier islands.

Factors affecting Western Atlantic red knots (*Calidris canutus rufa*) and their prey during spring migration on Virginia's barrier islands

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GENERAL AUDIENCE ABSTRACT

Red knots (*Calidris canutus rufa*) are imperiled shorebirds that migrate thousands of kilometers each year from wintering grounds as far south as Argentina to breeding grounds in the Canadian Arctic. To migrate such long distances, red knots use stopover habitat, like Virginia's barrier islands, to regain the fat required to continue flights to breeding grounds. Climate change is causing the ranges of important red knot prey, such as blue mussels (*Mytilus edulis*), to shift. Red knots may be unable to quickly adapt to changes in prey abundances on stopover grounds, making it more challenging for them to gain the weight required for successful migration and breeding. Thus, understanding the factors that may affect red knots in Virginia are important for successful management of this shorebird. Here, we determined what prey red knots consume in Virginia, addressed the factors that affect red knots in Virginia, and predicted the origin of blue mussels, an important prey resource for red knots in Virginia.

We collected sand and peat substrate samples from Virginia's ocean intertidal zone to determine what prey were available to red knots and collected red knot feces to determine what prey red knots consume. Red knots consumed clams, mussels, fly larvae, and crustaceans in Virginia and showed preference for clams and mussels over the more abundant crustaceans. Red knots selected foraging locations that had higher prey abundances than unused sites. The relationship between red knots and prey was affected by the tidal cycle, with the highest abundance of prey available and most birds foraging at low tide when peat banks were exposed.

After confirming that red knots preferentially consume blue mussels, which is experiencing a northward range contraction due to increasing ocean temperatures, we analyzed how blue mussel numbers have changed over time in Virginia. Blue mussel numbers declined over the duration of our study. We also analyzed blue mussel shells to help determine where the blue mussels were spawned, as shells contain environmental clues that relate to the conditions within which the shell forms. The juvenile blue mussels red knots consumed on Virginia's peat banks likely originated in ocean waters between Delaware and Virginia, but potentially in waters as far north as New York, possibly using ocean currents to travel to Virginia's intertidal zone.

Because migratory stopovers, like Virginia's barrier islands, support a large percentage of the total red knot migratory population, any changes in factors that affect red knots during their spring stopover, like prey availability, may negatively affect red knots. Because red knots need to quickly consume large quantities of prey to gain the weight needed for continued migration and breeding, managers should continue to prevent factors that negatively affect prey in Virginia. For example, we found that peat banks support high quantities of red knot prey; thus, continuing to ensure that peat banks are able to form on the islands is paramount. However, peat banks form through shoreline erosion and overwash, two natural processes which are often portrayed negatively. Beach nourishment and stabilization projects are commonly used in other coastal areas to prevent shoreline erosion and overwash along coastal beaches. If Virginia's barrier island beaches are nourished, natural processes like shoreline erosion may be less likely to occur. The absence of shoreline erosion on Virginia's barrier islands would preclude the formation of peat banks, likely decreasing prey availability for red knots. Beach nourishment also buries invertebrate prey, as sand is deposited along the beaches' intertidal zone. The disturbance caused by nourishment combined with the reduction in prey caused by it could negatively affect red knots in Virginia. Managers should continue to protect both peat and sand substrates by further allowing natural processes, like shoreline erosion, to occur on Virginia’s barrier islands.

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ATTRIBUTIONS

This section outlines a brief description of the contributions made by coauthors for each of my three Chapters.

Chapter 1: "Red knot (*Calidris canutus rufa*) diet reconstruction using DNA metabarcoding analyses of feces collected in Virginia"

Sarah Karpanty (Department of Fish and Wildlife Conservation, Virginia Tech) provided research question development support and substantially edited the manuscript. Joseph Craine (Jonah Ventures, Inc.) conducted all of the DNA metabarcoding analyses, provided methodology, granted much feedback on how to analyze and interpret the results presented in the manuscript, and edited the manuscript. Daniel Catlin (Department of Fish and Wildlife Conservation, Virginia Tech) provided statistical support and substantially edited the manuscript. James Fraser (Department of Fish and Wildlife Conservation, Virginia Tech) provided research question development support and substantially edited the manuscript.

Chapter 2: "Factors that affect migratory Western Atlantic red knot (*Calidris canutus rufa*) and their prey during spring stopover on Virginia's barrier islands"

Sarah Karpanty (Department of Fish and Wildlife Conservation, Virginia Tech) provided support and substantially edited and helped conceptualize the manuscript. Daniel Catlin (Department of Fish and Wildlife Conservation, Virginia Tech) provided analytical support and substantially edited the manuscript. Jonathan Cohen (Department of Environmental and Forest Biology, College of Environmental Science and Forestry, The State University of New York) provided analytical support, helped collect data, provided data from the early years of the research study, and substantially edited the manuscript. Daniel Gibson (Department of Fish and Wildlife Conservation, Virginia Tech) provided analytical support and will edit the manuscript. Shannon Ritter (Department of Fish and Wildlife Conservation, Virginia Tech) provided logistical and geospatial support and edited the manuscript. James Fraser (Department of Fish and Wildlife Conservation, Virginia Tech) provided support and substantially edited and helped conceptualize the manuscript.

Chapter 3: "Abundance and origin of blue mussels (*Mytilus edulis*) on Virginia's peat banks with implications for the conservation of the red knot (*Calidris canutus rufa*)"

Sarah Karpanty (Department of Fish and Wildlife Conservation, Virginia Tech) provided support and substantially edited the manuscript. Benjamin Gill (Department of Geosciences, Virginia Tech) helped run all of the stable isotope analyses, provided analytical support, and edited the manuscript. Shannon Ritter provided support with all ArcMap files and analyses and edited the manuscript. James Fraser (Department of Fish and Wildlife Conservation, Virginia Tech) provided support and substantially edited the manuscript.

INTRODUCTION

Every spring, diverse species of Nearctic shorebirds migrate from southern hemisphere wintering grounds to northern hemisphere breeding grounds (Morrison 1984, Watts and Truitt 2000, Piersma and Lindstrom 2004). These migrants rely on stopover regions to gain fat stores before continuing their migrations northward to breeding grounds (Watts and Truitt 2000, Atkinson et al. 2007, Cohen et al. 2011). Since gaining fat reserves at these stopovers is important to the successful completion of migration and breeding, understanding the factors that affect migrants' prey in stopover habitats is paramount (Leyrer et al. 2006, Niles et al. 2008). The Western Atlantic red knot (*Calidris canutus rufa*; 'red knot') is an example of a Nearctic shorebird that relies on several important stopover sites as it migrates over 15,000 km from wintering grounds as far south as Tierra del Fuego, Argentina, to breeding grounds in the Canadian Arctic (D'Amico et al. 2014, Watts and Truitt 2015). The red knot, listed as federally threatened in the United States (U.S.) and endangered in Canada, travels one of the longest migrations in the western hemisphere (Federal Registrar 2014) and is representative of the numerous challenges long-distance migrant shorebirds face.

Migratory shorebirds must adapt to habitat changes and subsequent variations in food availability as they stop along migration routes (Guglielmo et al. 2005). Anthropogenic factors have led to the loss of stopover grounds, particularly along North America's Atlantic Coast, amplifying the importance of remaining stopover habitat in Virginia, the Delaware Bay, and elsewhere (e.g., Florida, Georgia, South Carolina, and North Carolina; USFWS 2014). The loss of stopover grounds likely challenges migratory shorebirds' efforts to gain weight as competition for food and habitat increases (MacKay 1893, Myers et al. 1979, Galbraith et al. 2002, United

States Commission on Ocean Policy 2004, Thomas et al. 2006). Red knots, in particular, use at least 6 known stopovers in the Southern Hemisphere during spring migration, from Río Gallegos, Argentina, to French Guiana (Ferrari et al. 2002, Dey et al. 2011). While red knots historically used numerous spring stopover sites in large numbers on coastal beaches between Florida and Massachusetts in the United States (Wilson and Bonaparte 1832, MacKay 1893), red knots now predominantly use two North American stopover grounds: the Delaware Bay and Virginia's barrier islands (MacKay 1893, United States Commission on Ocean Policy 2004, Thomas et al. 2006, Cohen et al. 2010a, USFWS 2015). However, Florida, Georgia, South Carolina, and North Carolina continue to support a total estimated 13% of the migrating red knot population each year (A. Dey personal communication April 24, 2014, USFWS 2014), with sites in South Carolina and Georgia appearing to serve as final stopover locations for at least some red knots before they continue their migrations to Arctic breeding grounds (Harrington 2005a, b, SCDNR 2013, USFWS 2014).

Many stopover grounds, such as the Delaware Bay and Virginia's barrier islands for red knots, support high percentages of the entire population of particular migratory species (Brown et al. 2001, Chipley et al. 2003). Consequently, large changes in historic prey abundances may have important implications for these species, particularly those that are prey specialists (Tulp and Schekkerman 2008). Global climate change interacts with other anthropogenic factors (e.g., coastal development) to further complicate the creation and implementation of successful management plans for migrant shorebirds. The effects of climate change are vast and range from physical changes such as increasing ocean temperatures, shoreline erosion, and altered storminess to biological responses to these physical changes in the form of alterations in plant and animal ranges, distributions, and phenologies (Parmesan and Yohe 2003, Parmesan 2006, Bindoff et al. 2007, Perovich et al. 2008, Jonzen et al. 2011). Climate change results in distribution and range changes among species at varying rates, potentially causing temporal and spatial mismatches between predators and their prey (Edwards and Richardson 2004, Both et al. 2006, Durant et al. 2007, Post and Forchhammer 2008). The effects of climate change on these mismatches is especially evident in migratory shorebirds and their prey, as northward migration and subsequent breeding occur within a short period each year, and the availability of prey resources at the right time and in the right location is imperative to migration success (Schneider and Harrington 1981, Piersma et al. 1994).

Since red knots were listed as threatened under the U.S. Endangered Species Act in January 2015 (Federal Register 2014), understanding limiting factors in their U.S. migratory stopover habitat is even more important to the conservation of the subspecies. Specifically, the United States Fish and Wildlife Service (USFWS 2015, 2019a, b) has released research priorities for the red knot, several of which we addressed in this study. The priorities addressed in this study included, but were not necessarily limited to: (1) "better characterize the nonbreeding diet and document baseline conditions regarding food resources," (2) "support ongoing efforts to obtain regional population size estimates where needed to inform management decisions at major migration stopovers (e.g., Delaware Bay, Virginia, South Carolina, Georgia) and to expand such efforts to additional areas as important new stopovers are identified," and (3) "communicate scientific findings to partners and the public, emphasizing the red knot's linkages to climate change, other shorebirds, the Arctic, and other imperiled coastal species."

Because red knots are limited to a small spring stopover range and may occupy a narrow foraging niche at these locations, their ability to adapt to changing prey ranges and abundances could be limited (MacKay 1893, United States Commission on Ocean Policy 2004, Thomas et al.

2006, Cohen et al. 2010a, b). If red knots fail to adequately gain weight at final stopover sites, they may not survive migration or may be disadvantaged for refueling once at their breeding grounds, leading to potentially lower reproductive success and/or survival (Morrison et al. 2006; Hua et al. 2013). Therefore, further clarification of stopover habitat ecology in Virginia is needed, as little is known about how spatial and temporal variation in prey resources may affect red knot site selection and flock size, specifically in the presence of changing ocean temperatures and island geomorphological features.

Virginia Tech's long-term shorebird research on the Virginia Coast Reserve, of which this dissertation is a part, focuses on better understanding the role of the Virginia stopover in red knot ecology and conservation. Since Virginia's barrier islands are largely undisturbed by humans, large portions of beaches and marsh provide excellent stopover and breeding habitat for thousands of shorebirds, including, but not limited to, sanderlings (*Calidris alba*), whimbrels (*Numenius phaeopus*), dunlin (*Calidris alpina*), ruddy turnstones (*Arenaria interpres*), plovers (*Charadrius spp.*), and red knots (Watts and Truitt 2000, 2015, Cohen et al. 2011).

Despite former speculation that a small number of red knots used Virginia sporadically as a short-term intermittent stopover, Watts and Truitt (2000) found between 14,808 and 23,917 red knots used Virginia stopover in 1995 and 1996, respectively, while Cohen et al. (2009) and Duerr et al. (2011) found that between 7,224 and 14,688 red knots used Virginia stopover between 2006 and 2010. Cohen et al. (2009) also found that on average only 5% of red knots move between Virginia and the Delaware Bay within a year, supporting that Virginia is an independent stopover from the Delaware Bay for some migrating red knots. Since Virginia Tech began work in Virginia in 2006, the project has predicted that between 2,792 and 11,644 red knots use Virginia stopover during the peak migration period per year (May 21 – 27), estimates which are comparable to peak migration estimates obtained from aerial surveys conducted by Watts and Truitt between 1995 – 2014 (5,547 – 9,150; B. Watts personal communication November 15, 2012 and August 19, 2014, Watts 2013, USFWS 2014).

Although red knots consume horseshoe crab eggs in the Delaware Bay (Karpanty et al. 2006, 2011, Niles et al. 2009, Fraser et al. 2010, 2012, Smith and Robinson 2015), they consume hard-shelled bivalves in most other areas across their wintering and migratory ranges (Piersma 1991, Zwarts and Blomert 1992, Dekinga and Piersma 1993). This bivalve specialization appears to be exhibited on their Virginia stopover, where coquina clams (*Donax variabilis*) and blue mussels (*Mytilus edulis*) are abundantly found across the ocean shoreline (Watts and Truitt 2000, Cohen et al. 2010b). Large populations of coquina clams are found along sandy intertidal zones, which comprise the most abundant substrate along Virginia's barrier islands (Sperry 1940, Cohen et al. 2010b, Watts and Truitt 2015). Virginia's barrier islands also support intertidal peat banks. These banks are composed of partially decomposed marsh vegetation that forms when a barrier island migrates over tidal marshes on the bay side of the islands and ultimately beyond the buried marshes (Truitt et al. 2001, Bachmann et al. 2002, Cohen et al. 2010, Watts and Truitt 2015, Kreuzburg et al. 2018). While peat banks only encompass about 6% of the ocean shoreline each year in Virginia, many invertebrate organisms, like juvenile blue mussels, live within the banks (Smith et al. 2008, Cohen et al. 2010, Watts and Truitt 2015).

Several studies conducted by Watts and Truitt (2000, 2015) and Cohen et al. (2010b, 2011) suggest that coquina clams and blue mussels comprise most of the red knot's diet in Virginia. Nevertheless, much speculation remains about red knot site-usage and prey availability across Virginia's barrier islands. For example, Cohen et al. (2010b) suggested that coquina clams were the most dominant available prey item for red knots in Virginia. However, Watts and Truitt

(2015) suggested that by peak migration, red knots used peat banks, where blue mussels live, ten-times more than sand, where coquina clams live. The design of both studies likely contributed to their findings. Cohen et al. (2010b) sampled prey throughout the 12-hour tidal cycle, during which coquina clams were potentially available for 8 hours and blue mussels for only 4 hours. Watts and Truitt (2015) surveyed the islands only along falling tides when blue mussels were exposed along peat banks. If the abundance and/or availability of coquina clams and blue mussels in Virginia change in the future, red knots may have a more challenging time meeting daily energetic demands (Cohen et al. 2009). This may be especially relevant as global climate change is contributing to the blue mussel's range contraction (Hansen et al. 2006, Jones et al. 2010).

Previous studies have examined the availability and abundance of potential red knot prey species relative to red knot distribution and abundance (Watts and Truitt 2000, 2015, Cohen et al. 2010b, 2011). However, no study has confirmed that red knots are preferentially consuming these bivalve prey resources in Virginia or whether other invertebrates are being consumed or selected by red knots as well. Additionally, while Cohen et al. (2010a, b, 2011) and Watts and Truitt (2000, 2015) addressed some of the factors that may affect red knots in Virginia, no studies have addressed the factors that affect red knot site selection, red knot flock size, and prey abundances across the intertidal shoreline over an extended period (> 2 years), or where blue mussels, one potentially key prey resource, may originate. To address these knowledge gaps, we compiled and analyzed our data into three chapters.

In Chapter 1, "Red knot (*Calidris canutus rufa*) diet reconstruction using DNA metabarcoding analyses of feces collected in Virginia," we collected substrate core samples from Virginia's barrier island intertidal zones to determine which prey were available to foraging red knots. We conducted DNA metabarcoding analyses on red knot fecal samples to determine which prey red knots consumed in Virginia. We then combined these two data-sets to conduct use-availability analyses to determine which prey red knots select in Virginia.

In Chapter 2, “Factors that affect migratory Western Atlantic red knots (*Calidris canutus rufa*) and their prey during spring stopover on Virginia’s barrier islands,” we used zero-inflated negative binomial mixed-effects regression models to predict the number of red knots in Virginia during peak migration and to determine what factors affected red knot site selection and flock sizes. We used generalized linear mixed-effects regression models to determine how climatic and geomorphological factors affected prey abundances on Virginia’s barrier islands from 2007 – 2018. We used the prey that we determined red knots consumed in Chapter 1 to inform our statistical modeling approaches.

Finally, in Chapter 3, “Abundance and origin of blue mussels (*Mytilus edulis*) on Virginia’s peat banks with implications for the conservation of the red knot (*Calidris canutus rufa*),” we conducted oxygen stable isotope analyses on different portions of blue mussel shells to approximate where Virginia’s juvenile blue mussels originate and to determine how continued ocean temperature increases may affect the blue mussel’s range contraction, which could ultimately affect red knot abundances in Virginia.

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RED KNOT (*CALIDRIS CANUTUS RUFA*) DIET RECONSTRUCTION USING DNA METABARCODING ANALYES OF FECES COLLECTED IN VIRGINIA

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ABSTRACT

Understanding which prey wildlife use and how prey are selected relative to their availability is important to conservation planning. Available and abundant prey resources along stopovers are imperative to migratory shorebird migration and ultimately to breeding success. We determined which prey are available to foraging red knots (*Calidris canutus rufa*) using Virginia's barrier islands as spring stopover habitat by collecting substrate core samples containing prey on sand and peat substrates in May 2017 – 2019. We also collected red knot feces (*n* = 100) during the same period and used fecal DNA metabarcoding analyses to determine which invertebrate prey red knots consumed. We then compared prey availability data with prey use data to determine which prey red knots selected on these islands. Prey availability and use varied by foraging substrate, with more mussels generally available and used on peat and more clams generally available and used on sand. Red knots consumed bivalves (Orders Veneroida and Mytiloida), crustaceans (Orders Amphipoda and Calanoida), and insect larvae (Order Diptera). Red knots selected bivalves and used crustaceans and other prey less than expected given their abundance and relative use. Despite using non-bivalve prey less than expected, crustaceans may still be an important portion of the total caloric intake on Virginia's stopover given their abundance and use. It is imperative that coastal management practices within Virginia and the larger mid-Atlantic stopover region continue to be designed to promote natural barrier island movement which leads to the formation of the peat banks used by many prey.

KEY WORDS *Calidris canutus rufa*, diet, DNA metabarcoding, migratory stopover, red knot, refueling, Virginia

INTRODUCTION

Long-distance migration is energetically expensive (Jardine et al. 2015, Quinn et al. 2017). It typically requires animals to obtain substantial fat reserves pre-migration and efficiently regain energy along stopover grounds to successfully migrate and breed (Myers et al. 1987, Hebert et al. 2002, 2008, Blight 2011, Klaassen et al. 2012, Smith et al. 2012, Jardine et al. 2015, Novcic et al. 2015, Twining et al. 2016, Quinn et al. 2017). To efficiently regain energy stores, many migratory animals exhibit flexible generalist foraging strategies to exploit the variety of prey they may encounter across wintering, migratory, and breeding grounds (Recher 1966, Hicklin and Smith 1979, Pienkowski et al. 1984, Skagen and Oman 1996, Davis and Smith 2001, Skagen 2006, Novcic et al. 2015, Gerwing et al. 2016). Foraging theory states that generalist foragers consume prey based on what is most abundant, whereas specialists select food items that provide the most energy in the shortest time (i.e., “profitable” prey) given their foraging and digestive investments (Stephens and Krebs 1986, Ydenberg et al. 2007, López-Vidal et al. 2014). Therefore, animals exhibiting specialist foraging strategies consume profitable prey more than expected, given their abundance, while less-profitable prey are consumed less than expected, given their abundance (Pyke et al. 1977, Ydenberg et al. 2007, López-Vidal et al. 2014).

Migratory shorebirds are among the most-studied animals in regards to migration and foraging strategies (Piersma and van Gils 2011, Piersma 2012, Onrust et al. 2013), as they are quickly able to travel long distances in a short period and typically are easily observed and counted (Dekinga and Piersma 1993, Onrust et al. 2013). The impact of the loss and degradation of stopover grounds required to refuel and rest mid-migration also stimulates interest in migratory shorebirds (MacKay 1893, Myers et al. 1979, Galbraith et al. 2002, U.S. Commission on Ocean Policy 2004, Thomas et al. 2006). The Western Atlantic red knot (*Calidris canutus rufa*; ‘red knot’) is a long-distance migrant shorebird that travels approximately 15,000 km twice a year from wintering grounds primarily in South America to breeding grounds in the Canadian Arctic (Gonzalez et al. 1996, D’Amico et al. 2014, Watts and Truitt 2015). To migrate such long distances, red knots use coastal beaches on the United States’ (U. S.) Atlantic coast to refuel and rest mid-migration (Dey et al. 2011, Dey 2014, United States Fish and Wildlife Service 2015, 2019). Most spring migrant red knots stop on the Delaware Bay in New Jersey and Delaware and Virginia’s barrier islands during their spring migration (MacKay 1893, U.S. Commission on Ocean Policy 2004, Cohen et al. 2010a, Lyons et al. 2018).

While red knots using the Delaware Bay stopover forage predominantly on Atlantic horseshoe crab eggs (*Limulus polyphemus*; Karpanty et al. 2006, 2011, Niles et al. 2009, Fraser et al. 2010, 2012, Novcic et al. 2015, Smith and Robinson 2015, Burger et al. 2018), red knots generally are considered to be bivalve specialists and feed upon hard–shelled organisms throughout much of their wintering and migratory grounds (Piersma 1991, Zwarts and Blomert 1992, Dekinga and Piersma 1993, Tulp and Schekkerman 2008). Red knots ingest bivalves whole, crush them in their relatively large gizzards (Dekinga and Piersma 1993, Piersma et al. 1993, van Gils et al. 2003, Quaintenne et al. 2009, Onrust et al. 2013), and rapidly pass shells through their adaptively large colons (Piersma et al. 1993, 1999, Battley and Piersma 2004, Quaintenne et al. 2009). This bivalve specialization is thought to include their Virginia stopover, where coquina clams (*Donax variabilis*) and blue mussels (*Mytilus edulis*) are some of the most abundantly available prey resources along ocean sand and peat substrates, respectively, where red knots and other shorebirds regularly forage (Watts and Truitt 2000, Cohen et al. 2010b).

Due to the abundance of both coquina clams, found in sandy ocean intertidal substrate, and blue mussels, found in peat ocean intertidal substrate, on Virginia's barrier islands, numerous studies have suggested that these two prey resources comprise the majority of the red knot's diet in Virginia (Watts and Truitt 2000, 2015, Cohen et al. 2010b, 2011). However, populations of these bivalves are highly variable among sampling points and across years (Heller et al. unpublished data), yet red knots consistently stop in Virginia in large numbers and feed along the intertidal zone. It is possible that red knots feed on a variety of organisms.

Incomplete and inaccurate diet assessments decrease our ability to understand how an animal will respond to changing environmental conditions (Bowser et al. 2013). Diet analyses can help us better understand what animals are consuming during different parts of their life cycles (Dufft et al. 2007, Valentini 2008, Bowser et al. 2013). More specifically, understanding the foraging ecology of threatened and endangered species, like the red knot, is important, as precise knowledge of these species' diets may elucidate what environmental factors are important to key prey and need to be of focus when designing and implementing management and conservation strategies (Marrero et al. 2004, Cristóbal-Azkarate and Arroyo-Rodriquez 2007, Valentini 2008, Jardine et al. 2015).

Previously, confirming shorebird dietary compositions required bird sacrifices to examine stomach contents (Sperry 1940, Recher 1966, Holmes and Pitelka 1968, Schneider and Harrington 1981, Worrall 1984, Davis and Smith 2001, Niles et al. 2008, Novcic et al. 2015). For example, Sperry (1940) examined 219 red knot stomachs (representing birds predominantly collected on the Atlantic and Gulf Coasts of the United States) and found that almost 70% of the stomach contents were composed of mollusks, though crustaceans and insects also were present. Due to the red knot's threatened and endangered statuses in the U. S. and Canada, respectively (Federal Registrar 2014, USFWS 2015, 2019), sacrificing individuals would not only be impossible and irresponsible (Novcic et al. 2015), but also would preclude any estimation of the effects prey have on red knot survival and breeding success (Sullins et al. 2018). Non-invasive microhistological analysis of feces is another method used to provide inference on diet; however, these analyses may underestimate prey items that are easily digested, overestimate prey that are less-digestible, lead to misidentification of similar-appearing prey, and completely miss prey not easily seen (Bartolome et al. 1995, Litvatis 2000, Valentini et al. 2008, Bowser et al. 2013, Craine et al. 2015, Sullins et al. 2018). Further complicating dietary inferences, not all crop contents reach the intestines, as birds regurgitate (Jordan 2005, Sullins et al. 2018). Biochemical techniques, including fatty acid and stable isotope feather analyses, commonly are used for diet determination as well; however, these methods provide insight only on animals' long-term diet, both seasonally and annually, as opposed to information on what the animal is eating at a given point in time (Bowser et al. 2013). Additionally, these methods require *a priori* knowledge of the animal's diet (Bowser et al. 2013, Gerwing et al. 2016) and animal capture is required. Thus, using biochemical methods to determine if unknown prey items are being consumed in an animal's diet during a short period (such as during migratory stopover) would not be practical.

Fecal genetic DNA metabarcoding provides a more reliable way to determine diet by analyzing prey DNA in feces (Casper et al. 2007, Deagle et al. 2007, Barnett et al. 2010, Braley et al. 2010, Bowser et al. 2013). DNA metabarcoding of fecal material is non-invasive, as fecal matter may be obtained with little to no disturbance (Valentini et al. 2009, Gerwing et al. 2016). This method is less likely to over- or under-estimate prey presence and abundance based on digestibility (Bartolome et al. 1995, Litvatis 2000, Valentini 2008, Craine et al. 2015, Sullins et al. 2018), can analyze prey items that are degraded (Kohn and Wayne 1997, Deagle et al. 2006, Pompanon et al. 2012, Gerwing et al. 2016), and can be used for *de novo* diet determination (Bowser et al. 2013). To conduct these analyses, a standardized DNA region (i.e., barcode) is identified, amplified from fecal samples, and then compared to sequences from a publicly-available reference database (Valentini et al. 2008, Bowser et al. 2013). From these data, the presence (frequency of occurrence; FOO) and relative contribution of different food items to all prey detected within red knot feces (relative read abundance; RRA) can be estimated using the presence and frequency of different sequences (Ratnasingham and Hebert 2007, Valentini et al. 2008, Zeale et al. 2011, Craine et al. 2015, Sullins et al. 2018). Both FOO and RRA may be biased by the number of primers used to amplify DNA. While FOO data is often considered a more reliable and conservative approach to determining diet, occurrence data is more likely to overestimate the importance of prey eaten in small quantities. Relative read data are more sensitive to detectability and recovery issues and may be misinterpreted as absolute abundance. Therefore, both approaches serve only as surrogates for true diet (Deagle et al. 2018).

Determining prey availability and how it relates to what red knots are using may help elucidate if the red knot’s diet in Virginia reflects a specialist or generalist strategy and thus how susceptible red knots may be to changes in prey abundances and distributions in the future. Although several studies have been conducted pertaining to red knot prey in Virginia (Cohen et al. 2010b, 2011, Watts and Truitt 2015), these studies showed what prey are *potentially* available to red knots but confirm neither what red knots actually consume in Virginia nor how prey consumption may vary by foraging substrate (i.e., sand vs. peat). Here, we address 1) how foraging substrate (sand vs. peat) affects which prey are available to red knots, 2) how foraging substrate is related to which prey are consumed by red knots, and 3) if red knots are selecting certain prey items over others.

STUDY AREA

We studied red knots on barrier islands off the coast of the Eastern Shore of Virginia on the Virginia Coast Reserve Long Term Ecological Research site, from Assawoman Island in the north to Fisherman Island in the south (Fig. 1; 37°23.7’N, 75°42.5’W; Cohen et al. 2010). The site contains eleven islands that are bounded by the Atlantic Ocean on the east and a shallow lagoon system with open water, mudflats, and marsh to the west (‘Virginia’s barrier islands;’ Cohen et al. 2009, 2010, 2011). The predominantly undeveloped islands are separated from each other by a series of channels that follow along approximately 100 km of Eastern Shore of Virginia coastline (Smith et al. 2008, Cohen et al. 2011, Watts and Truitt 2015). Red knots using Virginia’s barrier island habitat primarily foraged within the ocean intertidal zone, which contains two substrates: sand and peat (Cohen et al. 2011). Peat banks, comprising about 6% of the shoreline each year, form when ocean beaches erode over low elevation island segments and transgress over bay tidal marsh (Truitt et al. 2001, Bachmann et al. 2002, Cohen et al. 2010, Watts and Truitt 2015, Watts and Truitt 2015, Kreuzburg et al. 2018).

METHODS

Field Methodology

Prey Availability

We collected core samples of the local substrate at randomly generated sampling points along the islands' ocean intertidal zones to sample prey availability during the red knot stopover in Virginia (May 14 – 27) in 2017 and 2018 (n = 191 sand samples, n = 113 peat samples). We also collected core samples in 2019, but due to financial constraints, 2019 availability samples have not been analyzed. Sampling points were generated using the Hawth's Tools extension (Beyer 2004) in ArcGIS 10.1 (ESRI 2012) and the Geospatial Modelling Environment extension (Beyer 2009) in ArcGIS 10.5 (ESRI 2017) using The United States Department of Agriculture (USDA) Farm Service Agency's National Agriculture Imagery Program (NAIP) orthophotography imagery (USDA-FSA-APFO Aerial Photography Field Office 2020). To determine peat bank locations, we used orthophotography imagery to stratify random sampling points by substrate type.

We used a section of PVC piping (275 cm^3) cut to the approximate length of a red knot's bill (10 cm diameter x 3.5 cm deep; Tomkovich 1992) to sample prey that red knots could obtain within sand and peat substrates. Core samples were frozen and later sorted in the lab. We counted the number of invertebrates in each sample and separated them by category using a series of sieves, with the smallest mesh size #40 (0.32 mm holes), and a dissecting microscope. Later, we classified prey items into the Orders determined by DNA metabarcoding analyses for comparison of prey availability vs. prey use (see *Prey Availability vs. Use* section below). For prey availability analyses, we could not visually distinguish crustaceans between Orders Amphipoda (malacostracan crustaceans) and Calanoida (copepod crustaceans) and thus combined the two Orders. Prey that could not be visually classified into Amphipoda + Calanoida, Mytiloida (true mussels), or Veneroida (marine and freshwater bivalve mollusks/clams) Orders were grouped into a “miscellaneous” category. Because we know that coquina clams and blue mussels are found in Virginia, we assumed that Order Veneroida reflected the presence of coquina clams, while Order Mytiloida demonstrated the presence of blue mussels.

Prey Use - Fecal Sample Collection

We opportunistically collected 100 fecal samples (n = 84 on sand, n = 16 on peat) from red knots during their spring stopover period (May 14 – 27) on Virginia’s barrier islands in 2017 (n = 16), 2018 (n = 46), and 2019 (n = 38; Appendix 1). Samples were collected only from flocks consisting of at least 90% red knots to reduce cross-species fecal contamination potential and from the locations where individual red knots were observed defecating. We collected each sample using sterilized forceps (WinnerMedical Sterile Alcohol Prep Pads, 70% – 75% isopropyl alcohol), touching as little substrate as possible (Gerwing et al. 2016). We placed each sample in a separate 14.8 mL vial, labeled with the date, time, island, sample ID, and substrate (sand vs. peat). Red knots have fast digestive turnovers (~ 56 min; Van Gils 2004, Quaintenne et al. 2009), meaning that the composition of their fecal matter likely varies during the course of the day dependent upon where they foraged during the past hour. Therefore, we assumed that the sample’s collection site corresponded to the red knot’s foraging location. Vials were stored in freezers at the Virginia Coast Reserve, LTER's, Anheuser-Busch Coastal Research Center and at Virginia Tech before being transferred to barcoded vials and shipped frozen overnight for laboratory analyses at Jonah Ventures, LLC (Boulder, CO).

Lab Methodology

Prey Use - Sequencing

Each well within a 96-well plate was assigned a unique sample index. Within a laminar flow hood, sterile cotton swabs (Fisher, cat# 23-400-100) were dipped in nuclease free water and coated each sample. Swabs were placed in the corresponding well or tube. Before extraction, the wooden ends of the cotton swabs were removed using sterile tweezers or pliers. Plates were immediately stored in a -20°C freezer until the extraction process could be performed.

Genomic DNA was extracted from fecal samples using the PowerSoil htp-96 well Isolation Kit (Cat#12955-4, MO BIO Laboratories, Carlsbad, California, USA) according to the manufacturer's protocol. Approximately 0.25 grams of sample was used for genomic DNA extraction. Genomic DNA was eluted into 100µl and frozen at -20°C. An 18S Eukaryotic, 2-step PCR standard protocol was used with forward primer: GTACACACCGCCCGTC and reverse primer: TGATCCTTCTGCAGGTTCACCTAC (Ramirez et al. 2014). A portion of the 18S rRNA gene was PCR amplified from each genomic DNA sample using eukaryotic-specific primers that amplified the V9 hypervariable region. Both forward and reverse primers also contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. Each 25 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI), with 12.5ul Master Mix, 0.5 µl of each primer, 1.0 µl of gDNA, and 10.5 µl DNase/RNase-free H_2O. DNA was PCR amplified using the following conditions: initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of 45 seconds at 94 °C, 1 minute at 57 °C, and 90 seconds at 72 °C, and a final elongation at 72 °C for 10 minutes.

Amplicon size and PCR efficiency was determined using gel electrophoresis standard protocol on a 2% agarose gel with 5µl of each sample as input. Then, amplicons from each sample were cleaned by incubating them with ExoI/SAP for 30 minutes at 37 °C, followed by inactivation at 95 °C for 5 minutes, and storage at -20°C. A second round of PCR was performed to give each sample a unique 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 µM of each primer and 2 µl of template DNA (cleaned amplicon from the first PCR reaction). Conditions consisted of an initial denaturation of 95°C for 3 minutes followed by 8 cycles of 95°C for 30 sec, 55°C for 30 seconds and 72°C for 30 seconds. Five µl of indexing PCR product from each sample was visualized again on a 2% agarose gel to ensure the success of the metabarcoding PCR. Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA). 25µl of PCR amplicon is purified and normalize using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol before pooling them for sequencing on a MiSeq (Illumina, San Diego, California) in the University of Colorado BioFrontiers Sequencing Center using the v2 500-cycle kit (cat# MS-102-2003). Quality control measures were performed at the sequencing center prior to sequencing.

Prey Use - Bioinformatics Protocol

Sequences were filtered and operational taxonomic unit (OTU; here, proportion of each sample represented by Order grouping) picking was performed using the JAMP pipeline (https://github.com/VascoElbrecht/JAMP). Demultiplexing of raw data was done with "iudemultiplex" v2.3 (https://github.com/merenlab/illumina-utils). Forward and reverse reads from each sample were paired end merged with USEARCH v11. Primers were trimmed from both sides, and reads of 50 - 200 bp length retained using Cutadapt v1.15. Quality filtering used expected error filtering (max_ee=0.5) as implemented in USEARCH v11.

For OTU clustering, reads were pooled, dereplicated and singletons removed. Sequences were clustered using UEARCH within a 1% threshold (Sullins et al. 2018), and each sample (included singletons) was remapped against the generated OTUs using USEARCH_global with 99% similarity for OTU picking. Taxonomy assignment was based on the SILVA reference database (a public sequence database) and assign_taxonomy.py as implemented in QIIME. All samples with < 5 18S reads were excluded from analyses for invertebrates in diet. We limited assignment of OTU to Orders present at our study site, as estimated from core sampling (see prey availability details above; Sullins et al. 2018) and eliminated OTUs that were obtained from contamination (e.g., *Homo sapiens*).

For each fecal sample, we calculated the relative read abundance (RRA; percentages of all sequences assigned to a given OTU; Kartzinel et al. 2015, Sullins et al. 2018). We determined the frequency of occurrence (FOO; number of fecal samples that contained a given prey item, divided by the total number of fecal samples; Gerwing et al. 2016) for each prey Order detected. For a visual representation by substrate of the RRA and FOO of the Orders of invertebrates found using DNA metabarcoding analyses on red knot fecal samples, we provide heat maps in Appendix 2. Fifteen of the 100 samples did not contain DNA from any of the Orders for which we screened; however, we include these samples in analyses to avoid over representing an Order's relative importance.

Data Analyses

Prey Availability

We used Chi-square contingency tests of independence to determine if mean available prey abundances from core samples differed in their community make-up on peat vs. sand. Shapiro-Wilk normality tests indicated that availability data for mussels from Order Mytiloida ($W = 0.25$, $p < 0.001$), clams from Order Veneroida ($W = 0.46$, $p < 0.001$), crustaceans from Orders Amphipoda + Calanoida ($W = 0.28$, $p < 0.001$), and miscellaneous prey ($W = 0.44$, $p < 0.001$) were not normally-distributed. We used Wilcoxon rank sum tests, which are less sensitive to non-normal data, with Bonferroni correction to determine if there were differences in mean available prey abundances from core samples by substrate.

Prey Use

We used Chi-square contingency tests of independence to determine if mean FOO and RRA from red knot fecal samples differed in their community make-up on peat vs. sand. We used 2-sample Chi-square tests for equality of proportions with continuity correction to determine if there were differences in the proportion of fecal samples with invertebrate DNA by substrate.

Prey Availability vs. Use

To determine if red knots selected certain prey items over others, we compared our core sample abundance data (prey availability) to our red knot fecal data (relative prey use; RRA). We combined Orders Amphipoda and Calanoida for these analyses, as we could not visually distinguish between crustaceans in these Orders for prey availability analyses (see *Prey Availability* in the *Methods* section above).

We plotted the mean abundance of prey that were available to red knots from core samples with the mean relative read abundance of prey from fecal samples and used Wilcoxon rank sum tests with Bonferroni correction to determine if there were differences between the mean relative abundance of prey available to red knots by substrate and the relative read abundances of prey used by red knots. If the relative abundance of available prey was lower than the relative read abundance of used prey, we concluded that red knots selected that prey resource. If the abundance of available prey was higher than the relative read abundance of used prey, we concluded red knots selected against that prey resource. We used Program R package "base" for all analyses (R Core Team 2017, Wickham et al. 2018; R Version 3.4.1, www.r–project.org, accessed 1 October – 15 November 2019).

RESULTS

Prey Availability

The composition of invertebrate prey Orders available to red knots differed between the two substrates (Chi-square contingency test of independence; $\chi^2 = 5{,}526$, df = 3, $p < 0.001$). On sand (n = 191), crustaceans from Orders Amphipoda and Calanoida comprised 71%, clams from Order Veneroida 28%, mussels from Order Mytiloida 0.02% , and miscellaneous prey 1.5% of total available prey counted. On peat (n = 113), crustaceans from Orders Amphipoda and Calanoida comprised 75%, clams from Order Veneroida 0.8%, mussels from Order Mytiloida 18%, and miscellaneous prey 73% of total prey counted (Fig. 2). The mean abundance of invertebrate prey Orders available to red knots using Virginia's barrier islands also varied by substrate, with differences observed across all four prey Order categories. Clams from Order Veneroida were more abundant on sand (Wilcoxon rank sum test with Bonferroni correction; W

= 6,955, $p < 0.001$), while crustaceans from Orders Amphipoda and Calanoida (Wilcoxon rank sum test with Bonferroni correction; W = 13,312, $p < 0.001$), mussels from Order Mytiloida (Wilcoxon rank sum test with Bonferroni correction; W = 18,411, $p < 0.001$), and miscellaneous prey (Wilcoxon rank sum test with Bonferroni correction; W = 17,741, $p < 0.001$) were more abundant on peat (Fig. 2).

Prey Use

Amphipoda (malacostracan crustaceans), Calanoida (copepod crustaceans), Diptera (true flies), Mytiloida (true mussels), and Veneroida (marine and freshwater bivalve mollusks/clams) were identified in red knot fecal samples. The frequency of occurrence of invertebrate prey Orders used by red knots differed between the two substrates (Chi-square contingency test of independence; $\chi^2 = 40$, df = 4, $p < 0.001$). Frequency of occurrence (FOO) of clams from Order Veneroida on sand was higher than that of clams on peat (2-sample Chi-square test for equality of proportions; $\chi^2 = 5$, df = 1, $p = 0.02$), while FOO of crustaceans from Order Amphipoda (2-sample Chi-square test for equality of proportion; $\chi^2 = 10$, df = 1 , $p = 0.001$) and mussels from Order Mytiloida (2-sample Chi-square test for equality of proportion; $\chi^2 = 36$, df = 1, $p < 0.001$) were higher on peat. No differences in FOO on sand vs. peat were observed for crustaceans from Order Calanoida or for insects from Order Diptera (Fig. 3).

Relative read abundances of prey Orders found in red knot fecal samples varied by substrate (Chi-square contingency test of independence; $\chi^2 = 46,646$, df = 4, $p < 0.001$). On sand ($n = 84$ samples, $n = 51,807$ sequences), crustaceans from Order Amphipoda comprised 0.00%, crustaceans from Order Calanoida 80%, insects from Order Diptera 0.02%, mussels from Mytiloida 0.57%, and clams from Order Veneroida 19% of all sequences. On peat, ($n = 16$

samples, *n* = 23,596 sequences), crustaceans from Order Amphipoda comprised 47%, crustaceans from Order Calanoida 18%, insects from Order Diptera 0.00%, mussels from Mytiloida 32%, and clams from Order Veneroida 3% of all sequences (Fig. 4).

Prey Availability vs. Use

Relatively more individuals from Orders Amphipoda and Calanoida (crustaceans) were available to red knots than found as used in red knot fecal samples on both sand (Wilcoxon rank sum test with Bonferroni correction; W = 12,836, $p < 0.001$) and peat (Wilcoxon rank sum test with Bonferroni correction; W = 1,277, $p = 0.008$). Relatively more miscellaneous prey were available than used in fecal samples on sand (Wilcoxon rank sum test with Bonferroni correction; W = 9,174, $p < 0.001$) and peat (Wilcoxon rank sum test with Bonferroni correction; W = 1,560, $p < 0.001$). Red knots used mussels from Order Mytiloida on sand more than expected based on availability (Wilcoxon rank sum test with Bonferroni correction; W = 7,777, $p = 0.05$). Red knots used clams from Order Veneroida on both sand (Wilcoxon rank sum test with Bonferroni correction; W = 2,996, $p < 0.001$) and peat (Wilcoxon rank sum test with Bonferroni correction; W = 653, $p = 0.03$) more than expected based on availability, though selection for prey in Order Veneroida was higher on sand (Fig. 5).

DISCUSSION

Migratory shorebirds like red knots are highly susceptible to habitat alteration, loss, and destruction as they spend much of the year migrating and therefore are likely affected by habitat changes on migratory as well as wintering and breeding grounds (Newton 2001, Novcic et al. 2015). Shorebirds’ reliance on predictable stopover grounds, such as Virginia’s barrier islands, to provide the space to rest and refuel augments this susceptibility (Brown et al. 2011, Novcic et al. 2015). Red knots consumed more types of prey in Virginia than indicated in previous studies (Watts and Truitt 2000, 2015, Cohen et al. 2010b, 2011). Red knots extensively use horseshoe crab eggs 100 km north in the Delaware Bay stopover, but few horseshoe crab eggs were available or used by red knots in Virginia. Red knots using Virginia stopover habitat primarily consumed bivalves, as they do throughout much of their wintering and migratory grounds (Piersma 1991, Zwarts and Blomert 1992, Dekinga and Piersma 1993, Tulp and Schekkerman 2008).

Here, the prey with the highest RRA (i.e., Orders Mytiloida and Veneroida; true mussels and clams) in red knot feces were not the same as the most abundantly available prey (i.e., Orders Amphipoda + Calanoida; crustaceans). If red knots were generalist foragers and consuming prey according to availability, we would expect them to consume crustaceans most, on both sand and peat. However, red knots selected coquina clams and blue mussels. These data suggest that red knots selected bivalves and are bivalve specialists during their spring migratory stopover in Virginia. We suggest that bivalves are important food sources for migrating red knots in Virginia for three reasons. First, the availability of these prey each year (Cohen et al. 2009, 2010, 2011, Heller et al. In Prep) support that bivalves are a predictable food source that red knots can rely on each spring. Virginia's barrier islands and the Delaware Bay are currently the last two large spring stopovers for these birds before reaching Arctic breeding grounds, though historically thousands of red knots also used spring stopover grounds from Massachusetts to Florida (Wilson and Bonapart 1832, MacKay 1893, Shriner 1897, Morrison et al. 1980, Myers et al. 1987, Cohen et al. 2009, Novcic et al. 2015). Sperry (1940) documented that red knots consumed large quantities of bivalves in Virginia, further supporting that bivalves have been a consistent prey resource for red knots using Virginia's barrier island stopover for over 80 years.

Second, red knots have strong gizzards and large colons that are able to crush (Dekinga and Piersma 1993, Piersma et al. 1993, van Gils et al. 2003, Quaintenne et al. 2009, Onrust et al. 2013) and pass hard bivalve shells (Piersma et al. 1993, 1999, Battley and Piersma 2004, Quaintenne et al. 2009) respectively. Other shorebirds may be less adapted to digest and process bivalves and may therefore expend more energy processing bivalve prey. Red knots may capitalize on their ability to digest and process prey that other shorebirds cannot process as efficiently, ensuring that red knots occupy a less utilized niche.

Third, the large size of intact bivalves in comparison to crustaceans may make it easier for red knots to detect and capture bivalve prey, as they are tactile and visual foragers (Martin and Piersma 2009). Red knots rely predominantly on tactile foraging along wintering and stopover grounds, using corpuscles, receptors located within sensory pits at the end of the bill, to quickly and efficiently grasp and consume prey buried in soft substrate (Piersma et al. 1998, Nebel et al. 2005, Martin and Piersma 2009). Using tactile foraging and search imaging approaches may explain why red knots select bivalve prey (optimal foraging theory; Martin and Piersma 2009, Garay et al. 2018). Previous studies estimated that the caloric value of crustaceans from genus Corophium (Order Amphipoda) being consumed by redshank (*Tringa totanus*), a shorebird of comparable size to red knots, to be between 4 – 54 Kcals/g ash-free dry mass (AFDM; Wood personal communication, Goss-Custard 1977). J. Cohen (unpublished data) found that coquina clams and blue mussels readily available to red knots in Virginia contain 1 – 28 Kcals/g AFDM and 0.5 – 31 Kcals/g respectively, less than that found in similarly-sized crustaceans. Therefore, bivalve prey may be less energetically dense per gram, but the size of these prey items and their propensity to aggregate together may make these prey more available to red knots and may enable red knots to search for and detect bivalve prey more quickly than crustaceans and other prey which do not aggregate in the same manner (Goss-Custard 1977, Garay et al. 2018). The trade-off of red knot's using a search image approach may be the increase in handling time associated with consuming larger prey (Cook and Cockrell 1978); however, as bivalve specialists throughout much of their range, red knots are likely adapted to locate, handle, consume, and digest bivalves efficiently (Piersma 1991, Zwarts and Blomert 1992, Dekinga and Piersma 1993, Tulp and Schekkerman 200). While we found that red knot select bivalves in Virginia, we do not suggest that crustaceans, and other prey, are unimportant to red knots, as they may be more abundant and energetically dense per gram than available bivalves.

The red knot's apparent bivalve specialization may be concerning from a conservation context. Shorebird populations in general are declining, with the steepest declines being seen in long-distance migrants, like the red knot (North American Bird Conservation Initiative Canada 2019). Because red knots select clams specifically in Virginia suggests that they could be more susceptible to changing prey abundances and distributions than more generalist species. Changes in the distribution and abundance of bivalves may affect red knot population size and distribution along Virginia's barrier islands (Cohen et al. 2009), especially as global climate change contributes to the rapid range contraction of blue mussels (Hansen et al. 2006, Jones et al. 2010). As recently as fifty years ago, adult blue mussels bred in intertidal zones as far south as Cape Hatteras, North Carolina; however, their range has contracted northward, most likely due to warming ocean temperatures, and adult blue mussels currently do not breed in intertidal zones south of Delaware (Jones et al. 2010, Wethey et al. 2011). The blue mussel's range contraction is concerning to red knots using the Virginia stopover, as red knots appear to track the availability of their bivalve prey resources along stopover sites and use different substrates at different times during the tidal cycle (Evans et al. 1980, Evans 1981, Alerstam 1990, Piersma et al. 1994, Gonzalez et al. 1996, Heller et al. In Prep). Alternatively, coquina clams may be experiencing range expansion, as they prefer warmer ocean temperatures (Ruppert and Fox 1988, Laudien et al. 2003, Cobb et al. 2011). Therefore, coquina clams may become more abundant in Virginia should ocean temperatures continue to rise. It is possible, however, that as climate change continues to alter environmental conditions that affect prey distribution and abundance (Edwards and Richardson 2004, Both et al. 2006, Durant et al. 2007, Post and Forchhammer 2008), red knots, like other shorebirds, may be able to adopt an opportunistic and generalist foraging strategy, depending on local prey availability, in response to environmental change by selecting underutilized non-bivalve resources such as crustaceans (Recher 1966, Hicklin and Smith 1979, Pienkowski et al. 1984, Skagen and Oman 1996, Davis and Smith 2001, Skagen 2006, Novcic et al. 2015, Gerwing et al. 2016).

Because prey availability and abundance are important to red knots in Virginia during spring migration, protecting intertidal invertebrate habitat is important for successful management of the red knot. For example, to best encourage the formation of peat banks, important to both mussels and crustaceans, beach stabilization and nourishment projects should continue to be avoided in Virginia. Efforts used to stabilize barrier islands by preventing shoreline erosion and island movement reduce an island’s ability to transgress over bay-side marsh and form peat. Additionally, sand deposition on the ocean’s sand and peat intertidal zone may cause mass prey die-offs and/or bury prey beyond the point at which red knots can access them (Saloman 1974, Oliver et al. 1977, NRC 1995, Rakocinski et al. 1996, USACE 2001, Menn et al. 2003, Bishop et al. 2006, Rosov et al. 2016). Managers in Virginia should continue their ongoing protection that allows natural island processes to occur.

LIMITATIONS AND FUTURE RESEARCH

DNA metabarcoding analyses, while useful in serving as true diet surrogates, have limitations (Deagle et al. 2019). For example, these methods can detect the prey items of prey items ("secondary consumption;" Deagle et al. 2005, 2007, Barnett et al. 2007, Pompanon et al. 2012, Gerwing et al. 2016). However, it is unlikely that secondary consumption influenced our results, as there were no known predator-prey relationships among the prey items we found. Additionally, DNA metabarcoding analyses are limited by the number of primers used and by the presence of prey in the reference databanks used. Because we took a conservative approach and pooled prey to a relatively high taxonomic rank – Order – it is unlikely that our analyses would have benefited greatly by comparing data across reference databanks. We only used the 18S primer for this study, which may have decreased the detectability of certain prey in both FOO and RRA analyses. Future research should consider using taxa-specific primers know that we have confirmed *a priori* knowledge of what red knots consume in Virginia. More potential for reference database bias would have been present if we separated prey by species, rather than by Order, particularly for crustaceans from Orders Amphipoda and Calanoida. While we know that blue mussels from Order Mytiloida and coquina clams from Order Veneroida live in Virginia, we know less about which species of crustaceans, from either Order, live in Virginia. Future research should aim to gain a comprehensive understanding of which crustacean species live in Virginia's sand and peat banks and should also include estimating the caloric content of each major prey group used by red knots in Virginia, as this may further elucidate why red knots select certain prey over others.

We focused on the red knot's spring migration in Virginia. Future study should collect fecal samples across their entire spring migration range, during fall migration, and along wintering grounds to help us better understand how the red knot's diet varies throughout its full life cycle using modern molecular techniques. Additionally, some red knots travel from wintering grounds as far south as Tierra del Fuego, Argentina, while others winter in Northern South America and Florida (Baker et al. 2013, Red Knot *Calidris canutus*, In Neotropical Birds Online 2019). Future research should use bird resight methodology (bands are color-coded to indicate the country the bird was banded in) to address if longer-distance migrants use different prey resources than shorter-distance migrants, as the distance a bird migrates may impact its foraging strategy and thus the contents of its feces. Resight methodology may also provide insight into potential sex-specific foraging strategies (Elner and Seaman 2003, Placyk and Harrington 2004, Nebel 2005, Mathot et al. 2007, Jardine et al. 2015). Female red knots, among other sandpipers, have slightly longer bills on average than males (Tomkovich 1992, Baker et al. 1999), and therefore, may be able to consume invertebrates that are buried deeper in the substrate (Page and Fearis 1971, Carter 1984, Stein et al. 2008).

Additionally, while care was taken when collecting red knot fecal samples, it is impossible to rule-out the possibility of feces being contaminated by substrate material being mixed with fecal matter. Further study into the differences between fecal samples collected via this method and fecal samples collected directly from captured birds is recommended (Jardine et al. 2015).

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Research and Monitoring Special Use Permit # 2019-010, Virginia Department of Conservation and Recreation Division of Natural Heritage Natural Area Preserve Research and Collecting Permit # RCP-ESR01-19 (renewal for ESR03-18), The Nature Conservancy Research Permit, Commonwealth of Virginia Marine Resources Commission Permit # 19-040, and Virginia Tech Institutional Animal Care and Use Committee permit number 16-244 (FWC).

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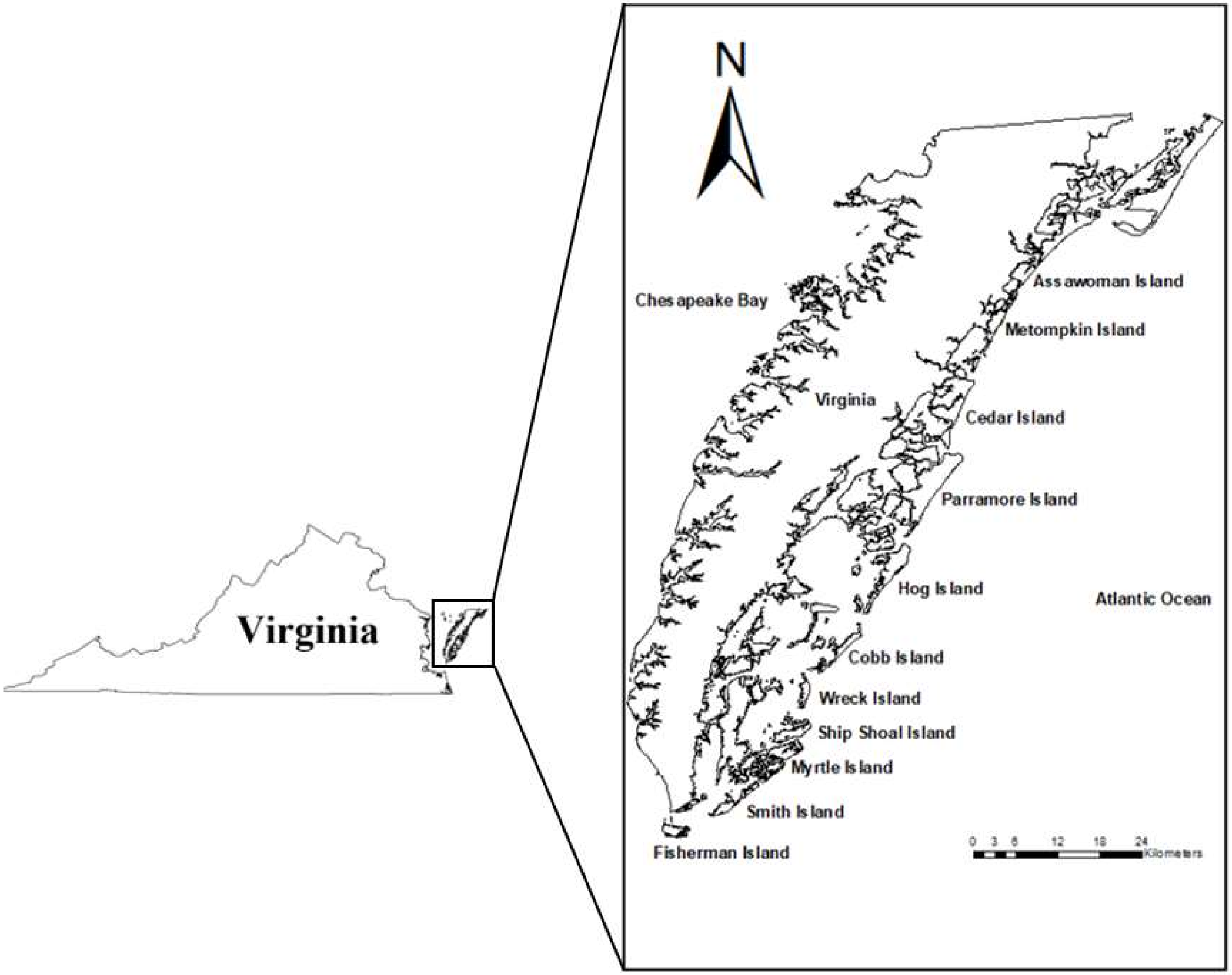
FIGURES


Virginia
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Cedar Island
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Hog Island
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Ship Shoal Island
Myrtle Island
Smith Island
Fisherman Island
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Figure 1. Study area on the Virginia Coast Reserve Long Term Ecological Research Site on the Eastern Shore of Virginia, including Virginia's barrier islands from Assawoman Island in the north to Fisherman Island in the south. We collected red knot fecal samples (*n* = 100) on the Atlantic Ocean intertidal shoreline on peat banks and sand, Virginia's barrier islands, May 14 – 27, 2017 – 2019.

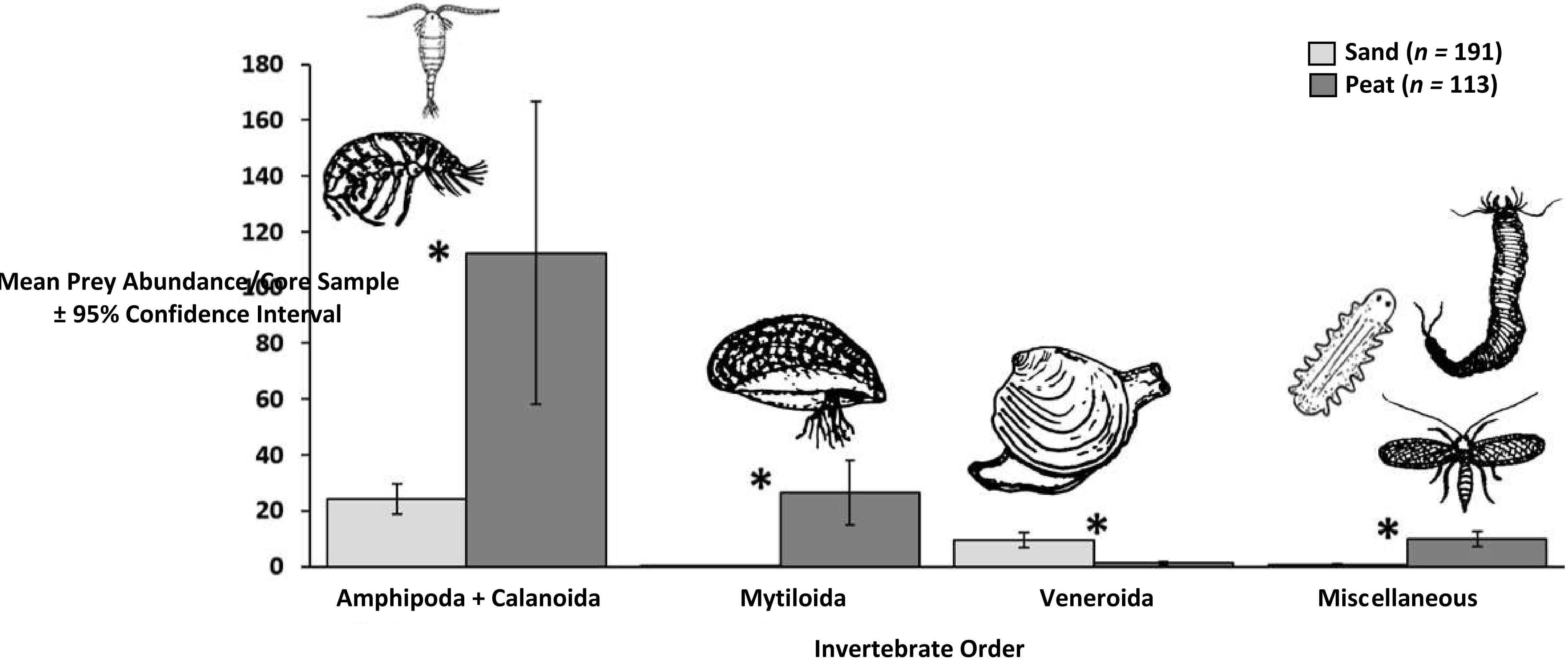


Figure 2. Mean abundance (organisms/275 cm^3 ± 95% CI) of invertebrates captured in 10 cm diameter x 3.5 cm deep cores by prey Order available to red knots on sand (*n* = 191) and peat (*n* = 113), Virginia's barrier islands, May 14 – 27, 2017 – 2018. * Indicates a difference ($p < 0.05$) in means between Orders found in sand and peat based on Wilcoxon rank sum tests with Bonferroni correction.

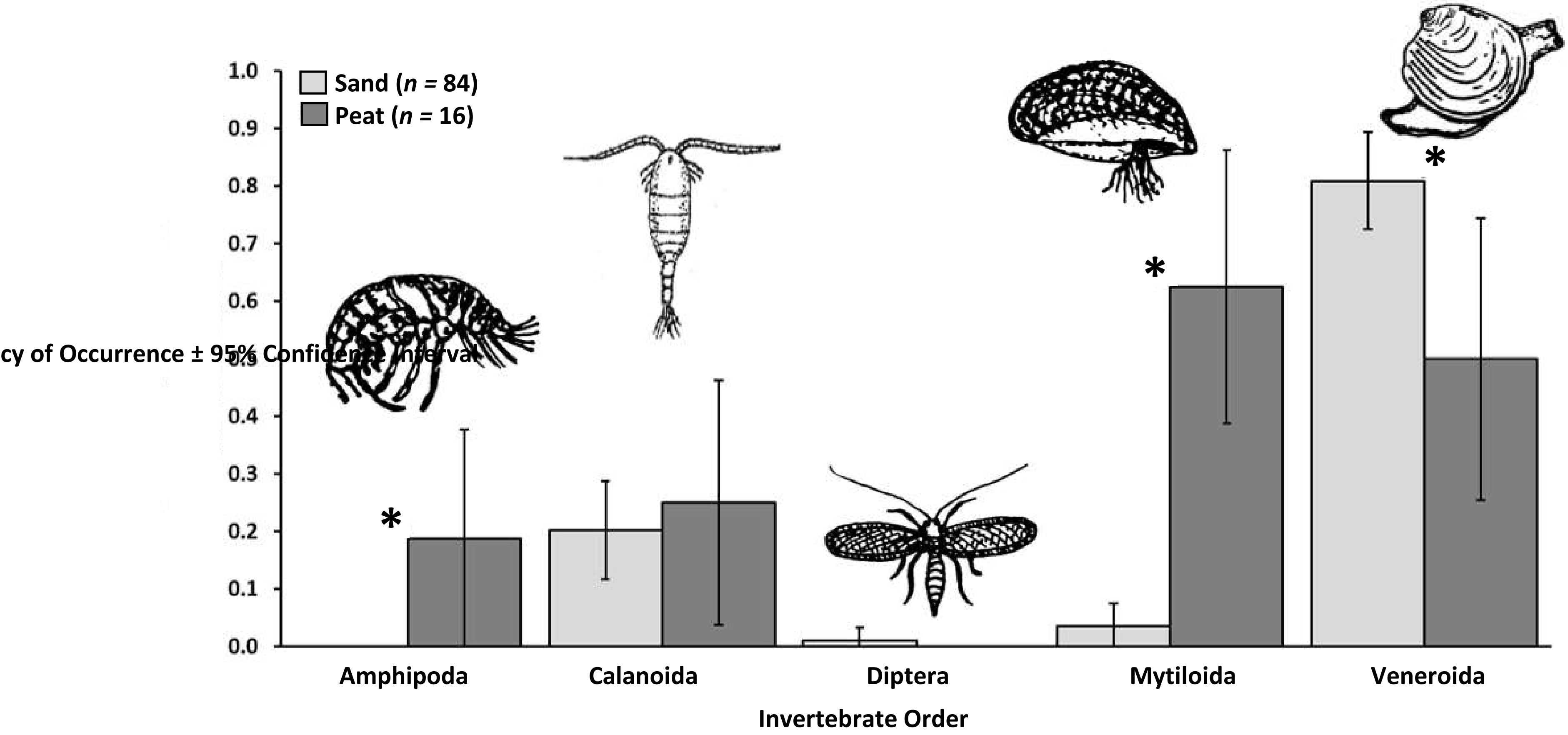


Figure 3. Frequency of occurrence (FOO ± 95% CI) of prey Orders found in red knot fecal samples collected on sand (*n* = 84) and peat (*n* = 16), Virginia's barrier islands, May 14 – 27, 2017 – 2019. Fecal samples contained DNA from Orders Amphipoda (malacostracan crustaceans), Calanoida (copepod crustaceans), Diptera (true flies), Mytiloida (true mussels), and Veneroida (marine

and freshwater bivalve mollusks/clams). * Indicates a difference ($p < 0.05$) between Orders found in peat and sand based on 2-sample Chi-square tests for equality of proportions with continuity correction on sand and peat banks.

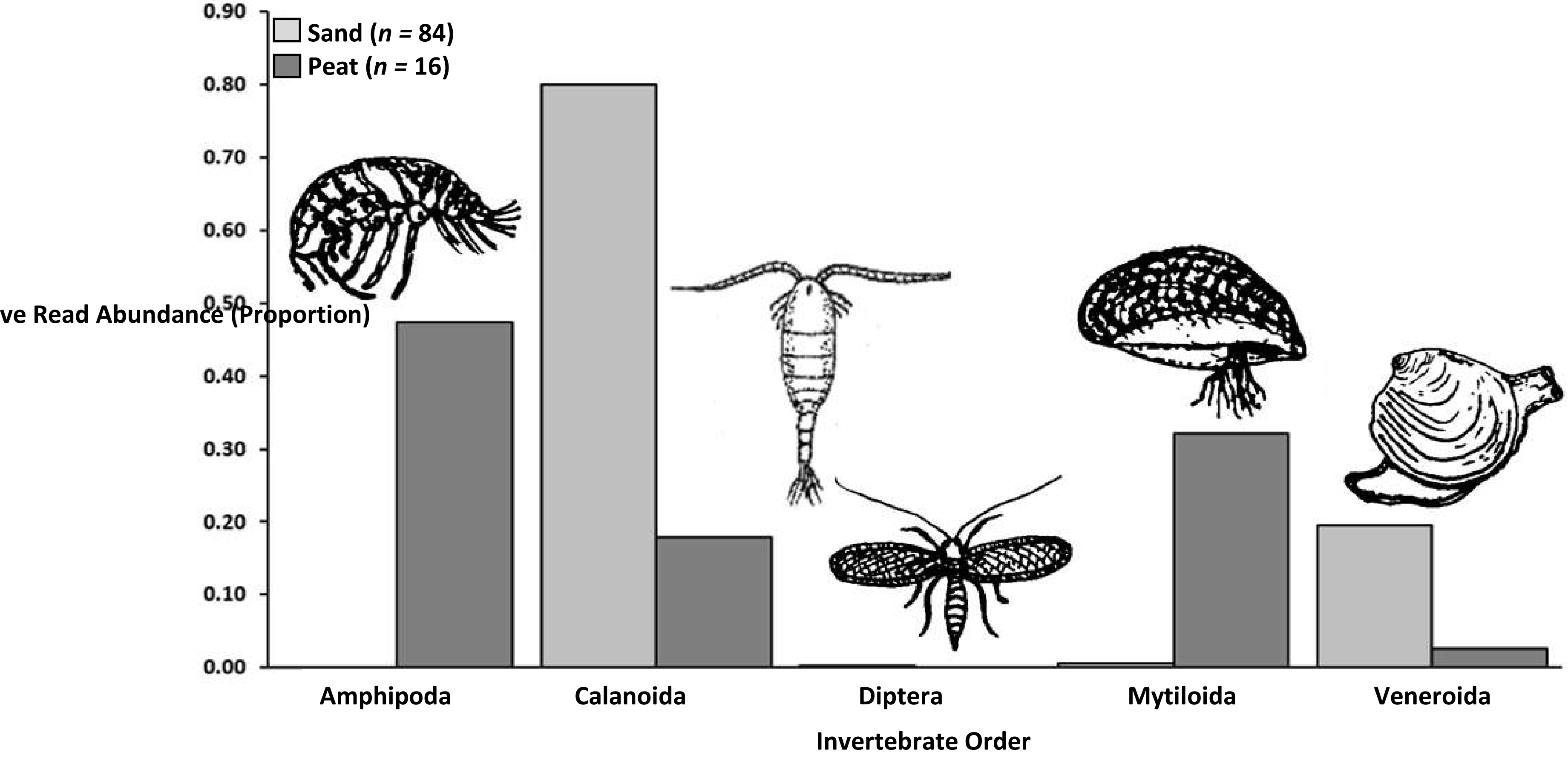


Figure 4. Relative read abundance (RRA) of invertebrate Orders detected using DNA metabarcoding in red knot fecal samples collected on sand (*n* = 84 samples; *n* = 51,807 sequences) and peat (*n* = 16 samples; *n* = 23,596 sequences), Virginia's barrier islands, May 14 – 27, 2017 – 2019.

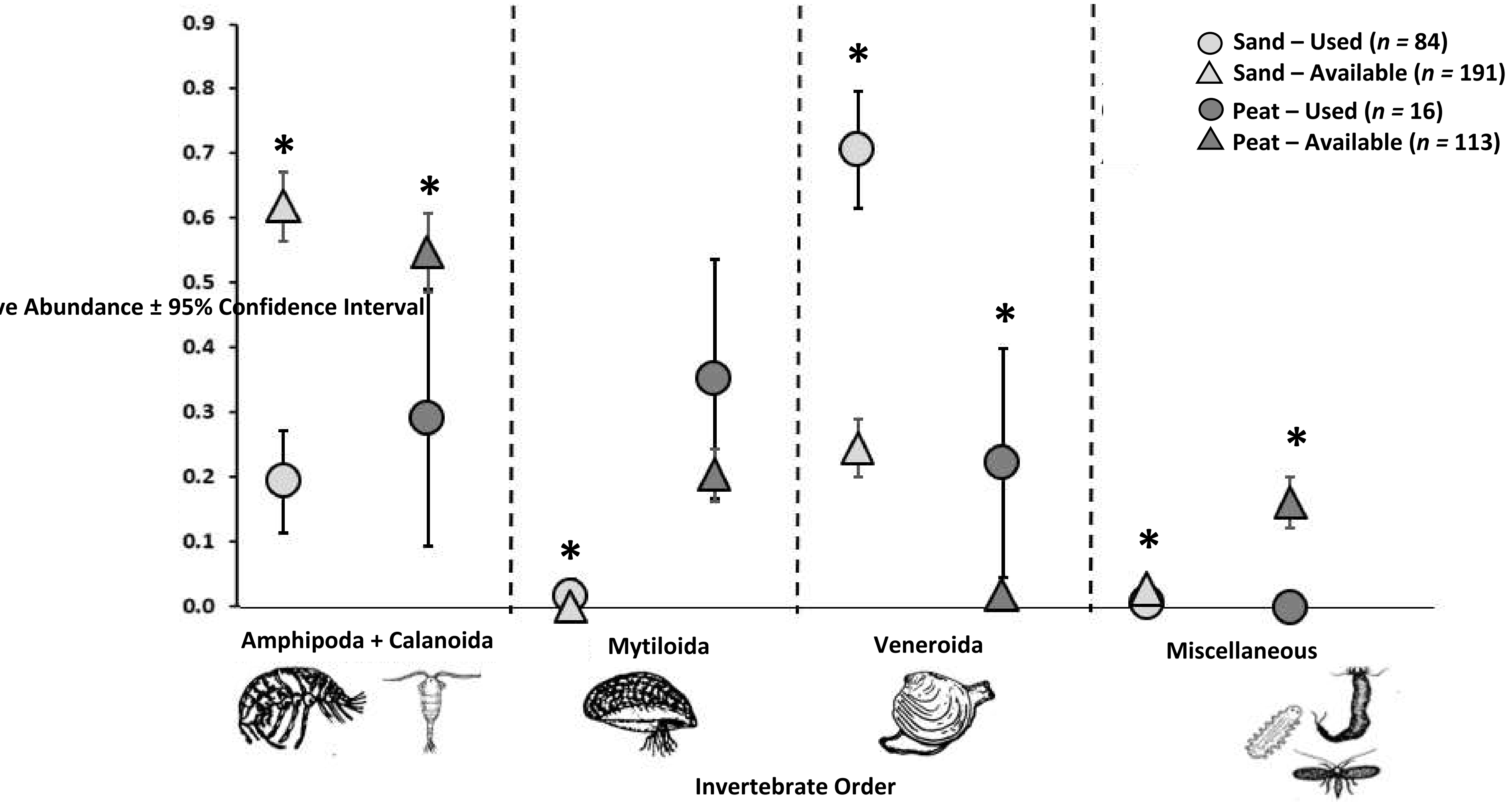


Figure 5. Mean relative abundance (± 95% CI) of prey captured in 10 cm diameter x 3.5 cm deep cores (‘Available’) and the mean relative read abundance (RRA; ± 95% CI) of invertebrate Orders detected using DNA metabarcoding in red knot fecal samples (*n* =

100) collected ('Used') on sand (Used: *n* = 84 fecal samples, *n* = 51,807 sequences; Available: *n* = 191) and peat (Used: *n* = 16 samples, *n* = 23,596 sequences; Available: *n* = 113), Virginia's barrier islands, May 14 – 27, 2017 – 2019. * Indicates a difference ($p < 0.05$) in means between Used and Available prey found on sand and peat based on Wilcoxon rank sum tests with Bonferroni correction.

APPENDICES

Appendix 1. Number of red knot fecal samples collected on sand and peat with the number of reads from samples containing readable invertebrate DNA, Virginia's barrier islands, May 14 – 27, 2017 – 2019.

Substrate (Sample Size)	Year (Sample Size)	Order	# Reads from Readable Samples (# Readable Samples)[a]
Sand (n = 84)	2017 (n = 11)	Amphipoda	0 (0)
		Calanoida	157 (2)
		Diptera	10 (1)
		Mytiloida	240 (2)
		Veneroida	360 (6)
	2018 (n = 35)	Amphipoda	0 (0)
		Calanoida	22,491 (7)
		Diptera	0 (0)
		Mytiloida	0 (0)
		Veneroida	1,285 (27)

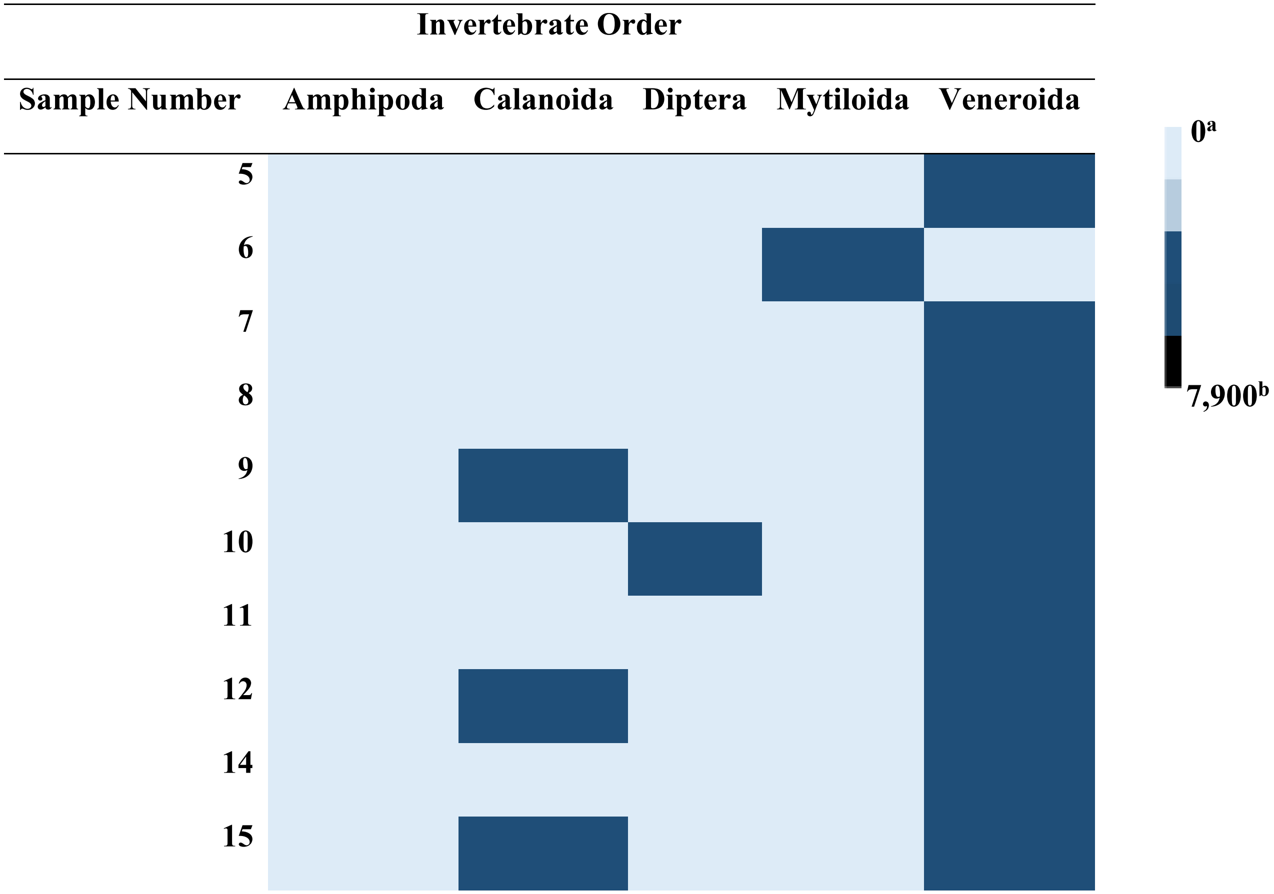
	2019 (*n* = 38)	Amphipoda	0 (0)
		Calanoida	11,749 (8)
		Diptera	0 (0)
		Mytiloida	6 (1)
		Veneroida	6,735 (35)
Peat (*n* = 16)	2017 (*n* = 5)	Amphipoda	0 (0)
		Calanoida	4,213 (4)
		Diptera	0 (0)
		Mytiloida	2,293 (3)
		Veneroida	0 (0)
	2018 (*n* = 11)	Amphipoda	11,206 (3)
		Calanoida	0 (0)
		Diptera	0 (0)
		Mytiloida	5,264 (7)
		Veneroida	622 (8)
	2019 (*n* = 0)	Amphipoda	0 (0)

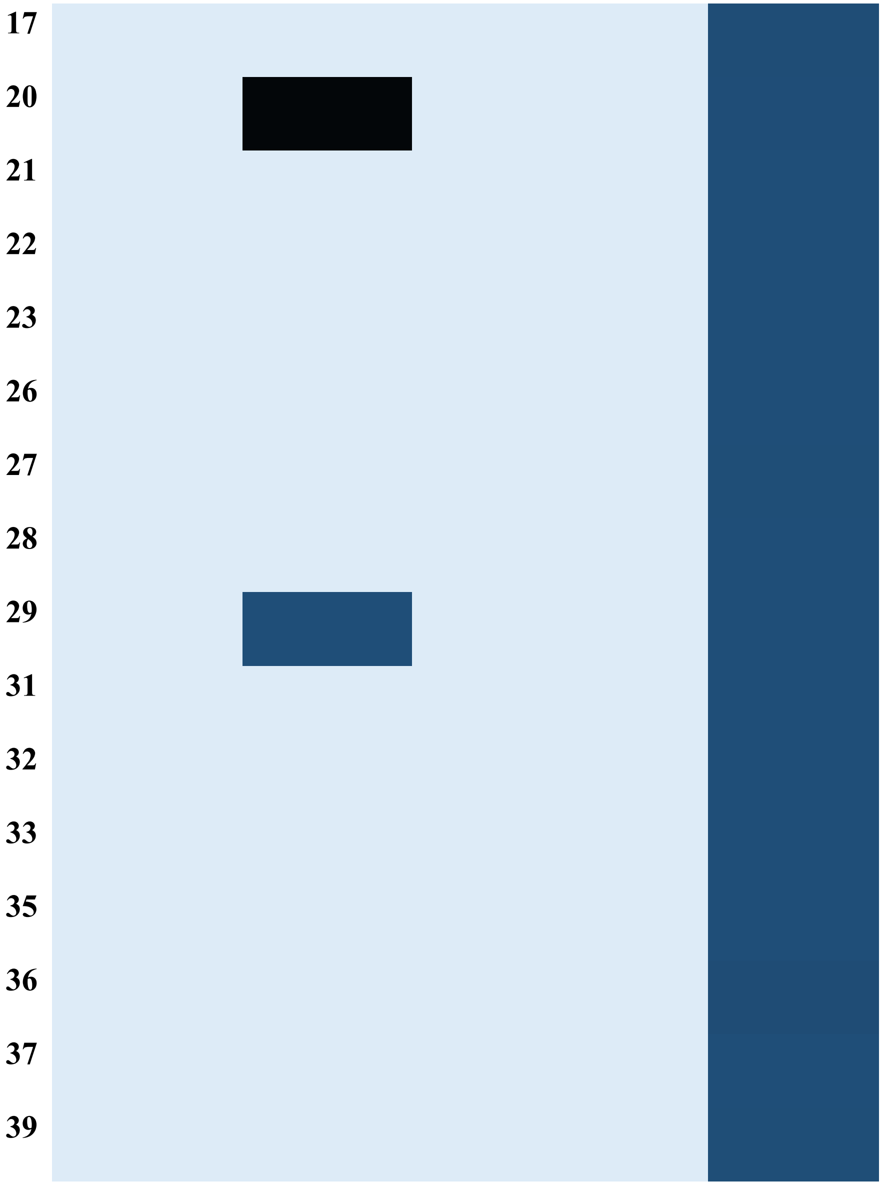
Calanoida	0 (0)
Diptera	0 (0)
Mytiloida	0 (0)
Veneroida	0 (0)

[a] Readable DNA signifies ≥ 5 reads from one fecal sample.

Appendix 2. Heat maps demonstrating the relative read abundances (RRA) and frequency of occurrence (FOO) of five Orders of invertebrates found using DNA metabarcoding analyses on red knot fecal samples collected on (a) sand (*n* = 84) and (b) peat (*n* = 16), Virginia's barrier islands, May 14 – 27, 2017 – 2019.

(a)

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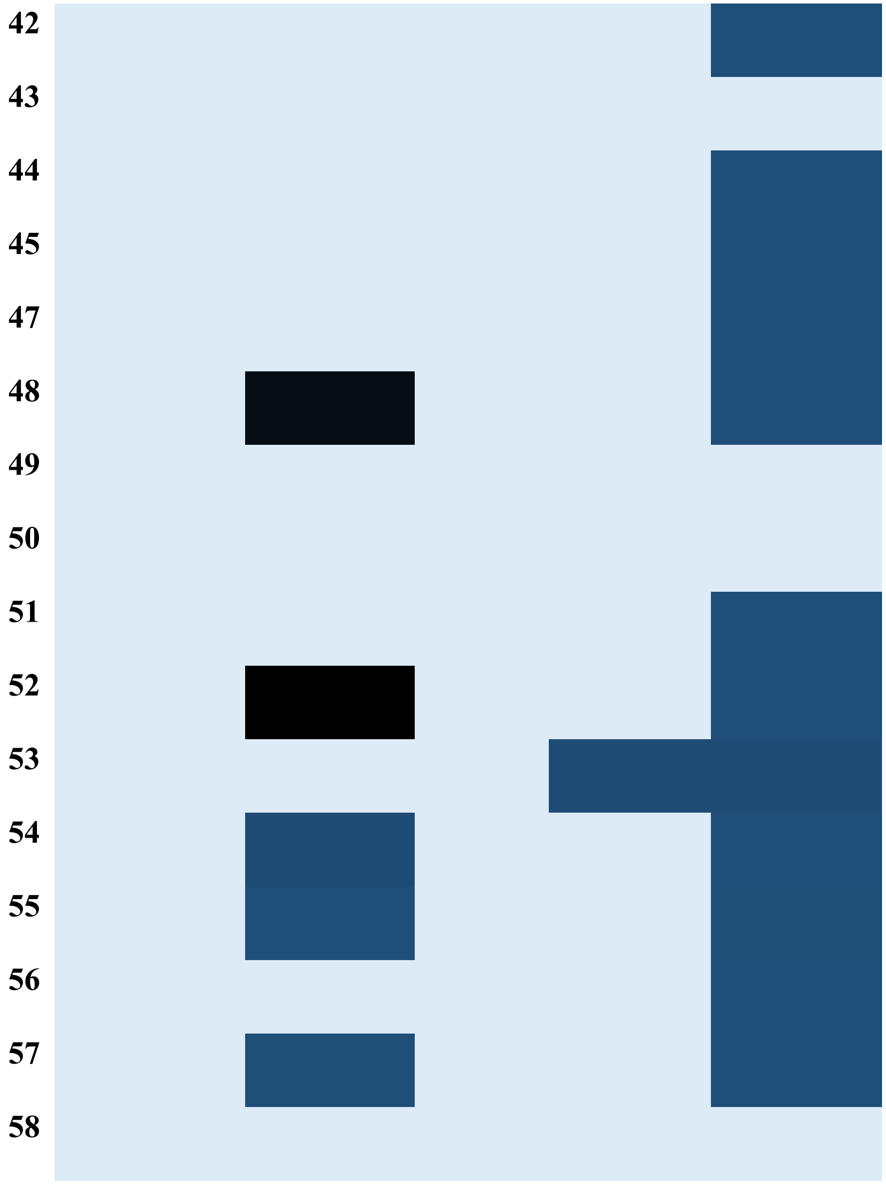
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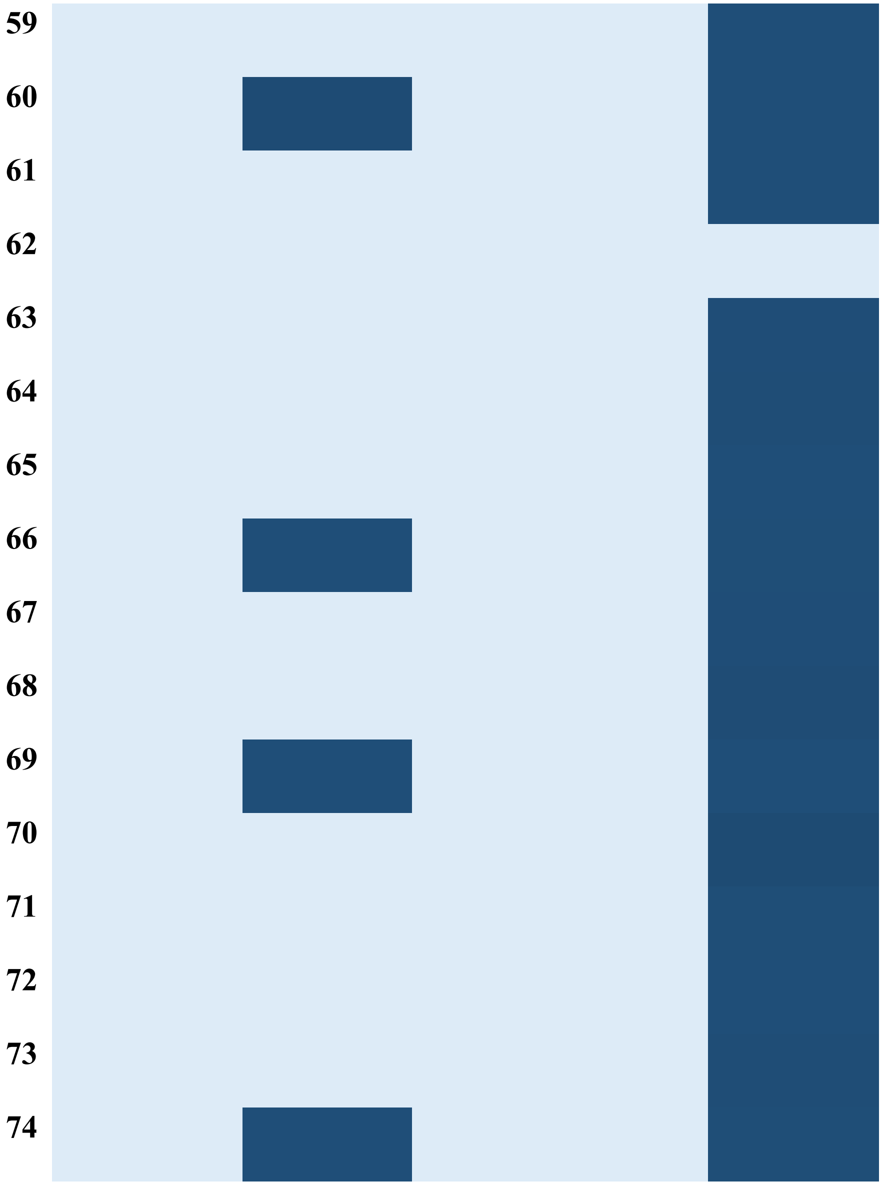
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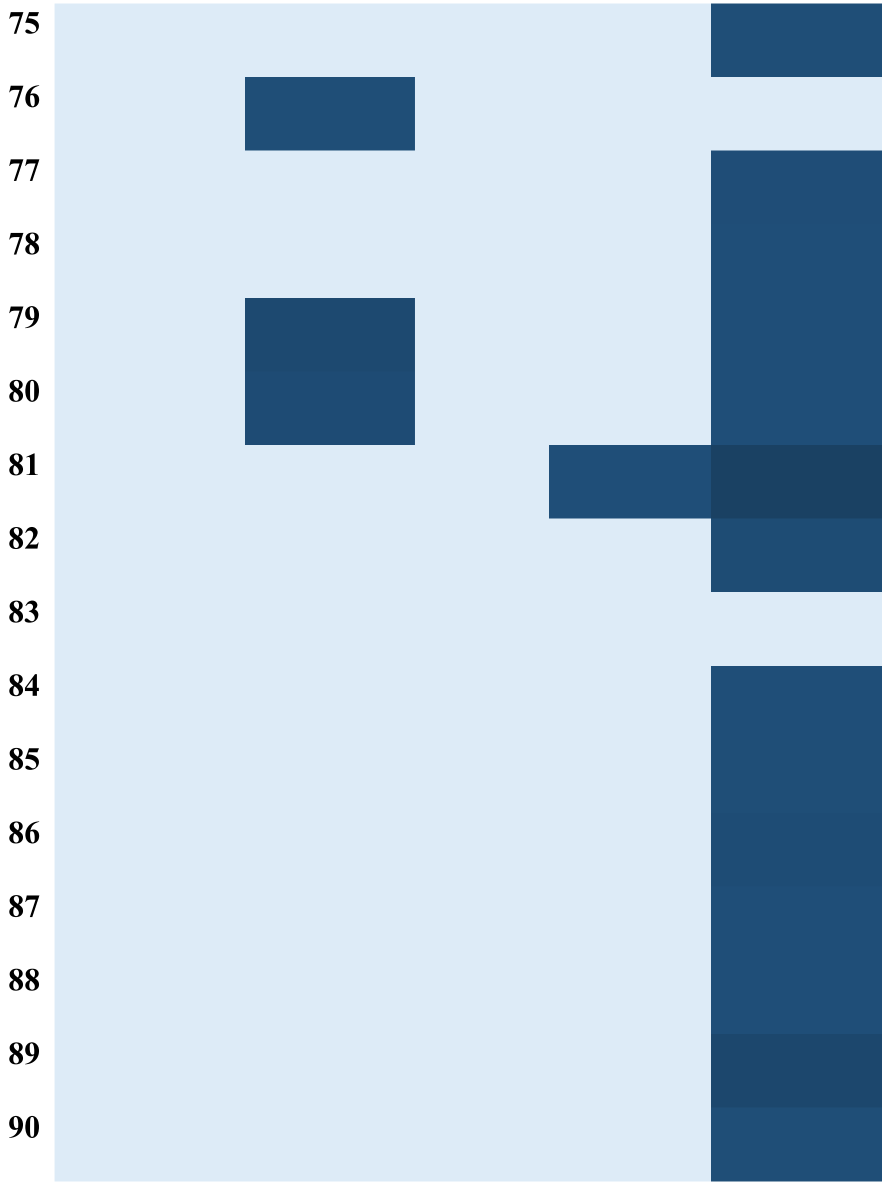
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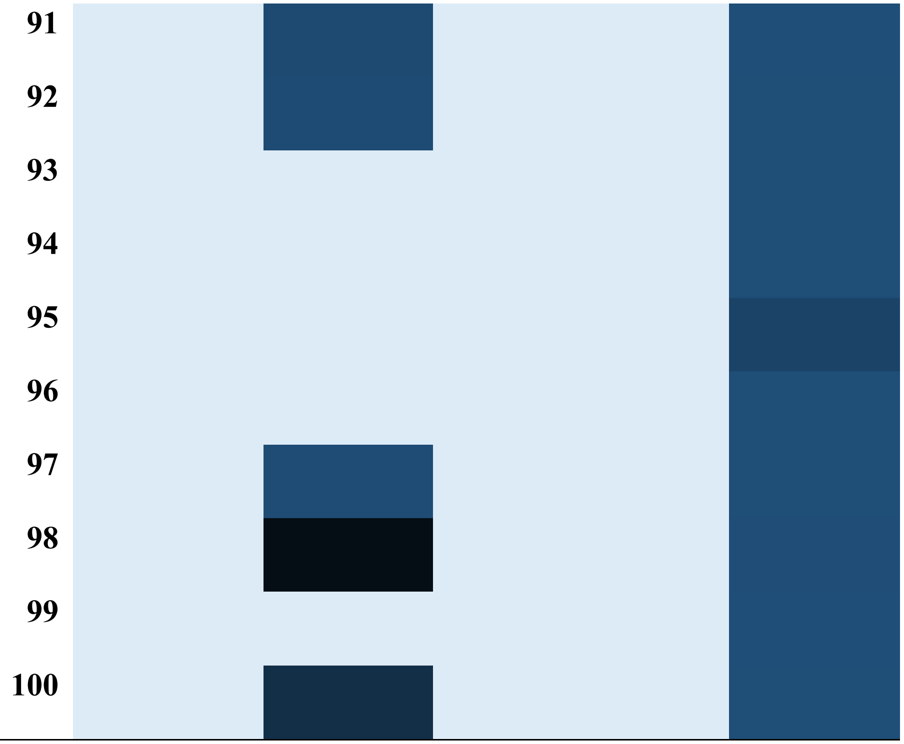
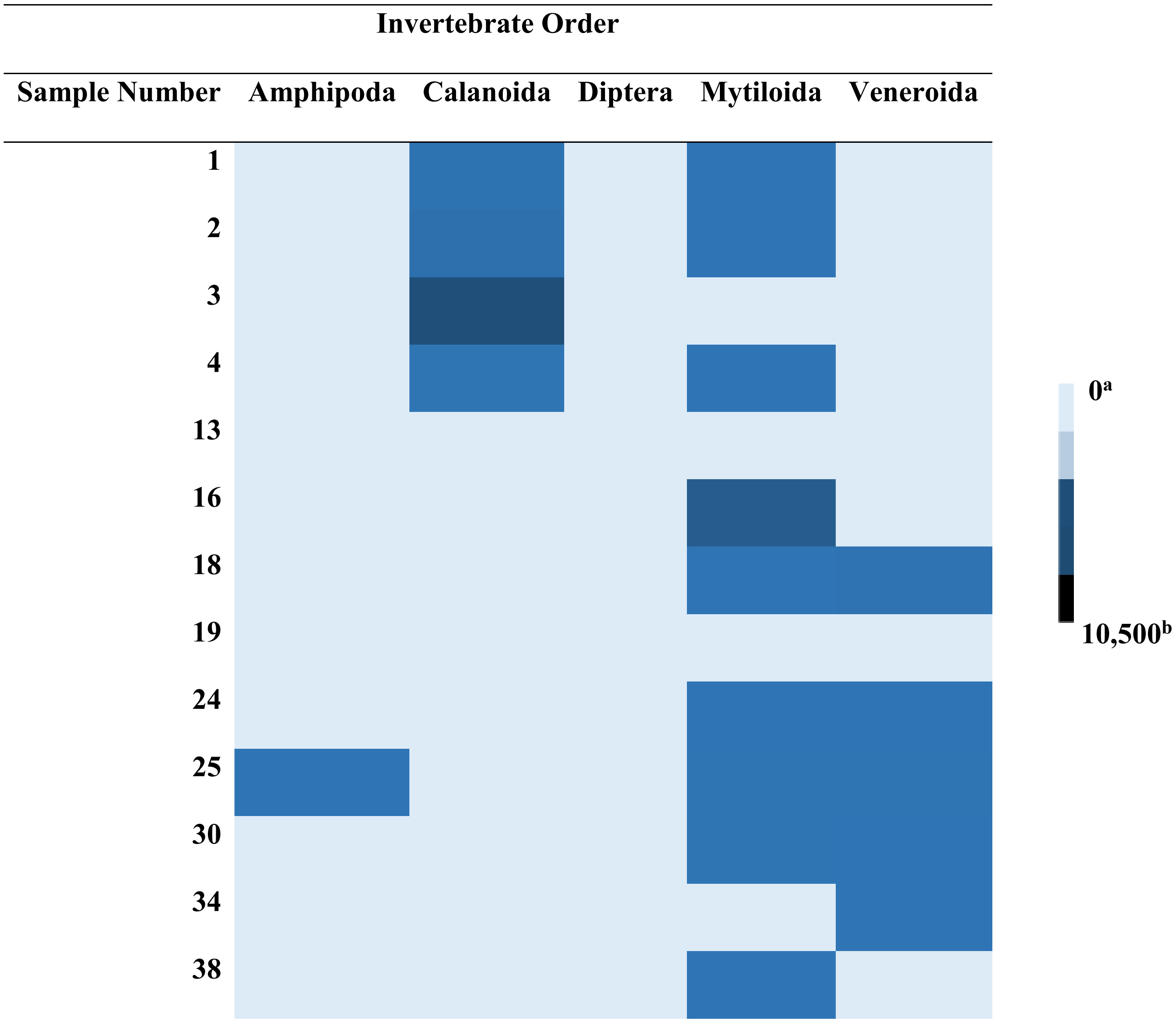
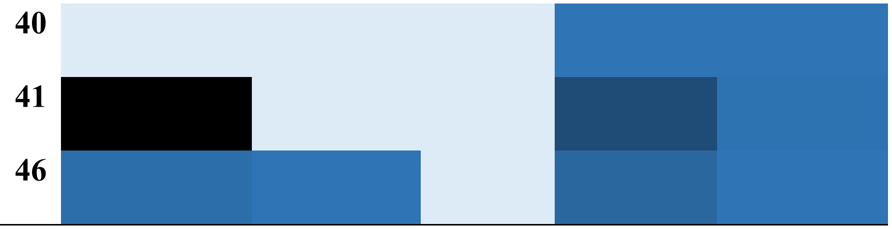


RRA	0.00	0.80	0.00	0.01	0.20
FOO	0.00	0.23	0.01	0.04	0.81

(b)


Invertebrate Order
Sample Number
Amphipoda
Calanoida
Diptera
Mytiloida
Veneroida
1
2
3
4
13
16
18
19
24
25
30
34
38
0[a]
10,500[b]



RRA	**0.48**	**0.18**	**0.00**	**0.32**	**0.03**
FOO	**0.19**	**0.25**	**0.00**	**0.63**	**0.38**

[a] Light blue indicates no detection (denoted by < 5 reads).

[b] Blues get darker as the number of reads increases.

FACTORS THAT AFFECT MIGRATORY WESTERN ATLANTIC RED KNOTS (*CALIDRIS CANUTUS RUFA*) AND THEIR PREY DURING SPRING STOPOVER ON VIRGINIA'S BARRIER ISLANDS

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ABSTRACT

Understanding factors that influence a species' distribution and abundance across the annual cycle is required for planning range-wide conservation interventions. Thousands of federally threatened (U.S.A.) and endangered (Canada) red knots (*Calidris cantus rufa*) stop on Virginia's barrier islands each year to replenish fat stores during spring migration. We investigated the variation in red knot distribution and flock size, the potential effects of prey on this variation, and factors influencing prey abundance on Virginia's barrier islands. We counted red knots and collected potential prey samples at randomly selected sites from 2007 – 2018 during a two-week period, the first week during early migration and the second week during peak migration. Core samples contained crustaceans (Orders Amphipoda and Calanoida), blue mussels (*Mytilus edulis)*, coquina clams (*Donax variabilis)*, and miscellaneous prey (horseshoe crab eggs (*Limulus polyphemus*), angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae, snails, worms)). Estimated red knot numbers in Virginia during peak migration were highest in 2012 (11,644) and lowest in 2014 (2,792; 12-year peak migration $\bar{x}$ = 7,055, SD = 2,841). Red knot and prey numbers per sampling unit varied across sampling periods and substrates (i.e., peat and sand). Red knots generally used sites with more prey. Miscellaneous prey ($\bar{x}$ = 18.85/core sample, SE = 0.88) were important in determining red knot presence at a site early in migration, when we only sampled on peat banks. Coquina clams ($\bar{x}$ = 11/core sample, SE = 0.30) and blue mussels ($\bar{x}$ = 0.94/core sample, SE = 0.04) were important in determining red knot presence at a site during peak migration, when we sampled both substrates. Few clear relationships between prey and red knot flock size existed, suggesting that other unmeasured factors played a role in determining red knot numbers at occupied sites. Counts of red knots at Tierra del Fuego wintering grounds within the same year were positively related to the likelihood of red knot presence at individual sites but were negatively related to red knot flock size in Virginia. Tide and mean daily water temperature were important in determining prey abundance. Maximizing the diversity, availability, and abundance of prey for red knots on barrier islands requires management that encourages the presence of both sand and peat bank intertidal habitats. Continued management that allows the natural processes of overwash and island transgression to occur in Virginia is important for the creation and maintenance of both sand and peat bank habitats.

KEY WORDS *Calidris canutus rufa*, prey, red knot, stopover ecology, Virginia

INTRODUCTION

Species conservation requires an understanding of the factors that influence a species' distribution and abundance (Lawton 1993, Ehrlén and Morris 2015). Understanding the factors that affect migratory species throughout their annual cycles is challenging, though of great importance (Rushing et al. 2016). Stopover sites enable animals to migrate long distances in short periods by providing food so migrants can replenish fat stores and rest mid-migration (Burger et al. 1997, Schwilch and Jenni 2001, Atkinson et al. 2007, Cohen et al. 2011, McGuire et al. 2011, Maxted et al. 2016). Migratory shorebirds must be adaptable to variation in food availability on stopovers (Guglielmo et al. 2005, Martínez-Curci et al. 2016). Anthropogenic and climate–related factors have led to the rapid loss and degradation of stopover grounds, amplifying the importance of remaining stopover habitat and food availability for migratory shorebirds (MacKay 1893, Myers et al. 1979a, Placyk and Harrington 2004, United States Commission on Ocean Policy 2004, Aharon-Rotman et al. 2016, Murray et al. 2018, Studds et al. 2017).

The Western Atlantic red knot (*Calidris canutus rufa*; 'red knot'), listed as federally threatened in the United States (U. S.) and endangered in Canada, has one of the longest migrations in the western hemisphere, travelling from wintering grounds as far south as Tierra del Fuego, Argentina, to breeding grounds in the Canadian Arctic (Federal Registrar 2014, Lyons et al. 2018, USFWS 2015, 2019). Due to its imperiled status, recognized after large declines in the mid-1990s (Morrison et al. 1989, 2004, Dey et al. 2011, 2015), and long migration, red knot stopover ecology often is viewed as an exemplar of the challenges faced by long–distance migrant shorebirds (Piersma and Lindstrom 2004, Dey et al. 2011, 2015, Federal Registrar 2014, Watts and Truitt 2015).

Large numbers of red knots historically frequented North American stopover sites on coastal beaches from Florida to Massachusetts (Wilson and Bonaparte 1832, MacKay 1893). However, while thousands of red knots continue to use coastal beaches on the United States' Atlantic Coast (Dey et al. 2011, Dey 2014, USFWS 2015, 2019), most of them now stop at two sites each spring migration: the Delaware Bay and Virginia's barrier islands (MacKay 1893, U.S. Commission on Ocean Policy 2004, Cohen et al. 2010a, Lyons et al. 2018). Despite the relatively short distance between the two sites (~125 km), only about 5% of red knots move between these sites in a given year, and red knots remaine at both sites for approximately 2 weeks (Cohen et al. 2009). Additionally, Cohen et al. (2009) found that radiotagged red knots rarely left the site where they were tagged before May to early June, the time when most red knots on the Atlantic Coast leave for the Arctic. These findings suggest that Virginia and the Delaware Bay are primarily independent stopovers within years.

Historically, much attention was given to red knots using the Delaware Bay spring stopover (Smith et al. 2008, Cohen et al. 2009, Niles et al. 2009, Watts 2013, Watts and Truitt 2015), as it consistently supports between about 50 – 70% of the annual stopover population of red knots, at higher densities compared to Virginia's barrier islands (on average 291 red knots/km in Delaware Bay vs. 81 red knots/km in Virginia; Watts and Truitt 2000, Cohen et al. 2010a, 2011, Federal Registrar 2014, USFWS 2015, 2019). The large number of red knots using the Delaware Bay stopover each spring usually is attributed to the abundance of Atlantic horseshoe crab eggs (*Limulus polyphemus*; Karpanty et al. 2006, 2011, Niles et al. 2009, Fraser et al. 2010, 2012, Smith and Robinson 2015, Burger et al. 2018).

Although red knots feed primarily on horseshoe crab eggs in the Delaware Bay (Karpanty et al. 2006, 2011, Niles et al. 2009, Fraser et al. 2010, 2012, Smith and Robinson 2015, Burger et al. 2018), they feed on hard-shelled organisms throughout most of their migration and wintering ranges (Piersma 1991, Zwarts and Blomert 1992, Dekinga and Piersma 1993, Dufour 2018). The red knot's reliance on bivalves likely includes its Virginia spring stopover, where coquina clams (*Donax variabilis*) and blue mussels (*Mytilus edulis*) have been described as the most abundant and used prey resources by red knots (Watts and Truitt 2000, 2015, Cohen et al. 2010b, 2011). Confirming the importance of numerous prey resources in Virginia, Heller et al. (In Prep) used fecal DNA metabarcoding analyses to confirm that red knots consumed bivalves (Orders Veneroida and Mytiloida) in Virginia and found that red knots also consumed crustaceans (Orders Amphipoda and Calanoida) and insect larvae (Order Diptera).

Numerous studies have demonstrated positive correlations between waterbird numbers and invertebrate prey (Murkin and Kadlec 1986, Colwell and Landrum 1993, Safran et al. 1997, Placyk and Harrison 2004, Cohen et al. 2010b). Because red knots likely track prey resources in space and time (Piersma et al. 1994, Karpanty et al. 2006) and different prey species may be available in different habitats at different tides, red knots may shift foraging locations throughout the day to maximize foraging efficiency. Other factors, such as ocean temperature, may differentially affect prey availability and survival. For example, blue mussels require water temperatures between 5 and 20°C to survive and breed. Repeated exposure to air and to temperatures above 30°C causes high blue mussel mortality (Ansell 1983, Jones et al. 2009). Historically, adult blue mussels were found in intertidal waters from the Arctic through North Carolina (Dall 1889, Wells and Gray 1960). Currently, Virginia's barrier islands are on the southern edge of juvenile blue mussels' intertidal range, while Delaware is the southern edge of adult blue mussels' intertidal range. Coquina clams, however, require warmer ocean waters and are found breeding in sandy intertidal zones from Virginia through the Gulf of Mexico, where ocean temperatures can exceed 30°C (Ruppert and Fox 1988, Laudien et al. 2003, Cobb et al. 2011, NCEI 2020). Temperature variations, therefore, may affect the presence and abundance of prey resources in Virginia for migratory birds like red knots.

Some uncertainty remains concerning the influence that prey may have on red knot distribution and flock size on Virginia's barrier islands. For example, Cohen et al. (2010b) suggested that coquina clams were the dominant prey item for red knots in Virginia, whereas Watts and Truitt (2015) suggested by peak migration, red knots used peat banks, where blue mussels and other prey primarily live, ten-times more than sand, where coquina clams primarily live. Although studies have been conducted on the prey that red knots potentially consume in Virginia (Cohen et al. 2010b, 2011, Watts and Truitt 2015), these were limited to short–term datasets (≤ 2 years) and did not fully address all potential prey resources. Therefore, the factors that may affect red knots in Virginia over extended periods need further study (Karpanty et al. 2006, 2011, Niles et al. 2009, Fraser et al. 2010, 2012, Smith and Robinson 2015).

To gain a better understanding of how many red knots use Virginia's barrier islands each spring migration and how numerous factors affect both red knot and prey abundance, our objectives were to determine: 1) the predicted number of red knots using Virginia's barrier islands during peak migration (May 21 – 27) from 2007 – 2018, 2) if prey other than coquina clams and blue mussels were available to red knots over an extended (> 2 years) time, 3) if and how red knot and prey numbers varied between sampling periods and substrates, 4) if there were differences in prey abundances between sites used and unused by red knots, 5) if red knot presence and flock size were influenced by prey in Virginia and the number of red knots in the flyway (i.e., wintering red knot counts in Tierra del Fuego), and 6) what factors affected prey abundance in Virginia over time and space.

STUDY AREA

We studied red knots on eleven barrier islands in the Virginia Coast Reserve Long-Term Ecological Research site from Assawoman Island in the north to Fisherman Island in the south (Fig. 1; 37°23.7'N, 75°42.5'W; Cohen et al. 2010b). The combined barrier island shoreline extends approximately 82 km and is bounded by the Atlantic Ocean on the east and a shallow lagoon system with open water, mudflats, and *Spartina spp*. marsh on the open bays to the west, between the barrier islands and the Delmarva Peninsula mainland (Cohen et al. 2009, 2010b, Bachmann et al. 2012). The islands are separated from each other by a series of channels and shallow marshes that follow Virginia's coastline within the Delmarva Peninsula (Smith et al. 2008). The islands are predominantly uninhabited and undeveloped, accessible primarily by boat, and experience little anthropogenic activity.

The islands provide foraging habitat for migratory shorebirds preparing to breed or preparing to continue migrating to breeding grounds farther north. These shorebirds forage principally on two ocean intertidal substrates: sand and peat (Nelson 1986, Schlacher et al. 2008, Cohen et al. 2011, Watts and Truitt 2015). Large populations of coquina clams are found in sandy intertidal zones, the most common intertidal substrate on Virginia's barrier islands (Sperry 1940, Cohen et al. 2010b). Intertidal peat banks, which comprise about 6% of the shoreline in a given year, are formed when outer beaches erode along low and narrow island segments that transgress over tidal marshes on the bay side of the islands (Truitt et al. 2001, Bachmann et al. 2002, Cohen et al. 2010a, b, Kreuzburg et al. 2018). Although these peat banks are found sporadically across only some islands, they may support dense invertebrate populations (Smith et al. 2008, Cohen et al. 2010a, b, Watts and Truitt 2015). The islands vary in their susceptibility to overwash and landward-movement based partly on morphological characteristics, such as elevation. Low elevation islands, in general, are narrow and lack vegetated dunes. These islands are thus prone to overwash, enabling the formation of peat banks on the islands' ocean side over time. Higher elevation islands have well-developed, more vegetated dunes that help resist overwash events and thus generally lack extensive peat banks (Wolner et al. 2013, Durán and Moore 2014); however, high elevation islands can sustain peat banks on lower elevation, narrow island segments.

METHODS

Survey Methods

Early and Peak Migration Sampling

We collected data along Atlantic Ocean intertidal zones on Virginia's barrier islands each year from 2007 – 2018 (Fig. 1). Using the Hawth's Tools extension (Beyer 2004) in ArcGIS 10.1 (ESRI 2012), the Geospatial Modelling Environment extension (Beyer 2009) in ArcGIS 10.5 (ESRI 2017), and the most recently available United States Department of Agriculture (USDA) Farm Service Agency's National Agriculture Imagery Program (NAIP) orthophotography imagery (USDA Farm Service Agency 2018), we generated and then sampled random points along each island each year. The locations of randomly generated points varied by year. To determine the location of peat banks, we walked along the shore at low tide when peat substrate was exposed and recorded the location of the north and south boundaries of peat banks greater than 1 m in length using hand-held GPS devices. These data were cross-referenced with orthophotography imagery and were jointly used to stratify random sampling points by substrate type. Data collection occurred May 14 – 27, 2007 – 2018, corresponding to the two weeks of the red knot stopover period during which red knots most abundantly use Virginia's barrier islands.

We designed our study to first sample only peat banks around low tide, when peat is exposed, and then to sample both available substrates regardless of tide. We began sampling peat banks exclusively during the red knot's early migration (May 14 – 20; 'early migration period') in 2008 to ensure that we more adequately sampled peat, given its tide-dependent and limited distribution across the islands. Data from 2010 data are not presented for the early migration period as GPS locations of each sampling point were not retained. We sampled peat banks from two hours before low tide to two hours after low tide, encompassing the last hour of falling tide, low tide, and the first hour of rising tide, when peat was most exposed.

During the approximate peak of red knot migration (May 21 – 27; 'peak migration period'), we sampled approximately 100 randomly chosen locations each year. During this period, we sampled both peat and sand regardless of tide, dependent upon which substrate was found at each random point; Clark et al. 1993, Watts and Truitt 2000, Cohen et al. 2009, Watts 2013). Sampling was neither stratified by substrate type nor tide during this sampling period. Not all islands contained peat banks in any given year, and the number of points per island varied by island length. We counted the number of non-flying red knots within a 100 m radius semicircle of each point placed on the water line.

After red knots were counted, we sampled prey availability by collecting a core sample of the substrate at the water-line at each sampling point using a section of PVC piping (10 cm diameter x 3.5 cm deep; core volume = 275 cm^3). The cores' depth represented the approximate length of a red knot's bill (Tomkovich 1992), so that we sampled only prey that red knots would be able to access while foraging. The PVC was pushed into the substrate until the top was even with the substrate's surface, then a trowel was slipped under the PVC to prevent the substrate sample from falling out as the PVC and core sample were retrieved. Samples were placed in a zipper-lock plastic bag, returned to the lab, and frozen for future identification. We used a series of sieves, with the smallest mesh size #40 (0.32 mm holes), and a dissecting microscope to sort and count the number of invertebrates in each sample. We sorted prey by category (i.e., crustacean [Orders Amphipoda and Calanoida (Heller et al. In Prep)], blue mussel, coquina clam, miscellaneous – horseshoe crab eggs, angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae, snails, worms) that we were unable to identify to species). We grouped these organisms as "miscellaneous" prey due to the relatively low number of animals of each type collected.

Sand and Peat Tidal Sampling

To address how red knots and prey varied at the same location over the tidal cycle, we sampled red knots and prey at the water line as the tide advanced and receded on sand (2013 – 2018) and peat banks (2015 – 2018). Sampling dates fell between May 17 – 28. For sand tidal sampling, we counted the number of non-flying red knots within a 100 m radius semicircle on the water line every hour for 12 hours, coinciding to the full tidal cycle. After counting red knots, we collected a core sample at the water line. Each core sample was collected at a point directly in line with the previous point perpendicularly from the water's edge, accounting for the changing water line as the tide receded or advanced. The same methods were used for peat tidal sampling within the four-hour period during which peat banks were most exposed. Thus, peat tidal sampling started two hours before low tide and ended two hours after low tide.

Prey Spatial Tidal Sampling on Peat and Sand

To address spatial variation of prey across the tidal cycle, we conducted spatial tidal sampling on peat banks (May 17, 2019; Myrtle Island) and sand (May 20, 2019; Hog Island). We collected core samples from a central location (0 m) and 5 m north, 5 m south, 10 m north, and 10 m south of the central location, horizontal to the water line, every hour. Peat spatial tidal sampling occurred within the four hour period during which peat banks were exposed, from two hours before to two hours after low tide. Due to time constraints, sand spatial tidal sampling was conducted for only 5 hours between the rising and falling tides, as opposed to during the full 12-hour tidal cycle.

Data Analyses

Predicted Red Knot Numbers

To estimate the predicted number of red knots using the entire length of Virginia's barrier islands during peak migration (May 21 – 27) each year, based on our observations at random sampling points across the island chain (Raw Data Expansion), we used the following equation to linearly expand our predictions from our sampling points:

$$\text{Predicted \# Red Knots Per Year}_{2007\text{-}2018} = \frac{\text{Total Shoreline Length (m)}}{\sum(\text{n1} + \text{n2} + \ldots \text{n11}) * 200\text{ m}} * (\textstyle\sum k_1 + k_2 \ldots k_{11}),$$

where, $n_{1\text{-}11}$ = number of sampling points on each of the 11 islands, and

$k_{1\text{-}11}$ = sum of all red knots counted in all sampling points on each of the 11 islands

Additionally, to account for non-normality and variation in island use, we used zero-inflated negative binomial regression models (ZINB Model Expansion; Red Knot ~ Island + Year) using Program R package pscl (Zeileis et al. 2008) to predict the number of red knots per sampling point for each island-year combination. We used this type of model because our data contained more 0s than expected based on the negative binomial distribution and were overdispersed, having a response variable with mean < variance ($\bar{x}$ red knot flock size range for peak migration: 9 – 29, variance range: 778 – 16,247; Green 1994, Yau et al. 2003; Appendices 1, 2). We calculated the predicted number of red knots using Virginia's barrier islands each year, by expanding from our sampling points using the ZINB model, as:

$$\text{Predicted \# Red Knots Per Year}_{2007\text{-}2018} = \sum\left(\frac{\text{Island1 Length (m)}}{200\text{ m}} * r_1\right) + \left(\frac{\text{Island2 Length (m)}}{200\text{ m}} * r_2\right) + \ldots\left(\frac{\text{Island11 Length (m)}}{200\text{ m}} * r_{11}\right),$$

where, $\text{Island}_{1\text{-}11}$ Length = length (m) of each of the 11 islands sampled, and

$r_{1\text{-}11}$ = ZINB mean predicted number of red knots in all sampling points on each of the 11 islands

For both methods, we estimated the length of each island and the total shoreline using aerial imagery from USDA's Farm Service Agency's NAIP orthophotography imagery (USDA Farm Service Agency 2018) and our own GPS estimates from walking the islands during the study. We compared the predictions from both methods (Raw Data Expansion and ZINB Model Expansion) using a paired t-test over all years, as the predictions for both methods were normally distributed (Shapiro-Wilk; W = 0.98, p = 0.98 and W = 0.95, p = 0.57, respectively). We used a Freidman test to determine if there were differences in red knot numbers by year (2007 – 2018), controlling for island. We used Pearson correlation to determine the strength and direction of any potential trends in the predicted number of red knots in the Virginia over time (2007 – 2018) and of any relationship between predicted red knot numbers in Virginia and the Delaware Bay stopover (Shapiro-Wilk; W = 0.93, p = 0.36; USFWS 2015; A. Deys pers. comm. April 21, 2014).

The resulting predicted numbers of red knots in Virginia during peak migration using both methods may be biased by three assumptions. First, we assumed that the number of birds entering and exiting Virginia's barrier islands were equal during peak migration. While we would need resighting data to formally test this assumption, red knot behavior demonstrates that the majority of red knots arrive by the start (May 21) of the peak migration period (May 21 – 27) and that red knots typically remain on the islands for at least two weeks, reducing the likelihood of immigration and emigration during this time (Clark et al. 1993, Watts and Truitt 2000, Cohen et al. 2009, Watts 2013). Second, we assumed that red knot detectability and identification were perfect over the 12-years of this study. While ZINB models do not account for imperfect detection or identification (Wenger and Freeman 2008), our field methodology was conducted using only observers trained extensively in shorebird counting and identification, reducing the likelihood of missing or incorrectly identifying red knots. Third, our predictions assumed that no birds were double counted within a given year's peak migration. We minimized the potential bias of this assumption by moving linearly down the beach's oceanfront when counting red knots and staying at least 100 meters away from flocks to prevent dispersal.

Red Knots over Time and Space

Shapiro-Wilk normality tests indicated that red knot ($W = 0.43$, $p < 0.001$) and all prey abundance ($W = 0.53$, $p < 0.001$) data were not normally-distributed during early migration. Shapiro-Wilk normality tests also indicted that red knot ($W = 0.23$, $p < 0.001$) and all prey abundance ($W = 0.37$, $p < 0.001$) data were not normally-distributed during peak migration. Therefore, we used Wilcoxon rank sum tests with Bonferroni correction to determine if there were differences in red knot and prey numbers (i.e., crustacean, blue mussel, coquina clam, miscellaneous prey, all prey) between 1) early and peak migration periods and 2) sites used vs. unused by red knots. We used Chi-square contingency tests of independence to determine if mean available prey abundances from core samples differed in their community make-up on peat vs. sand during the peak migration period. Then, we used Wilcoxon rank sum tests with Bonferroni correction to determine if any differences existed in mean prey abundances on peat and sand substrates within the peak migration period.

Early and Peak Migration Periods – Red Knot Models

We used zero-inflated negative binomial mixed-effects regression models to determine factors affecting red knot presence and flock size in Virginia, as our data contained more 0s than expected based on the negative binomial distribution and were overdispersed (had a response variable with mean < variance) during both early and peak migration periods ($\bar{x}$ red knot flock size range: 0.14 – 29, variance range: 0.17 – 16,247; Green 1994, Yau et al. 2003; Appendix 2). These models included two processes. The zero-inflated process addressed the likelihood of observing more 0s (red knot absences) than expected given the covariates under the assumed negative binomial distribution. The count process measured the flock size of red knots (i.e., the number of red knots at a given sampling point), which can include 0, conditional on the zero-inflated part of the model (Lewis et al. 2011). We analyzed the effects of covariates on the likelihood of 1) more site absences by red knots than expected based on the negative binomial distribution (Zurr et al. 2009) and 2) the abundance of red knots per 100 m radius semicircle on the water line at the sampling point ('flock size') during both early (2008 – 2018, except 2010) and peak migration (2007 – 2018) periods.

Explanatory variables considered included prey abundance (number/core sample), tide, distance to roost (m), and counts of red knots at the Tierra del Fuego wintering grounds within the same year (i.e., as an index for the total number of red knots in the flyway; Morrison and

Ross 1989, Morrison et al. 2004, Morrison 2014, Dey et al. 2015, USFWS 2015, Western Hemisphere Shorebird Reserve Network 2017, 2018). We characterized tide (i.e., high, falling, low, rising) from each sampling observation as: high within 1 hour of the predicted high, falling from 1 hour after the predicted high to 1 hour before the predicted low, low within 1 hour of the predicted low, and rising from 1 hour after the predicted low to 1 hour before the predicted high (Cohen et al. 2010a). High tide was not sampled during the early migration period, as peat banks typically were not exposed at high tide. Distance to roost was calculated as the distance between each sampling point and presumed red knot night roosts on Chimney Pole and Wreck Island (Cohen et al. 2010b). The closer of the two distances/sample point was used for our analyses. We compared all continuous covariates using Pearson correlation coefficients to identify highly correlated covariate combinations (represented by values > |0.7| Booth et al. 1994, Anderson et al. 2001). Highly correlated combinations for early migration period and peak migration period were not included in our model subsets (Appendix 3). For all models, we included island and year as random effects to prevent pseudoreplication of intra-island sampling units over time (Hurlbert 1984).

We used an information-theoretic approach (i.e., Akaike information criterion; Burnham and Anderson 2002) by building an *a priori* candidate model set of sixty-eight models. We ranked these zero-inflated negative binomial mixed-effects models using Akaike's Information Criterion for small sample sizes (AIC_c), with lower AIC_c values indicating better-supported models (Burnham and Anderson 2002). Here, we report all models with $\Delta AIC_c < 4$ (Arnold 2010). Full model sets are in Appendix 4. Three models did not converge during early migration period and were thus removed (Appendix 4). We calculated goodness-of-fit values based on Nagelkerke (1991).

Early and Peak Migration Periods – Prey Models

We used generalized linear mixed-effects regression models to determine factors affecting potential red knot prey resources. We analyzed the effects of covariates on different prey abundances per core sample during both early (2008 – 2018, except 2010) and peak migration (2007 – 2018) periods. Explanatory variables included tide, mean daily water temperature (°C), substrate (i.e., sand or peat), and island type (i.e., high elevation or low elevation). We characterized tide (i.e., high, falling, low, rising) from each sampling observation as described above for red knot models (Cohen et al. 2010a). Mean daily water temperatures were collected from buoy 44009 (Delaware Bay, DE; 38.457°N, 74.702°W), maintained by National Oceanic and Atmospheric Administration's (NOAA) National Data Buoy Center (NDBC; NOAA 2020). This buoy is located south of the Delaware-Maryland boundary (46 km southeast of Cape May, New Jersey) and was selected as it was the closest buoy to our sampling area that had ocean temperature data representing the entirety of our sampling years (2007 – 2018). The buoy was 98.01 km north of Assawoman Island and 188.29 km north of Fisherman Island. Substrate was not included in early migration period modeling, as only peat substrate was sampled. Island type (i.e., high vs. low) was based off classifications done in Wolner et al. (2013) and Durán and Moore (2014) and our own visual assessments: Assawoman Island (high), Metompkin Island (low), Cedar Island (low), Parramore Island (high), Hog Island (high), Cobb Island (low), Wreck Island (low), Ship Shoal Island (low), Myrtle Island (low), Smith Island (high), and Fisherman Island (high). For all models, we included island and year as independent random effects to prevent pseudoreplication of intra-island sampling units over time (Hurlbert 1984).

We used an information-theoretic approach (i.e., Akaike information criterion; Burnham and Anderson 2002) by building an *a priori* candidate model set of 8 models for early migration period and 16 models for peak migration period. We ranked these models using Akaike's Information Criterion for small sample sizes (AIC_c), with lower AIC_c values indicating better-supported models (Burnham and Anderson 2002). Here, we report all models with $\Delta AIC_c < 4$ (Arnold 2010). Full model sets by prey type and sampling period are in Appendix 5. We calculated goodness-of-fit values based on Nakagawa and Schielzeth (2013).

Peat and Sand Tidal Sampling

Shapiro-Wilk normality tests indicated that red knot and all prey abundance data during tidal sampling were not normally-distributed on peat (red knot: $W = 0.60$, $p < 0.001$; all prey: $W = 0.75$, $p < 0.001$) and sand (red knot: $W = 0.60$, $p < 0.001$; all prey: $W = 0.79$, $p < 0.001$). Therefore, we used Kruskal-Wallis tests to determine if there were differences in red knot and prey abundances (i.e., crustacean, blue mussel, coquina clam, miscellaneous prey, all prey) by tide (i.e., high, falling, low, rising).

Spatial Tidal Sampling

Shapiro-Wilk normality tests indicated that all prey abundance data during spatial tidal sampling were not normally-distributed on peat ($W = 0.42$, $p < 0.001$) and sand ($W = 0.86$, $p < 0.001$). Therefore, we used Kruskal-Wallis and Dunn tests to determine if there were differences in prey numbers (i.e., crustacean, blue mussel, coquina clam, miscellaneous prey, all prey) by distance to central sampling point (0 meters, 5 meters, 10 meters) throughout the tidal cycle. We used Program R packages base, stats, dplyr, glmmTMD, lme4, and dunn.test for the analyses described above (Dunn 1964, Bates et al. 2015, Brooks et al. 2017, Wickham et al. 2018, R Core Team 2013, R Version 3.4.1, www.r-project.org, accessed 6 May 2019 - 19 February 2020).

RESULTS

Early Migration Period – Overview

We collected and analyzed 457 core samples on Virginia's barrier islands during early migration period from 2008 – 2018 (except 2010), with a mean of 46 points per year (range = 39 – 61). Mean red knot flock size per sampling point was 7 (SE = 0.33). Compared to unused sites on peat banks during early migration period, red knots used sites with more crustaceans (Wilcoxon rank sum test with Bonferroni correction; W = 15,229, $p < 0.001$), blue mussels (Wilcoxon rank sum test with Bonferroni correction; W = 15,322, $p < 0.001$), miscellaneous prey (Wilcoxon rank sum test with Bonferroni correction; W = 13,822, $p < 0.001$), and all prey (Wilcoxon rank sum test with Bonferroni correction; W = 13,128, $p < 0.001$; Fig. 2a).

Within the barrier islands' peat banks during early migration, crustaceans ($\bar{x}$ = 135/core sample, SE = 6.31) were the most abundant prey item, followed by blue mussels ($\bar{x}$ = 103/core sample, SE = 4.83). Coquina clams were the least abundant prey ($\bar{x}$ = 0.94/core sample, SE = 0.04) and were only present in 21% of core samples. Miscellaneous prey, while not the most abundant prey ($\bar{x}$ = 19/core sample, SE = 0.88), were the most likely to be present in core samples, with 79% of samples containing miscellaneous prey. Red knots did not use 65% of sampled peat bank locations during early migration, while 9% of sampling locations contained no prey. Crustacean, blue mussel, miscellaneous prey, and all prey abundances were greater on peat banks sampled during the early migration period than combined sand and peat substrates sampled during the peak migration period (Appendix 1). Miscellaneous prey during early migration were comprised of angel wing clams (63%) and other organisms (37%).

Early Migration Period – Red Knot Models

The top model containing crustaceans, coquina clams, miscellaneous prey, and tide (falling, low, rising) best explained the variation in red knot presence and flock size on peat banks during early migration (AIC_c weight = 0.30; Table 1; Appendix 4). As the number of miscellaneous prey increased ($\beta = -2.15$, SE = 0.80) and as the tide transitioned from rising to low ($\beta = -1.53$, SE = 0.57), the probability of a zero count (red knot absence) decreased. As the number of crustaceans increased, red knot flock size increased ($\beta = 0.23$, SE = 0.11; Table 2).

Early Migration Period – Prey Models

Tide (falling, low, rising) and mean daily water temperature best explained the variation in crustacean (AIC_c weight = 0.61), coquina clam (AIC_c weight = 0.71), and miscellaneous prey (AIC_c weight = 0.71) abundances on peat banks during early migration (Table 3; Appendix 5). Crustacean, coquina clam, and miscellaneous prey abundances were highest at low tide (Table 4). As the mean daily water temperature increased, crustacean and miscellaneous prey abundances increased, while coquina clam abundance decreased (Table 4). One other model was supported for each prey type ($\Delta AIC_c < 4$; Table 3; Appendix 5); however, the second ranked models contained an additional parameter, suggesting that the additional parameter (island type) was uninformative (Arnold 2010); thus, we only considered the most parsimonious model as supported for each prey type.

Peak Migration Period – Overview

We collected and analyzed 1,322 samples on Virginia's barrier islands during the peak migration period (*n* = 71 peat samples, *n* = 1,251 sand) from 2007 – 2018, with a mean of 110 points per year (range = 93 – 129; Appendix 1). Predicted numbers of red knots in Virginia did not vary between expansion methods over the study's duration (paired t-test; t = -0.26, df = 11, p = 0.80), and thus we present results on the ZINB Model Expansion, as it better accounts for the raw count data's non-normal distribution. On average, we predicted that 7,055 (SD = 2,841) red knots used Virginia's barrier islands during peak spring migration each year. Predicted red knot numbers in Virginia were highest in 2012 (11,644) and lowest in 2014 (2,792; Fig. 3); however, predicted red knot numbers in Virginia showed no linear trend over time (Pearson correlation; t = 1.05, df = 10, p = 0.32), and there were no differences in predicted mean red knot numbers over time (Freidman chi-squared = 18.01, df = 11, p = 0.08). There was no correlation between the predicted numbers of red knots in Virginia and the Delaware Bay stopover numbers (Pearson correlation; t = -0.43, df = 10, p = 0.67)

Mean red knot flock size per sampling point ($\bar{x}$ = 17 red knots/point, SE = 0.46) was higher during peak migration than during the early migration period ($\bar{x}$ = 7 red knots/point, SE = 0.33; Appendix 1). Sites used by red knots during peak migration had more crustaceans (Wilcoxon rank sum test; W = 120,030, p < 0.001), coquina clams (Wilcoxon rank sum test; W = 88,004, p < 0.001), miscellaneous prey (Wilcoxon rank sum test; W = 140,030, p = 0.003), and all prey (Wilcoxon rank sum test; W = 95,570, p < 0.001) than unused sites (Fig. 2b).

Across the barrier islands' intertidal shoreline (including both peat banks and sand), crustaceans were the most abundant prey ($\bar{x}$ = 21/core sample, SE = 0.57; Fig. 4a) and were collected in 80% of samples (Appendix 1). Blue mussels were the least abundant prey ($\bar{x}$ =

6/core sample, SE = 0.17; Fig. 4a) and were only collected in 4% of samples (Appendix 1). Red knots did not use 77% of sampling locations, while 10% of sampling locations did not contain any prey (Appendix 1). With both substrates included, miscellaneous prey was comprised of other organisms (96%), angel wing clams (2%), and horseshoe crab eggs (2%). When separating sites by substrate, the composition of prey differed between sand and peat substrates (Chi-square contingency test of independence; $\chi^2 = 15{,}793$, df = 4, $p < 0.001$). Blue mussels (Wilcoxon rank sum test; W = 68,288, $p < 0.001$) and miscellaneous prey (Wilcoxon rank sum test; W = 57,534, $p < 0.001$) were more abundant on peat than on sand, while coquina clams were more abundant on sand than on peat (Wilcoxon rank sum test; W = 29,832, $p < 0.001$; Fig. 4b). When separated by substrate, miscellaneous prey were as follows: peat – other organisms (69%) and angel wing clams (31%); sand - other organisms (97%), horseshoe crab eggs (2%), and angel wing clams (1%).

Peak Migration Period – Red Knot Models

The top ranked model containing crustacean, blue mussel, and coquina clam abundances in Virginia and wintering counts of red knots in Tierra del Fuego best explained the variation in red knots in Virginia during peak migration (AIC_c weight = 0.24; Table 1; Appendix 4). Probability of a zero count decreased with increasing blue mussel abundance ($\beta = -8.87$, SE = 4.11), coquina clam abundance ($\beta = -3.08$, SE = 0.69), and the number of red knots using Tierra del Fuego wintering grounds ($\beta = -0.49$, SE = 0.17; Table 2). However, red knot flock size decreased with increasing blue mussel abundance ($\beta = -0.30$, SE = 0.10), crustacean abundance ($\beta = -0.24$, SE = 0.09), and the number of red knots using Tierra del Fuego ($\beta = -0.26$, SE = 0.13; Table 2). While six other models had ΔAIC_c values less than 4, the second ranked model contained ten additional parameters, suggesting that the additional parameters were uninformative (Arnold 2010; Appendix 4); thus, we only considered the most parsimonious model as supported.

Peak Migration Period – Prey Models

Tide (i.e., high, falling, low, rising), substrate, and mean daily water temperature best explained the variation in crustacean (AIC_c weight = 0.56), coquina clam (AIC_c weight = 0.69), and blue mussel (AIC_c weight = 0.49) abundances during peak migration (Table 3; Appendix 5). Crustacean and blue mussel abundances were highest at low tide, while coquina clam abundances were highest at falling and rising tides. Crustaceans and blue mussels were more abundant on peat, while coquina clams were more abundant on sand. As the mean daily water temperature increased, crustacean and blue mussel abundances increased, while coquina clam abundance decreased (Table 4). While the blue mussel model with tide, substrate, mean daily water temperature, and island type (AIC_c weight = 0.51; Appendix 5) carried the most weight, because there was no difference in blue mussel abundance on low vs. high elevation islands, we considered the second ranked model described above as most parsimonious (Arnold 2010).

Peat and Sand Tidal Sampling

Crustacean abundance varied by tide during peat tidal sampling (Kruskal-Wallis; $\chi^2 = 13$, df = 2, p = 0.001), with crustacean abundance being highest at low tide (Fig. 5a). Red knot flock size (Kruskal-Wallis; $\chi^2 = 37$, df = 3, $p < 0.001$) and coquina clam (Kruskal-Wallis; $\chi^2 = 20$, df = 3, $p < 0.001$) and blue mussel (Kruskal-Wallis; $\chi^2 = 13$, df = 3, p = 0.003) abundances varied by tide during sand tidal sampling, with red knot flock size and blue mussel and coquina clam abundances being highest at rising tide (Figs. 5b, c).

Tidal Spatial Sampling

Only all prey abundance varied by distance (5 m, 10 m) from a central sampling point (0 m) during peat tidal spatial sampling conducted in 2019 (Kruskal-Wallis; $\chi^2 = 7$, df = 2, p = 0.03). Dunn tests with Bonferroni correction found that all prey abundance differed between 0 m and 10 m, with all prey being higher at 0 m (Dunn test; z = 2.63, p = 0.01; Fig. 6; Appendix 6).

DISCUSSION

Site selection by red knots and prey availability varied by substrate and tide. While most (~ 90%) sampling locations contained prey, red knots did not use 64 – 77% of all sampling locations, suggesting that red knots use a small proportion of habitat containing prey at any given time in Virginia. The sites that red knots used contained higher prey abundances than unused sites, supporting the work of previous studies (Watts and Truitt 2000, 2015, Piersma et al. 2003, van Gils and Piersma 2004, Karpanty et al. 2006, 2011, Cohen et al. 2010b, 2011). These relationships suggest that red knots decrease energy expenditure and maximize foraging efficiency by foraging in locations that are most profitable (i.e., those that provide the highest energy intake rates in the shortest period; van Gils et al. 2004, Cohen et al. 2010b) and that red knots likely feed on prey that are most digestible (Krebs et al. 1978, Cowie and Krebs 1979, Martin 1985, Charnov 1986, Martin and Karr 1986, van Gils et al. 2004). Our findings are similar to observed red knot behavior on the Dutch Wadden Sea, an area that closely resembles the intertidal habitat on Virginia's barrier islands. At the Wadden Sea site, red knots selected juvenile edible cockles (*Cerastoderma edule*) that had thinner shells and proportionately high flesh content to maximize energy intake rates and reduce processing time (i.e., shell digestion;

van Gils et al. 2003, Bijleveld et al. 2015). Other studies have also demonstrated that shorebirds such as sanderling (*Calidris alba*), Eurasian oystercatchers (*Haematopus ostralegus*), and dunlin (*Calidris alpina*) select prey that maximize their caloric intake (Sameoto 1969, Myers et al. 1979b, Grant 1981, Rands and Barkham 1981). Additional study into the caloric content and digestibility of all prey available to red knots in Virginia would help further clarify the relationship among red knots, prey, and habitat selection.

While the probability of at least one red knot was greater at sites with more prey, red knot flock size did not consistently relate to prey abundance. Most prey abundances (blue mussels, crustaceans, miscellaneous, and all prey) were highest during the early migration period when only peat banks were sampled, while red knot flock size was highest during the peak migration period when both sand and peat were sampled. This relationship is likely an artifact of the different sampling approaches used during early migration, when we only sampled peat, and peak migration, when we sampled both peat and sand, as we found prey abundance to be higher in peat than in sand. Therefore, direct comparisons of red knot and prey numbers between early and peak migration periods, without accounting for substrate type, cannot be made. Additionally, while crustaceans positively influenced red knot flock size during the early migration period, crustaceans and blue mussels were negatively related to red knot flock size during the peak migration period, likely because these two prey live predominantly in peat banks. Although these red knot flock size - prey relationships may seem counterintuitive, as we would expect larger red knot flock sites at sites with more prey, the discrepancy that some prey negatively influenced red knot flock size may be influenced by five factors. First, we did not sample red knots at high tide or on sand substrate during the early migration period, which may have negatively biased our flock size estimates during early migration by not counting birds that roost, typically on sand, in large numbers at high tide (van Gils et al. 2005a, b, Cohen et al. 2011). Second, some of the relationships between red knot flock size and prey during peak migration (i.e., the negative correlation between red knot flock size and blue mussels and crustaceans) may be habitat correlates, related to the propensity of most prey to settle on peat banks that were sampled less during peak migration than early migration, rather than truly negative relationships (Karpanty et al. 2006). Third, flock size alone is not directly related to the overall number of birds using the islands. Forth, prey may have been depleted at some sites during peak migration before red knots were counted (Karpanty et al. 2006), and fifth, site suitability varies in the intertidal zone. For example, some peat banks are located far above the low-tide line and thus become dry throughout the tidal cycle. These banks do not support the same abundances of prey as peat that remains saturated throughout the tidal cycle. While the abundance of prey may not consistently affect flock size within a sampling period, consistent prey availability across the migration window may affect overall red knot abundance on the islands. For example, we designed our study to conduct sampling during early migration (May 14 – 20) and peak migration (May 21 – 27) using historical data that suggested these two weeks corresponded to early and peak migration for red knots using Virginia and Delaware Bay stopovers (Clark et al. 1993, Watts and Truitt 2000, 2015, Cohen et al. 2009, Watts 2013); thus, excluding any effects of prey, we would expect more red knots to use Virginia's barrier islands during peak migration period than during early migration period, regardless of flock size estimates.

Consideration of the multiple drivers of red knot flocking behavior also offers insight into our ability to explain red knot presence better than red knot flock size at a given point in Virginia. For example, Folmer et al. (2010) found that the predictive ability of resource-related (e.g., prey) models on the spatial distribution of foraging shorebirds decreased with the tendency of a species to flock. As social birds (Piersma et al. 2003, Quaintenne et al. 2011, Bijleveld et al. 2015), red knots prefer to forage in non-random groupings (flocks) and therefore likely base their foraging decisions at least partly on conspecific attraction and perceptions (Abrahams 1986, Folmer et al. 2010). These conspecific interactions may help red knots determine food availability and the presence of predators at a given site (Krause and Ruxton 2002, Folmer et al. 2010). Additionally, peregrine falcons (*Falco peregrinus*), predators of red knots, live on Virginia's barrier islands, where shorebirds provide an estimated 52% of the peregrine falcon's diet (Long 2009, Watts et al. 2015). Thus, conspecific interactions may also cause red knots to select a site simply because others do so to maximize predator detection, regardless of prey availability ("many eyes hypothesis;" Lima 1995, Beauchamp and Ruxton 2008).

During peak migration, red knot presence and flock size were also influenced by wintering red knot counts in Tierra del Fuego. The positive relationship between Tierra del Fuego counts and red knot presence suggests that the more birds there are in wintering grounds, and presumably the flyway, the more sites in Virginia will be occupied in a given year. The negative relationship between Tierra del Fuego wintering counts and flock size during peak migration period in Virginia suggests that red knots occupy more sites in Virginia, but in smaller flocks, when Tierra del Fuego counts are higher. Birds may separate into smaller flocks to exploit more foraging sites, while simultaneously decreasing competition within a site (Beauchamp 2002). However, that prediction is in contrast to numerous other studies that suggest that larger flock sizes may help animals determine site suitability based on the abundance of available prey (Barnard 1980, Rands and Barkham 1981, Guillemette et al. 1993, Nehls and Tiedemann 1993, Krause and Ruxton 2002, van Gils and Piersma 2004). Alternatively, this relationship may have been driven by a few very large red knot flocks observed in the early years of this study. Further research into the relationship between flock size and site suitability in Virginia could elucidate these conflicting findings related to Tierra del Fuego counts.

Blue mussel and miscellaneous prey abundances per core sample were higher in peat than in sand. These prey abundance-substrate relationships could be caused by the preference of these prey to live in peat versus sand, rather than on the time of the sampling period. Some prey, like blue mussels, require a substrate (e.g., peat) on which to attach (Rzepecki et al. 1992). Other prey that do not attach to the substrate may prefer to live in peat to more easily hide from predators in dense vegetation and/or because they consume various decaying plant and animal material found in the banks (Crisp and Heal 1959, Davidson et al. 2016, Able et al. 2018, URI EDC 2020). In contrast, coquina clam abundance per core sample was higher in sand than in peat. Coquina clams prefer sand substrate that enables them to migrate both vertically and horizontally across the shoreline throughout the tidal cycle, primarily migrating shoreward during rising tides, seaward during falling tides, and remaining idle during low and high tides (McArdle and McLachlan 1992, Ellers 1995). These migrations likely decrease predation risk by keeping the clams mobile, preventing them from becoming stranded at high tide and also by increasing the clams' foraging efficiency by reducing the risk of resource depletion within one area (Laudien et al. 2003, Cobb et al. 2011). However, these prey-substrate relationships do not account for the abundance of prey across the entire intertidal zone, only the abundance of prey found in collected core samples. Because peat banks comprise only ~ 6% of the intertidal zone each year, while sand comprises the remainder of the intertidal zone (Truitt et al. 2001, Bachmann et al. 2002, Cohen et al. 2010a, b, Watts and Truitt 2015), the abundance of prey in sand is likely much greater than the abundance of prey in peat on the entire barrier island chain. However, both substrates are important in supporting different types of prey and provide prey at different times in the tidal cycle.

The relationship between prey and substrate is inherently related to tide, as tide affects whether peat banks are exposed and prey availability on both sand and peat. For example, blue mussels and crustaceans were captured in the highest densities within two hours of low tide, corresponding to the time of greatest peat bank exposure, and coquina clams generally were most abundant during the falling and rising tides when clams engage in tidal migrations. However, tide was not a universally important predictor of red knot presence or flock size, as we might expect that red knots move throughout the tidal cycle to utilize available foraging habitats. More specifically, red knot presence and flock size were not affected by tide during peak migration when both sand and peat were sampled, though red knot presence was highest during the rising tide and flock size was highest at low tide during early migration when only peat was sampled. Red knot flock size and prey abundances were also generally highest around the rising tide during sand tidal sampling. While prey varied by substrate and tide, and despite other studies finding that invertebrate prey abundance is highly variable across space (Underwood and Chapman 1996, Benedetti-Cecchi 2001, Díaz-Tapia et al. 2013), the only difference in prey abundances within 10 meters of a central sampling point occurred for all prey during peat tidal sampling. The lack of consistent differences over space suggests that larger scale covariates (e.g., ocean temperature) may affect prey abundance and distribution throughout the tidal cycle or that the spacing we selected (5 m and 10 m from a central point) were inconsistent with prey spatial variation.

Crustaceans were abundant on both peat and sand. Heller et al. (In Prep) found that despite the high abundance of crustaceans across the intertidal landscape of Virginia’s barrier islands, red knots selected crustaceans less than expected given their availability. If ocean temperatures continue to warm (Hansen et al. 2006, Jones et al. 2010), causing further range contraction of blue mussels in Virginia, red knots may need to rely more heavily on abundant crustaceans or other prey that we grouped in the miscellaneous category (horseshoe crab eggs, angel wing clams, and other organisms (e.g., insect larvae, snails, worms)) in Virginia (Heller et al. In Prep). However, coquina clams may become larger and/or more abundant in Virginia due to ocean warming, as they grow faster and mature earlier in warmer water (Jones et al. 2005). Previous studies estimating the caloric value of crustaceans consumed by redshank (*Tringa tetanus*), a shorebird of similar size to red knots, found that crustaceans contained 4 – 54 Kcals/g ash-free dry mass (AFDM; Wood personal communication, Goss-Custard 1977), while bivalves consumed by red knots in Virginia contained between 0.5 – 31 Kcals/g AFDM (J. Cohen, unpublished data). These data demonstrate that crustaceans are more energetically dense than bivalves and are an energetically rich food resource for red knots; however, red knots' selection of bivalves over crustaceans during this study suggest that red knots select bivalves for other reasons. These reasons could potentially relate to the aggregative community structure of bivalves, to the tactile foraging approach red knots use and how that may relate to their ability to detect bivalves more easily than crustaceans, and/or to decreased competition for bivalves with other shorebirds without the anatomical and physiological adaptions required for bivalve specialization (Piersma 1991, Zwarts and Blomert 1992, Dekinga and Piersma 1993, Lourenço et al. 2016).

While prey models suggested that water temperature affects the abundance of prey resources, the mean daily temperature ranges we obtained from offshore buoys fell within known suitable temperature ranges of key prey resources. However, we suspect that local temperature fluctuations within the intertidal zone may be more predictive of impacts on prey than offshore buoy temperatures (Dall 1889, Wells and Gray 1960, Ansell 1983, Ruppert and Fox 1988, Laudien et al. 2003, Jones et al. 2009, Cobb et al. 2011, NCEI 2020). While we were limited by the availability of water temperature data coming from an offshore buoy around 100 km north of our study sites, we included these water temperature data as a proxy for general water temperature trends and prey responses to these trends. Because intertidal water temperatures typically are more variable and change more rapidly than offshore subtidal temperatures (Lathlean et al. 2011, Nguyen et al. 2011, Diederich et al. 2013), future studies should measure intertidal water temperatures over time specifically.

The predicted number of red knots using Virginia's barrier islands during peak migration was variable over the study's duration, though no positive or negative linear trend existed. We acknowledge that our approach to predict the total number of red knots in Virginia during peak migration was imperfect, as we assumed that 1) that the number of birds entering and exiting Virginia's barrier islands were equal during peak migration, 2) our detectability and identification were perfect, and 3) no birds were double counted. However, we minimized potential biases associated with these assumptions by sampling red knots during peak migration (May 21 – 27) after most have arrived on the islands and when they tend to stay for at least 2 weeks (Clark et al. 1993, Watts and Truitt 2000, Cohen et al. 2009, Watts 2013), by having only highly trained observers identify and count red knots, and by staying at least 100 meters away from flocks to prevent dispersal. Additionally, our average peak prediction of 7,055 red knots per year (2007 – 2018) was close to the average peak red knot count obtained using aerial surveys from 2007 – 2014 ($\bar{x}$ = 6,788 red knots per year; B. Watts personal communication November 15, 2012 and August 19, 2014, Watts 2013, USFWS 2014). Our average peak prediction of red knots each year ($\bar{x}$ = 6,386 red knots predicted per year; 2007 – 2014) was also close to aerial peak count estimates when we averaged only our ground count predictions for the years during which aerial counts were also made ($\bar{x}$ = 6,788 red knots per year, 2007 – 2014; B. Watts personal communication November 15, 2012 and August 19, 2014, Watts 2013, USFWS 2014). We are further encouraged by our ability to detect trends in red knot numbers using these ground count predictions as our observed decrease in predicted numbers of red knots from 11,644 in 2012 to 6,384 and 2,792 in 2013 and 2014 respectively also coincided to declines observed via aerial surveys in those years (8,482 in 2012 to 6,200 and 5,547 in 2013 and 2014 respectively; B. Watts personal communication November 15, 2012 and August 19, 2014, Watts 2013, USFWS 2014). Thus, our ground count predictions of red knot numbers and the annual aerial flight counts of red knots during the years of 2007 – 2014 (B. Watts personal communication November 15, 2012 and August 19, 2014, Watts 2013, USFWS 2014) showed a similar mean peak count and similar trends in numbers despite the varied methods and assumptions of each.

Cumulatively, our study suggests that the number of red knots in Virginia result from complex interactions both on the stopover site and beyond. Previous studies of red knot population trends (including *Calidris canutus rufa* and *Calidris canutus canutus*) demonstrated that red knot populations often cycle within 3 to 4-year periods. Years of high lemming population sizes resulted in high reproductive output of red knots and consequently higher red knot numbers the following 1 – 3 years (Sutherland 1988, Blomqvist et al. 2002, Fraser et al. 2013). However, in the late 1990s, lemmings stopped cycling in Europe (Kausrud et al. 2008, Brommer et al. 2010, Elmhagen et al. 2011). It is less clear whether lemming cycles changed in North America; however, if they did, any lemming cycle cessation may have contributed to the *rufa* red knot's population decline in the early 2000s (Fraser et al. 2013, Federal Register 2014).

Predicted red knot numbers in Virginia during this study did not visibly follow the same 3 – 4 year cycle as seen in Sutherland (1988) and Fraser et al. (2013), but there were repeated highs and lows. An examination of potential cyclic patterns may be warranted if long-term modelling continues to show variable trends over time.

The annual fluctuations in the red knot population that migrates through the mid-Atlantic region (Dey et al. 2011, Dey 2014, USFWS 2015, 2019) and the proportion of red knots that use the Virginia stopover may be related to the quality of Delaware Bay and Virginia stopover habitat within a given year. Our long-term findings regarding the relationship between red knots and their prey in Virginia only explain some of the variation in red knot site use and flock size. Because there is no evidence of extreme red knot population fluctuations since their decline in the mid-1990s (this study, Morrison et al. 1989, 2004, Dey et al. 2011, 2015), and because prey abundance varies over space and time, we speculate that any variation in the number of red knots using the Virginia barrier islands is at least partly due to the abundance and quality of prey at other locations across the annual cycle. The factors that affect the presence, flock size, and abundances of long-distance migrants using migratory stopover grounds are not straightforward, as factors across its wintering, breeding, and other stopover grounds likely affect birds year-round (Aharon-Rotman 2016, Murray et al. 2018). Thus, additional studies that link potentially relevant variables across each area within the range that red knots use during their life-cycle are warranted.

CONCLUSION

Red knots historically frequented a larger region in coastal North America from Florida to Massachusetts as migratory stopover habitat than they do today (Wilson and Bonaparte 1832, MacKay 1893). Red knot spring migratory stopovers now are primarily found on the Delaware Bay and Virginia's barrier islands (~ 50 – 70% of the annual stopover population; MacKay 1893, Watts and Truitt 2000, U.S. Commission on Ocean Policy 2004, Cohen et al. 2010a, 2011, Dey et al. 2011, Dey 2014, Federal Registrar 2014, USFWS 2015, 2019, Lyons et al. 2018). Because these stopover grounds support high percentages of the entire migratory population (Watts and Truitt 2000, U.S. Commission on Ocean Policy 2004, Cohen et al. 2010a, Dey et al. 2011, Dey 2014, Federal Registrar 2014, USFWS 2015, 2019, Lyons et al. 2018), any deviation in the historic norm of habitat and prey availabilities may have lasting population-wide implications for red knots and other long-distance migratory shorebirds (Tulp and Schekkerman 2008).

Our research suggests that although blue mussels and coquina clams are important prey resources for red knots, counts of red knots in wintering grounds and counts of other types of prey, such as crustaceans, may also be important predictors of red knot presence and flock size in Virginia. To continue maximizing the availability of red knot prey through protecting both sand and peat substrates, ongoing management on Virginia's barrier islands that discourages beach stabilization and nourishment projects and allows the natural processes of overwash and island transgression should continue. Beach nourishment buries invertebrate prey that live within the top layers of sand and peat, causing prey mortality, altered prey community assemblages, and/or a reduction in foraging shorebirds' ability to access prey (Saloman 1974, Oliver et al. 1977, NRC 1995, Rakocinski et al. 1996, USACE 2001, Menn et al. 2003, Bishop et al. 2006, Rosov et al. 2016). Beach stabilization and nourishment stall coastal shoreline erosion and are often used on barrier islands to prevent island transgression (Leatherman 1987, Charlier and de Meyer 1995, Lozán et al. 2001, Menn et al. 2003). However, peat banks cannot form when islands are unable to transgress over themselves onto back-side marsh (Truitt et al. 2001, Cohen et al. 2010a, b, Bachmann et al. 2012, Watts and Truitt 2015). Therefore, beaches, like most of those on the United States' Atlantic Coast, that are nourished or otherwise managed to prevent erosion generally lack peat banks. Over time, the loss of peat habitat would likely decrease the abundance of peat-reliant red knot prey species, like blue mussels and crustaceans.

Additionally, addressing how factors outside of Virginia may affect migrating birds demonstrates the need for managing species across their ranges. For example, Rushing et al. (2016) found that the relative impact of breeding habitat loss for the migratory wood thrush (*Hylocichla mustelina*) was between 3 – 6 times greater than that of equivalent non-breeding habitat loss. Thus, understanding the effects of important factors to migratory birds (e.g., habitat, prey) across the birds' annual cycles is necessary for successful management practices, developing conservation priorities, and setting resource allotments range-wide (Runge and Marra 2005).

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FIGURES

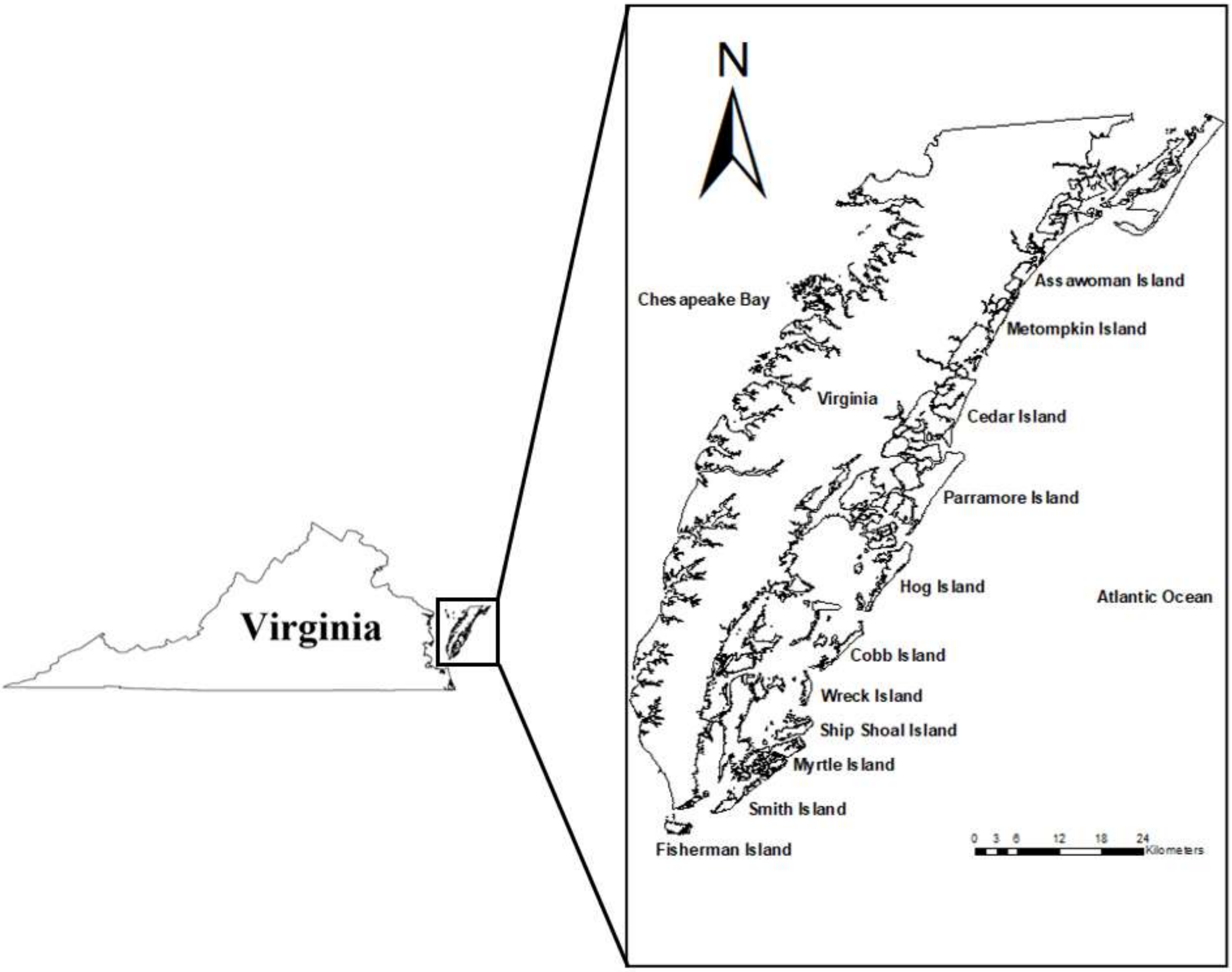


Figure 1. Study area on the Virginia Coast Reserve Long Term Ecological Research Site consisting of barrier islands off the Eastern Shore of Virginia. We sampled red knots and invertebrate prey on 11 of these barrier islands, which together comprise approximately 82 km of Atlantic Ocean intertidal shoreline, from Assawoman Island in the north to Fisherman Island in the south, 2007 – 2019.

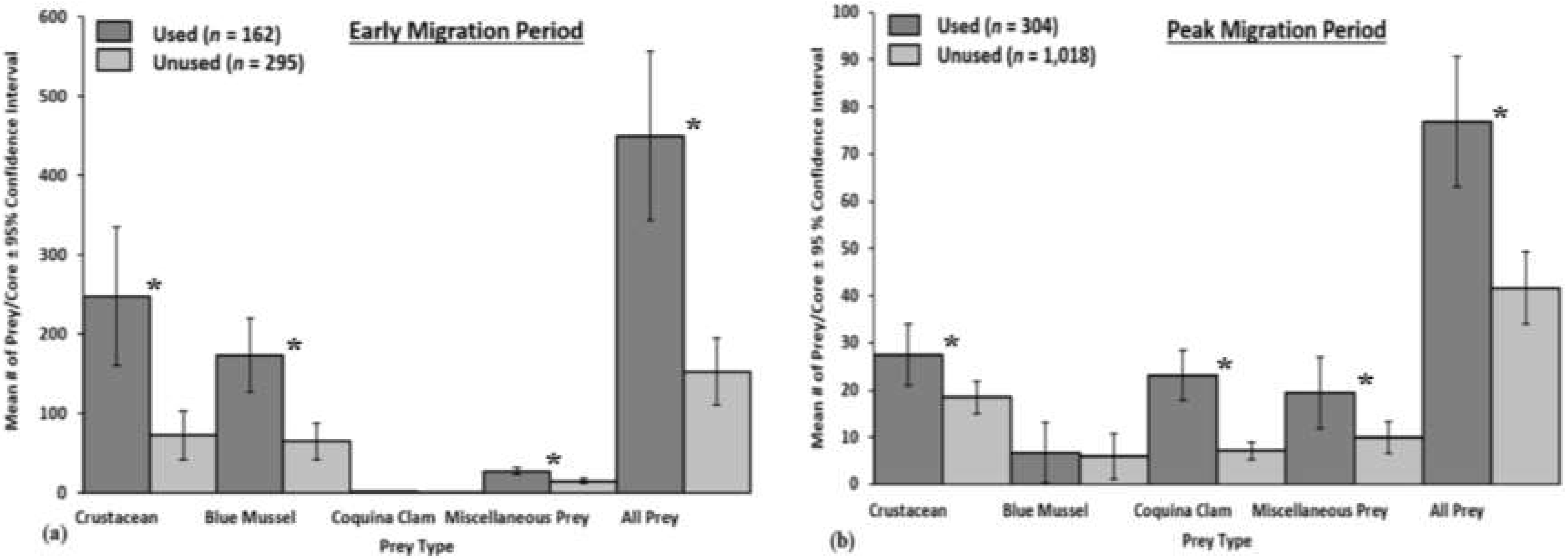


Figure 2. Mean abundance (organisms/275 cm^3 ± 95% CI) of crustaceans, blue mussels, coquina clams, miscellaneous prey, and all prey captured in 10 cm diameter x 3.5 cm deep cores in sites used and unused by red knots (a) on peat banks early in red knot migration (May 14 – 20, 2008 – 2018; *n* = 457; 'early') and (b) on sand and peat banks at the approximate peak of red knot migration (May 21 – 27, 2007 – 2018; *n* = 1,322; 'peak'), Virginia's barrier islands. * Indicates a difference between used and unused sites ($p < 0.05$) based on Wilcoxon rank sum tests with Bonferroni correction.

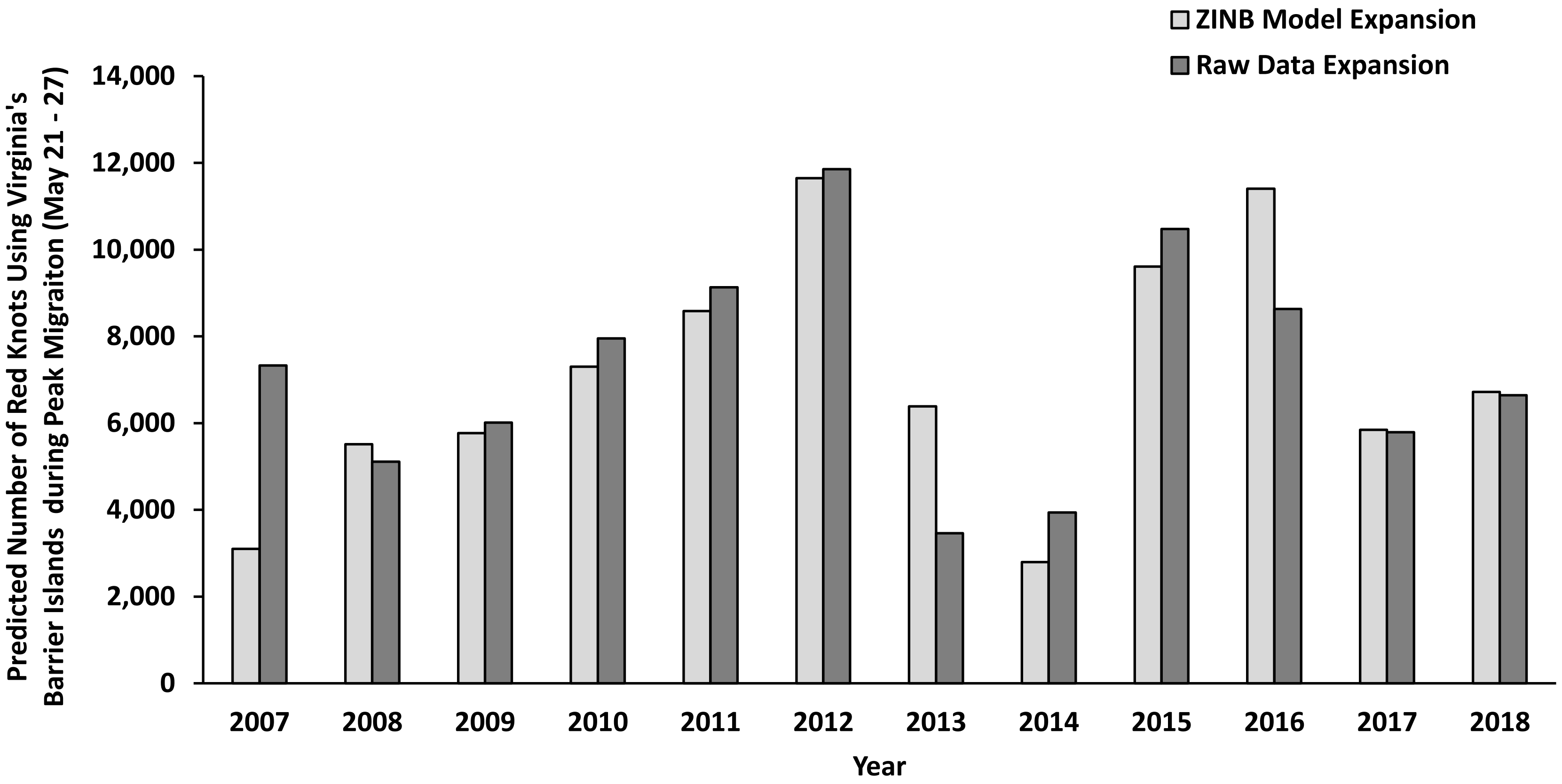


Figure 3. Predicted number of red knots as expanded from linear extrapolation of ground counts (dark gray) at randomly selected 100 m radius circles centered in the swash zone and as expanded from predictions made from a zero-inflated negative binomial mixed-effects model (Red Knot ~ Island + Year; light gray) from the same ground counts, at the approximate peak of red knot migration (‘peak migration period’), May 21 – 27, 2007 – 2018, Virginia’s barrier islands.

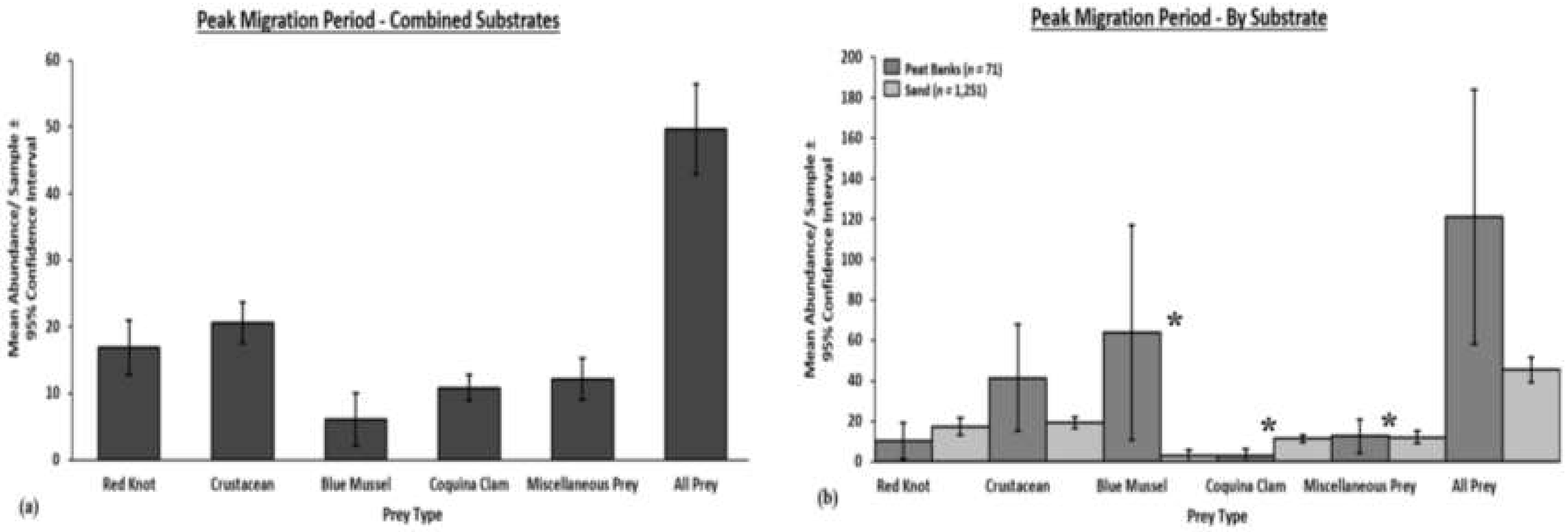
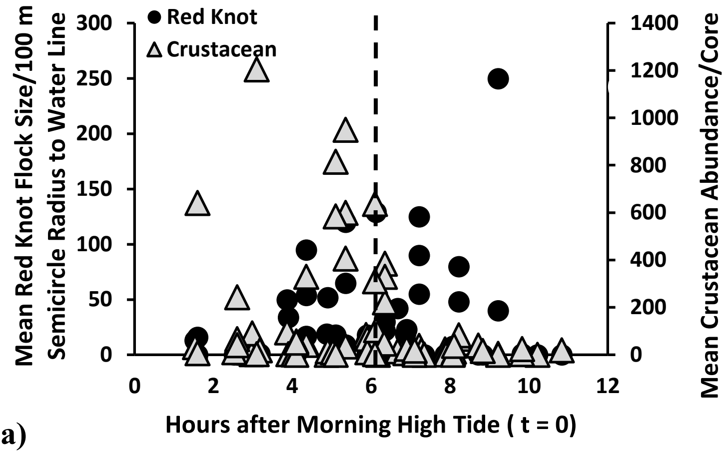
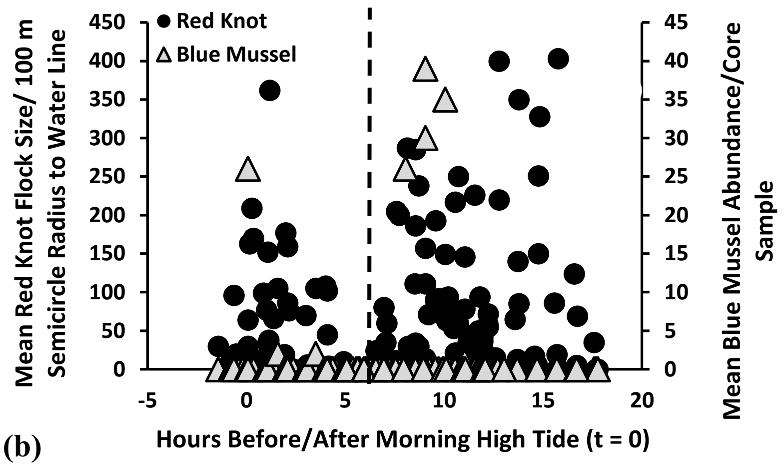


Figure 4. Mean red knot flock size per 100 m radius semicircle radius on the water line and mean abundance (organisms/275 cm^3 ± 95% CI) of crustaceans, blue mussels, coquina clams, miscellaneous prey, and all prey captured in 10 cm diameter x 3.5 cm deep cores on (a) on peat and sand substrates combined (*n* = 1,322) and on (b) peat banks (*n* = 71) and sand (*n* = 1,251) separately at the approximate peak of red knot migration ('peak migration period'), May 21 – 27, 2007 – 2018, Virginia's barrier islands. * Indicates a difference ($p < 0.05$) in red knot flock size and prey abundances between peat and sand substrates based on Wilcoxon rank sum tests with Bonferroni correction. Note: y-axis scales differ between (a) and (b).



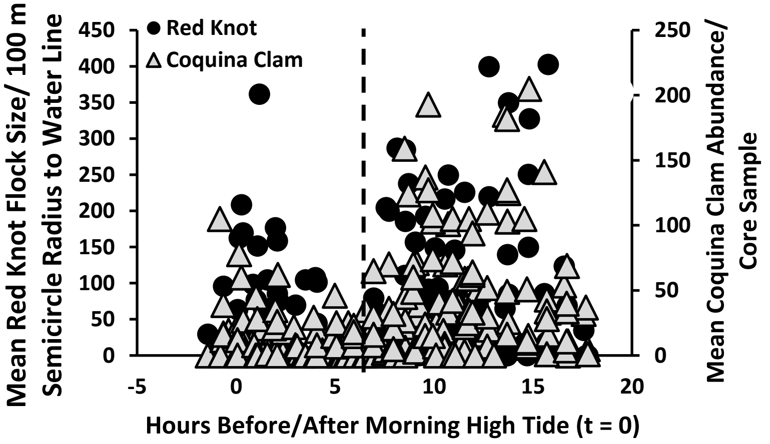
Peat Tidal: Red Knots and Crustaceans by Tide

(a)



Sand Tidal: Red Knots and Blue Mussels by Tide

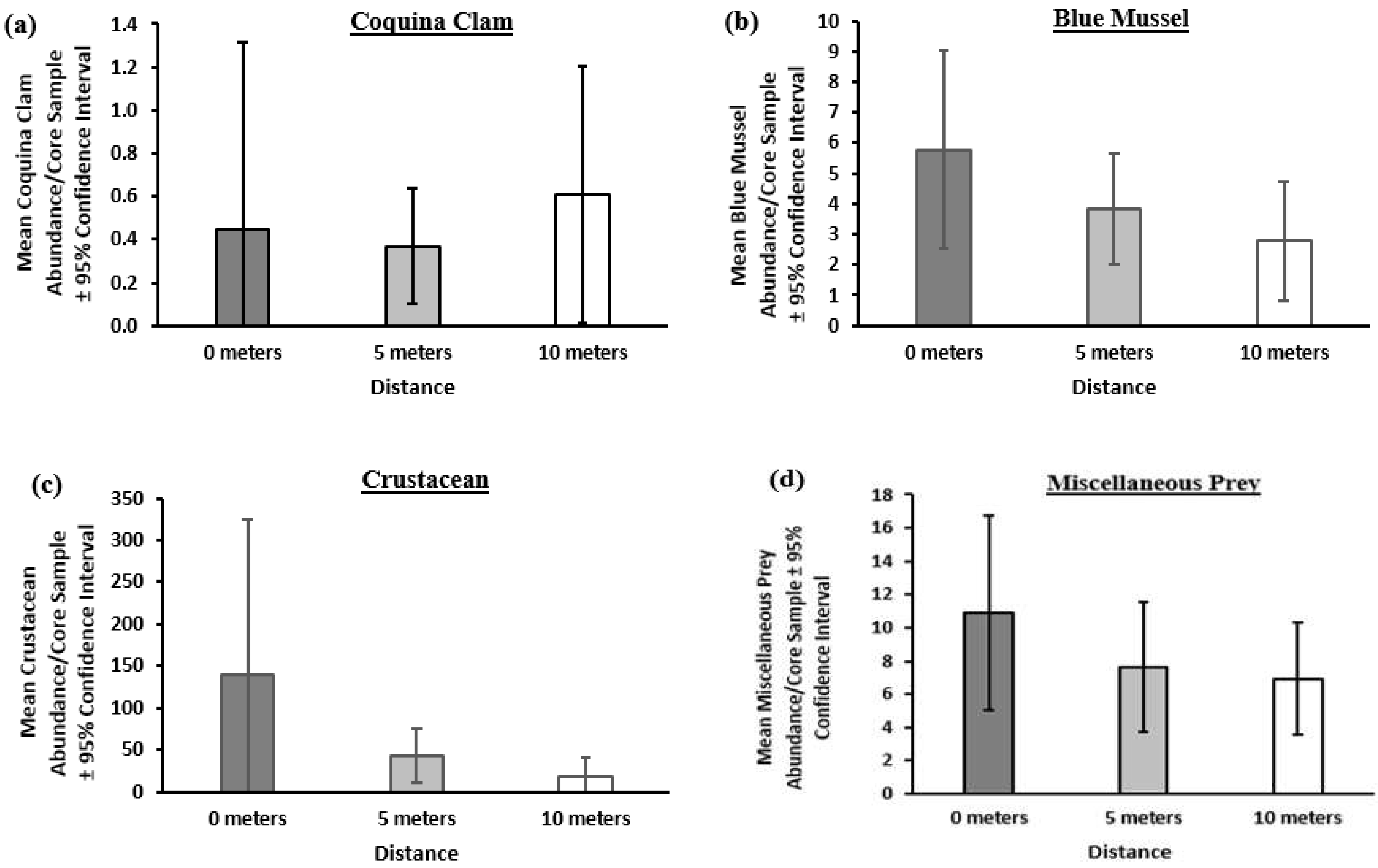
(b)



Sand Tidal: Red Knots and Coquina Clams by Tide

(c)

Figure 5. Mean red knot flock size per 100 m radius semicircle on the water line across the tidal cycle during peat and sand tidal sampling and the mean abundance (organisms/275 cm^3) of (a) crustaceans captured in 10 cm diameter x 3.5 cm deep cores during peat tidal sampling, May 17 – 25, 2015 – 2018, and the mean abundances of (b) blue mussels, and (c) coquina clams captured in 10 cm diameter x 3.5 cm deep cores during sand tidal sampling, May 17 – 27, 2013 – 2018, Virginia’s barrier islands. Dotted black line indicates low tide.


(a)
Coquina Clam
Mean Coquina Clam Abundance/Core Sample ± 95% Confidence Interval
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 meters
5 meters
10 meters
Distance
(b)
Blue Mussel
Mean Blue Mussel Abundance/Core Sample ± 95% Confidence Interval
10
9
8
7
6
5
4
3
2
1
0
0 meters
5 meters
10 meters
Distance
(c)
Crustacean
Mean Crustacean Abundance/Core Sample ± 95% Confidence Interval
350
300
250
200
150
100
50
0
0 meters
5 meters
10 meters
Distance
(d)
Miscellaneous Prey
Mean Miscellaneous Prey Abundance/Core Sample ± 95% Confidence Interval
18
16
14
12
10
8
6
4
2
0
0 meters
5 meters
10 meters
Distance

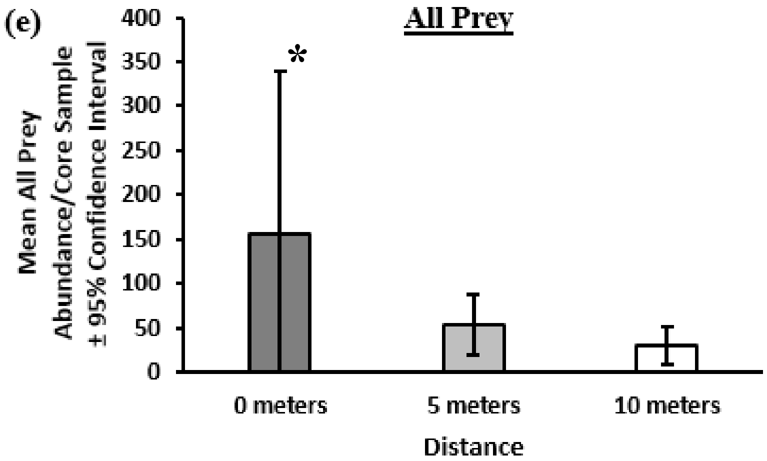


Figure 6. Mean (a) coquina clam, (b) blue mussel, (c) crustacean, (d) miscellaneous prey, and (e) all prey abundances (organisms/275 cm^3) captured in 10 cm diameter x 3.5 cm deep cores on peat banks during peat tidal spatial sampling, May 17, 2019, Myrtle Island, Virginia's barrier islands. Distance represents the distance from the center sampling point (0 meters). * Kruskal-Wallis and Dunn tests found a difference ($p < 0.05$) in all prey abundance between 0 m and 10 m.

TABLES

Table 1. Zero-inflated negative binomial mixed-effects regression models predicting red knot presence and flock size on peat banks early in red knot migration (May 14 – 20, 2008 – 2018, except 2010; *n* = 457; ‘early’), and on sand and peat substrates at the approximate peak of red knot migration (May 21 – 27, 2007 – 2018; *n* = 1,322; ‘peak’), Virginia’s barrier islands. All models had the same covariates on both the zero-inflated and count processes and contained “Island” and “Year” as random effects.

Period	**Model**	**K** [a]	**AIC_c** [b]	**ΔAICc** [c]	**w_i**[d]	**LL**[e]	**GOF**[f]
Early	Coquina Clam + Crustacean + Miscellaneous Prey + Tide	17	1,743.25	0.00	0.30	-853.93	0.08
	Crustacean + Miscellaneous Prey[g]	11	1,744.50	1.25	0.16	-860.96	0.07
	All Prey[h] + Tide	13	1,745.28	2.03	0.11	-859.23	0.07
	Coquina Clam + Blue Mussel + Crustacean + Miscellaneous Prey[g] + Tide	19	1,745.46	2.21	0.10	-852.86	0.08
	Blue Mussel + Crustacean + Miscellaneous Prey[g]	13	1,745.93	2.68	0.08	-859.55	0.07
	Crustacean + Miscellaneous Prey[g] + Distance to Roost	13	1,746.00	2.75	0.08	-859.59	0.07
	All Prey[h] + Distance to Roost + Tide	15	1,746.68	3.43	0.05	-857.79	0.07
Peak	Coquina Clam + Blue Mussel + Crustacean + TDF Count[i]	15	4,103.66	0.00	0.24	-2,036.65	0.07

Coquina Clam + Blue Mussel + Crustacean + Miscellaneous Prey[g] + Distance to Roost + TDF Count[i] + Tide	25	4,104.14	0.48	0.19	-2,026.57	0.08
Coquina Clam + Blue Mussel + Crustacean + Miscellaneous Prey[g] + TDF Count[i]	17	4,104.21	0.55	0.18	-2,034.87	0.07
Coquina Clam + Blue Mussel + TDF Count[i]	13	4,104.88	1.22	0.13	-2,039.30	0.07
Coquina Clam + Blue Mussel + Distance to Roost	13	4,106.03	2.37	0.07	-2,039.88	0.07
Coquina Clam + Blue Mussel + Crustacean + Distance to Roost	15	4,106.20	2.54	0.07	-2,037.92	0.07
Coquina Clam + Blue Mussel + Crustacean + Miscellaneous Prey[g] + Distance to Roost	17	4,106.70	3.04	0.05	-2,036.12	0.07

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[a] K = Number of parameters.

[b] AIC_c = Akaike's Information Criterion corrected for sample size.

[c] ΔAIC_c = Difference between a model's AIC and that of the best fitting model.

[d] w_i^d = Akaike model weight.

[e] LL = Log-Likelihood.

[f] GOF = Goodness of fit = [log-likelihood(null model) – log-likelihood(model)]/log-likelihood(null model).

[g] Miscellaneous Prey = Sum of horseshoe crab eggs (*Limulus polyphemus*), angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae, snails, worms).

[h] All Prey = Sum of coquina clams + blue mussels + crustaceans + miscellaneous prey.

[i] TDF Count = Tierra del Fuego Count = Counts of red knots using Tierra del Fuego wintering grounds by year (i.e., as an index for the total number of red knots in the flyway; Morrison and Ross 1989, Morrison et al. 2004, Morrison 2014, Dey et al. 2015, USFWS 2015, Western Hemisphere Shorebird Reserve Network 2017, 2018).

Table 2. Parameter estimates (β) from the most parsimonious zero-inflated negative binomial mixed-effect regression models predicting red knot presence (zero-inflated process) and flock size (count process) on peat banks early in red knot migration (May 14 – 20, 2008 – 2018, except 2010; *n* = 457; 'early'), and on sand and peat substrates near the peak of red knot migration (May 21 – 27, 2007 – 2018; *n* = 1,322; 'peak'), Virginia's barrier islands. All models contained "Island" and "Year" as random effects.

Period	**Model Process**	**Covariate**	**β [a]**	**SE [b]**	**LCI [c]**	**UCI [d]**	**z value**	**Pr(>|z|) [e]**	**Significance [f]**
Early	Zero-Inflated	Intercept	1.62	0.92	-1.19	2.42	0.67	0.51	
		Coquina Clam	-0.21	0.42	-1.02	0.61	-0.50	0.62	
		Crustacean	-1.13	1.10	-3.29	1.03	-1.03	0.30	
		Miscellaneous Prey	-2.15	0.80	-3.72	-0.57	-2.67	0.01	*
		Falling Tide	-1.03	0.64	-2.28	0.23	-1.60	0.11	
		Low Tide	-1.53	0.57	-2.64	-0.42	-2.70	0.01	*
	Count	Intercept	1.62	0.59	0.46	2.77	2.74	0.01	*
		Coquina Clam	-0.02	0.11	-0.24	0.20	-0.17	0.87	
		Crustacean	0.23	0.11	0.02	0.44	2.10	0.04	*
		Miscellaneous Prey	0.15	0.14	-0.13	0.43	1.04	0.30	
		Falling Tide	0.16	0.50	-0.81	1.13	0.32	0.75	

		Low Tide	0.67	0.42	-0.16	1.49	1.59	0.11	
Peak	Zero-Inflated	Intercept	0.05	0.51	-0.95	1.04	0.09	0.93	
		Blue Mussel	-8.87	4.11	-16.92	-0.81	-2.16	0.03	*
		Coquina Clam	-3.08	0.69	-4.42	-1.73	-4.49	<0.001	*
		Crustacean	-0.27	0.17	-0.62	0.07	-1.56	0.12	
		TDF Count[h]	-0.49	0.17	-0.82	-0.16	-2.91	0.004	*
	Count	Intercept	3.16	0.30	2.57	3.75	10.54	<0.001	*
		Blue Mussel	-0.30	0.10	-0.49	-0.11	-3.05	0.002	*
		Coquina Clam	0.02	0.09	-0.15	0.19	0.22	0.83	
		Crustacean	-0.24	0.09	-0.41	-0.07	-2.72	0.01	*
		TDF Count[h]	-0.26	0.13	-0.52	-0.01	-2.02	0.04	*

[a] β = Beta estimate.

[b] SE = Standard error.

[c] LCI = Lower 95% confidence interval.

[d] UCI = Upper 95% confidence interval.

[e] Pr(>|z|) = Significance level.

[f] Significance = * = $p \leq 0.05$.

[g] Miscellaneous Prey = Sum of horseshoe crab eggs (*Limulus polyphemus*), angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae, snails, worms).

[h] TDF Count = Tierra del Fuego Count = Counts of red knots using Tierra del Fuego wintering grounds by year (i.e., as an index for the total number of red knots in the flyway; Morrison and Ross 1989, Morrison et al. 2004, Morrison 2014, Dey et al. 2015, USFWS 2015, Western Hemisphere Shorebird Reserve Network 2017, 2018).

Table 3. Most parsimonious generalized linear mixed-effects regression models ($\Delta AIC_c < 4$) predicting crustacean, coquina clam, and miscellaneous prey abundances (organisms/275 cm^3) captured in 10 cm diameter x 3.5 cm deep cores on peat banks early in red knot migration (May 14 – 20, 2008 – 2018; *n* = 457; 'early') and on sand and peat banks at the approximate peak of red knot migration (May 21 – 27, 2007 – 2018; *n* = 1,322; 'peak'), Virginia's barrier islands. All models contained "Island" and "Year" as random effects.

Period	**Prey**	**Model**	**K** [a]	**AIC_c** [b]	**ΔAIC_c** [c]	**w_i** [d]	**LL** [e]	**MR2** [f]	**CR2** [g]
Early	Crustacean	Tide + Water Temperature	6	110,491.00	0.00	0.61	-55,239.42	0.02	0.57
		Tide + Water Temperature + Island Type	7	110,491.90	0.88	0.39	-55,238.83	0.06	0.56
	Coquina Clam	Tide + Water Temperature	6	1,952.17	0.00	0.71	-969.99	0.01	0.00
		Tide + Water Temperature + Island Type	7	1,954.23	2.06	0.25	-969.99	0.01	0.01
	Misc. Prey[h]	Tide + Water Temperature	6	8,721.57	0.00	0.71	-4,354.69	0.04	0.69
		Tide + Water Temperature + Island Type	7	8,723.40	1.84	0.29	-4,354.58	0.07	0.66

Peak	Crustacean	Tide + Substrate + Water Temperature	8	61,060.87	0.00	0.56	-30,522.38	0.02	0.22
		Tide + Substrate + Water Temperature + Island Type	9	61,061.39	0.51	0.44	-30,521.62	0.05	0.23
	Coquina Clam	Tide + Substrate + Water Temperature	8	35,893.06	0.00	0.69	-17,938.47	0.04	0.26
		Tide + Substrate + Water Temperature + Island Type	9	35,894.64	1.58	0.31	-17,938.25	0.05	0.26
	Blue Mussel	Tide + Substrate + Water Temperature + Island Type	9	22,816.70	0.00	0.51	-11,399.28	0.06	0.07
		Tide + Substrate + Water Temperature	8	22,816.77	0.07	0.49	-11,400.33	.	.

[a] K = Number of parameters.

[b] AIC_c = Akaike's Information Criterion corrected for sample size.

[c] ΔAIC_c = Difference between a model's AIC and that of the best fitting model.

[d] w_i^d = Akaike model weight.

[e] LL = Log-Likelihood.

[f] MR2 = Marginal r-squared = Considers only the variance of the fixed effects (Nagelkerke 1991).

[g] CR2 = Conditional r-squared = Considers the variance of both fixed and random effects (Nagelkerke 1991)..

[h] Misc. Prey = Miscellaneous Prey = Sum of horseshoe crab eggs (*Limulus polyphemus*), angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae, snails, worms).

Table 4. Parameter estimates (β) from the most parsimonious generalized linear mixed effects regression models predicting crustacean, coquina clam, and miscellaneous prey abundances (organisms/275 cm^3) captured in 10 cm diameter x 3.5 cm deep cores on peat banks early in red knot migration (May 14 – 20, 2008 – 2018, except 2010; *n* = 457; 'early'), and on sand and peat substrates near the peak of red knot migration (May 21 – 27, 2007 – 2018; *n* = 1,322; 'peak'), Virginia's barrier islands. All models contained "Island" and "Year" as random effects.

Period	**Prey**	**Covariate**	**β** [a]	**SE** [b]	**LCI** [c]	**UCI** [d]	**z value**	**Pr(>|z|)** [e]	**Significance** [f]
Early	Crustacean	Intercept	-4.75	0.81	-6.33	-3.17	-5.89	<0.001	*
		Low Tide	0.54	0.01	0.52	0.56	54.90	<0.001	*
		Rising Tide	-0.95	0.02	-1.00	-0.91	-41.34	<0.001	*
		Water Temperature	0.50	0.01	0.48	0.52	47.58	<0.001	*
	Coquina Clam	Intercept	2.33	1.24	-0.10	4.76	1.88	0.06	
		Low Tide	0.82	0.13	0.57	1.08	6.29	<0.001	*
		Rising Tide	0.74	0.18	0.39	1.09	4.17	<0.001	*
		Water Temperature	-0.24	0.08	-0.40	-0.08	-2.91	<0.001	*
	Misc. Prey[g]	Intercept	-0.70	0.63	-1.93	0.54	-1.10	0.27	*
		Low Tide	0.10	0.03	0.04	0.15	3.44	<0.001	*

		Rising Tide	-0.18	0.04	-0.26	-0.11	-4.65	<0.001	*
		Water Temperature	0.19	0.02	0.16	0.23	9.73	<0.001	*
Peak	Crustacean	Intercept	1.47	0.25	0.99	1.96	5.97	<0.001	*
		Falling Tide	0.16	0.02	0.12	0.21	6.95	<0.001	*
		Low Tide	0.76	0.02	0.71	0.80	31.66	<0.001	*
		Rising Tide	0.30	0.02	0.26	0.35	12.68	<0.001	*
		Sand Substrate	-0.90	0.02	-0.95	-0.86	-41.90	<0.001	*
		Water Temperature	0.12	0.01	0.10	0.14	10.66	<0.001	*
	Coquina Clam	Intercept	5.64	0.45	4.77	6.52	12.62	<0.001	*
		Falling Tide	0.84	0.03	0.78	0.90	26.61	<0.001	*
		Low Tide	0.83	0.04	0.76	0.91	22.93	<0.001	*
		Rising Tide	0.51	0.03	0.45	0.57	15.67	<0.001	*
		Sand Substrate	0.87	0.07	0.73	1.01	12.27	<0.001	*
		Water Temperature	-0.34	0.02	-0.37	-0.30	-19.14	<0.001	*
	Blue Mussel	Intercept	-13.54	1.60	-16.67	-10.40	-8.47	<0.001	*
		Falling Tide	1.05	0.18	0.69	1.41	5.73	<0.001	*

Low Tide	2.90	0.19	2.54	3.27	15.69	<0.001	*
Rising Tide	2.21	0.19	1.83	2.58	11.67	<0.001	*
Sand Substrate	-1.51	0.03	-1.56	-1.46	-55.06	<0.001	*
Water Temperature	0.59	0.07	0.46	0.72	8.85	<0.001	*

[a] β = Beta estimate.

[b] SE = Standard error.

[c] LCI = Lower 95% confidence interval.

[d] UCI = Upper 95% confidence interval.

[e] $Pr(>|z|)$ = Significance level.

[f] Significance = * = $p \leq 0.05$.

[g] Misc. Prey = Miscellaneous Prey = Sum of horseshoe crab eggs (*Limulus polyphemus*), angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae, snails, worms).

APPENDICES

Appendix 1. Mean red knot flock size per 100 m radius semicircle on the water line and mean abundance (organisms/275 cm^3 ± 95% CI) of coquina clams, blue mussels, crustaceans, miscellaneous prey, and all prey captured in 10 cm diameter x 3.5 cm deep cores on peat banks early in red knot migration (May 14 – 20, 2008 – 2018; *n* = 457; 'early') and on sand and peat banks at the approximate peak of red knot migration (May 21 – 27, 2007 – 2018; *n* = 1,322; 'peak'), Virginia's barrier islands.

Year	**Period**	***n***	***Calidris canutus rufa***		***Donax variabilis***		***Mytilus edulis***		**Crustacean**		**Misc. Prey**[b]		**All Prey**[c]	
			$\bar{x}$ ()[a]	**SE**	**$\bar{x}$ ()**[a]	**SE**	**$\bar{x}$ ()**[a]	**SE**	**$\bar{x}$ ()**[a]	**SE**	**$\bar{x}$ ()**[a]	**SE**	**$\bar{x}$ ()**[a]	**SE**
2007	Early	.	.	.	.	.	.	.	.	.	.	.	.	.
	Peak	96	9.26(71)	0.95	11.66(36)	1.19	0.39(93)	0.04	16.11(20)	1.64	15.42(45)	1.57	43.57(9)	8.27
2008	Early	61	5.05(42)	0.65	0.11(56)	0.01	24.97*(39)	3.20	3.72(44)	0.48	3.59*(32)	0.46	32.39*(25)	10.55
	Peak	98	12.03(72)	1.22	4.85*(50)	0.49	0.00(98)	0.00	9.77*(28)	0.99	1.68(67)	0.17	16.30(21)	3.88
2009	Early	42	3.79(28)	0.58	0.43(33)	0.07	182.05*(15)	28.09	40.19(13)	6.20	24.48*(6)	3.78	247.14*(4)	66.69
	Peak	99	14.14(69)	1.42	4.71*(50)	0.47	0.00(99)	0.00	20.56(22)	2.07	0.60(71)	0.06	25.86(14)	11.31
2010	Early	.	.	.	.	.	.	.	.	.	.	.	.	.
	Peak	95	19.36(66)	1.99	13.71(26)	1.41	5.58(90)	0.57	15.65(18)	1.61	2.61(53)	0.27	37.55(6)	11.66
2011	Early	50	2.78(29)	0.39	0.52(40)	0.07	153.88*(13)	21.76	442.68*(7)	62.60	22.34*(12)	3.16	619.42*(3)	112.63
	Peak	93	22.00(68)	2.28	15.12*(26)	1.57	0.00(93)	0.00	14.65(18)	1.52	2.17(68)	0.23	31.94(5)	4.57
2012	Early	42	0.14(37)	0.02	2.36(27)	0.36	22.19*(17)	3.42	38.60(11)	5.96	29.36(8)	4.53	92.50(1)	20.17
	Peak	125	28.56*(89)	2.55	14.77*(53)	1.32	0.07(124)	0.01	22.18(20)	1.98	86.98*(11)	7.78	124.00(0)	16.32
2013	Early	43	18.10*(19)	2.77	1.88(35)	0.29	325.19*(5)	49.59	151.67*(6)	23.13	23.21*(6)	3.54	501.95*(3)	84.62

	Peak	127	8.33(110)	0.74	12.32*(66)	1.09	56.18(110)	4.99	26.60(29)	2.36	19.84(56)	1.76	114.94(11)	24.46
2014	Early	50	6.44*(30)	0.91	0.32(46)	0.05	157.34*(5)	22.25	287.60*(5)	40.67	34.54*(6)	4.88	479.80*(3)	127.44
	Peak	129	9.60(113)	0.85	6.12*(65)	0.54	0.54(123)	0.05	25.20(24)	2.22	1.75(92)	0.15	33.61(14)	6.00
2015	Early	46	10.57(31)	1.56	1.09(33)	0.16	79.96*(6)	11.79	50.09(10)	7.38	17.70*(4)	2.61	148.83*(0)	43.47
	Peak	128	25.55(99)	2.26	22.57*(36)	1.99	0.14(126)	0.01	10.92(22)	0.97	0.16(117)	0.01	33.79(8)	4.18
2016	Early	39	7.67(30)	1.23	0.79(27)	0.13	29.87*(2)	4.78	39.85*(2)	6.38	12.08*(2)	1.93	82.59*(0)	13.14
	Peak	112	21.57(97)	2.04	5.95*(48)	0.56	0.14(109)	0.01	25.68(27)	2.43	0.29(103)	0.03	32.06()19	5.73
2017	Early	40	15.98*(18)	2.53	0.58(34)	0.09	36.90*(11)	5.83	104.08*(3)	16.46	17.48*(5)	2.76	159.03*(2)	39.62
	Peak	109	14.25(78)	1.36	9.58*(44)	0.92	0.57(103)	0.05	25.35(17)	2.43	0.52(86)	0.05	36.02(12)	4.01
2018	Early	44	2.95(31)	0.45	1.80(28)	0.27	28.89*(11)	4.35	159.43*(3)	24.04	7.00*(13)	1.06	197.11*(2)	66.10
	Peak	111	16.77(86)	1.59	7.13*(55)	0.68	1.77(98)	0.17	30.41(17)	2.89	1.43(88)	0.14	40.74(10)	7.40
TOTAL	Early	457	7.15(295)	0.33	0.94(359)	0.04	103.36*†(124)	4.83	134.81*†(104)	6.31	18.85*†(94)	0.88	257.96*†(43)	24.55
	Peak	1,322	16.88*(1,018)†	0.46	10.86*†(555)	0.30	6.11(1,266)	0.17	20.58(262)	0.57	12.13(857)	0.33	103.18(129)	3.45

[a] $\overline{x}$ () = Number in parentheses following each mean value represents the number of 0 counts for each category during its defined period and year.

[b] Misc. Prey = Miscellaneous Prey = Sum of horseshoe crab eggs (*Limulus polyphemus*), angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae, snails, worms).

[c] All Prey = Sum of coquina clams + blue mussels + crustaceans + miscellaneous prey.

[*] = Difference ($p < 0.05$) between means of early migration period vs. peak migration period based on Wilcoxon rank sum tests between peat and peak migration period within a given year. *placed with the larger mean.

[†] = Difference ($p < 0.05$) between mean red knot or prey abundances based on Wilcoxon rank sum tests during peat bank period and peak migration period across all years. [†] placed with the larger mean.

Appendix 2. Mean red knot flock size and standard errors per 100 m radius semicircle on the water line on peat banks early in red knot migration (May 14 – 20, 2008 – 2018; *n* = 457; 'early') and on sand and peat banks at the approximate peak of red knot migration (May 21 – 27, 2007 – 2018; *n* = 1,322; 'peak'), Virginia's barrier islands.

Period	**Year**	**Sample Size**	**Mean Red Knot Flock Size**	**Standard Deviation**	**Standard Error**	**Variance**
Early	2008	61	5.05	13.08	0.65	171.11
	2009	42	3.79	8.69	0.58	75.44
	2011	50	2.78	6.53	0.39	42.71
	2012	42	0.14	0.42	0.02	0.17
	2013	43	18.16	30.90	2.77	954.95
	2014	50	6.44	17.43	0.91	303.92
	2015	46	10.57	28.90	1.56	835.32
	2016	39	7.67	19.14	1.23	366.33
	2017	40	15.98	28.07	2.53	787.87
	2018	44	2.95	7.62	0.45	58.00
Peak	2007	96	9.26	27.90	0.95	778.45
	2008	98	12.03	36.37	1.22	1,322.79

2009	99	14.14	48.84	1.42	2,384.94
2010	95	19.36	60.50	1.99	3,660.30
2011	93	22.00	65.14	2.28	4,243.72
2012	125	28.56	127.47	2.55	16,247.46
2013	127	8.33	43.54	0.74	1,895.41
2014	129	9.60	56.96	0.85	3,244.44
2015	128	25.55	87.30	2.26	7,621.78
2016	112	21.57	115.76	2.04	13,401.47
2017	109	14.25	61.83	1.36	3,822.63
2018	111	16.77	88.09	1.59	7,760.11

Appendix 3. Pearson correlation coefficients for all continuous covariates on peat banks early in red knot migration (May 14 – 20, 2008 – 2018; *n* = 457; 'early') and on sand and peat banks at the approximate peak of red knot migration (May 21 – 27, 2007 – 2018; *n* = 1,322; 'peak'), Virginia's barrier islands. Highly correlated covariate combinations are represented by values > 0.7 and < -0.7 (Booth et al. 1994, Anderson et al. 2001).

Period	**Covariate**	**Red Knot[a]**	**Distance to Roost[b]**	**TDF Count[c]**	**Blue Mussel[d]**	**Crustacean[d]**	**Coquina Clam[d]**	**Miscellaneous Prey[d]**	**All Prey[d]**
Early	Red Knot	1.00	0.17	-0.07	0.23	0.17	0.04	0.21	0.25
	Distance to Roost	0.17	1.00	-0.02	0.37	0.13	0.01	0.35	0.30
	TDF Count	-0.07	-0.02	1.00	-0.05	-0.17	-0.07	0.06	-0.15
	Blue Mussel	0.23	0.37	-0.05	1.00	0.17	-0.03	0.50	0.63
	Crustacean	0.17	0.13	-0.17	0.17	1.00	0.12	0.13	0.87
	Coquina Clam	0.04	0.01	-0.07	-0.03	0.12	1.00	-0.01	0.09
	Miscellaneous Prey	0.21	0.35	0.06	0.50	0.13	-0.01	1.00	0.39
	All Prey	0.25	0.30	-0.15	0.63	0.87	0.09	0.39	1.00
Peak	Red Knot	1.00	-0.13	-0.01	-0.02	0.01	0.11	0.10	0.07
	Distance to Roost	-0.13	1.00	-0.03	0.10	-0.07	-0.06	-0.05	-0.01

TDF Count	-0.01	-0.03	1.00	-0.09	-0.05	-0.02	0.04	-0.06
Blue Mussel	-0.02	0.10	-0.09	1.00	0.21	-0.02	0.02	0.68
Crustacean	0.01	-0.07	-0.05	0.21	1.00	0.02	0.08	0.62
Coquina Clam	0.11	-0.06	-0.02	-0.02	0.02	1.00	0.03	0.29
Miscellaneous Prey	0.10	-0.05	0.04	0.02	0.08	0.03	1.00	0.51
All Prey	0.07	-0.01	-0.06	0.68	0.62	0.29	0.51	1.00

[a] Red Knot = At randomly generated sampling points on Virginia's barrier islands, we counted the number of non-flying red knots within a 100 m semicircle radius of each point.

[b] Distance to Roost = We calculated distance to roost as the distance between sampling points and locations presumed to be used for night roosting by red knots on Chimney Pole and Wreck Island (Cohen et al. 2010b).

[c] Tierra del Fuego Count = Counts of red knots using Tierra del Fuego wintering grounds by year (i.e., as an index for the total number of red knots in the flyway; Morrison and Ross 1989, Morrison et al. 2004, Morrison 2014, Dey et al. 2015, USFWS 2015, Western Hemisphere Shorebird Reserve Network 2017, 2018).

[d] Blue Mussel, Crustacean, Coquina Clam, Miscellaneous Prey, All Prey = At randomly generated sampling points on Virginia's barrier islands, we sampled prey availability by collecting a core sample of the substrate at the water-line at each sampling point using

a section of PVC piping (10 cm diameter x 3.5 cm deep). The cores' depth represented the approximate length of a red knot's bill (Tomkovich 1992), so that we sampled only prey that red knots would be able to access while foraging. Miscellaneous Prey = the sum of horseshoe crab eggs (*Limulus polyphemus*), angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae, snails, worms). All Prey = the sum of coquina clams + blue mussels + crustaceans + miscellaneous prey.

Appendix 4. Full model sets for zero-inflated negative binomial mixed-effects regression models predicting red knot presence and flock size on peat banks early in red knot migration (May 14 – 20, 2008 – 2018, except 2010; *n* = 457; ‘early’), and on sand and peat substrates at the approximate peak of red knot migration (May 21 – 27, 2007 – 2018; *n* = 1,322; ‘peak’), Virginia’s barrier islands. All models had the same covariates on both the zero-inflated and count processes and contained “Island” and “Year” as random effects.

Period	**Model**	**K**[a]	**AIC$_c$**[b]	**ΔAIC$_c$**[c]	w_i[d]	**LL**[e]	**GOF**[e]
Early	Coquina Clam + Crustacean + Miscellaneous Prey[g] + Tide	17	1,743.25	0.00	0.30	-853.93	0.08
	Crustacean + Miscellaneous Prey[g]	11	1,744.50	1.25	0.16	-860.96	0.07
	All Prey[h] + Tide	13	1,745.28	2.03	0.11	-859.23	0.07
	Blue Mussel + Coquina Clam + Crustacean + Miscellaneous Prey[g] + Tide	19	1,745.46	2.20	0.10	-852.86	0.08
	Blue Mussel + Crustacean + Miscellaneous Prey[g]	13	1,745.93	2.68	0.08	-859.55	0.07
	Crustacean + Miscellaneous Prey[g] + Distance to Roost	13	1,746.00	2.75	0.08	-859.59	0.07
	All Prey[h] + Tide + Distance to Roost	15	1,746.68	3.42	0.05	-857.79	0.07
	Coquina Clam + Crustacean + Miscellaneous Prey[g]	13	1,748.45	5.20	0.02	-860.82	0.07
	Miscellaneous Prey[g] + Tide	13	1,748.81	5.56	0.02	-860.99	0.07
	All Prey[h] + Tide + TDF Count[i]	15	1,749.02	5.76	0.02	-858.96	0.07

Blue Mussel + Coquina Clam + Crustacean + Miscellaneous Prey[g]	15	1,749.89	6.63	0.01	-859.40	0.07
Crustacean + Miscellaneous Prey[g] + Distance to Roost + TDF Count[i]	15	1,749.92	6.67	0.01	-859.42	0.07
Blue Mussel + Miscellaneous Prey[g] + Tide	15	1,749.94	6.68	0.01	-859.42	0.07
Coquina Clam + Crustacean + Miscellaneous Prey[g] + Distance to Roost	15	1,749.96	6.70	0.01	-859.43	0.07
All Prey[h] + Distance to Roost + TDF Count[i] + Tide	17	1,750.41	7.16	0.01	-857.51	0.07
All Prey[h]	9	1,751.01	7.75	0.01	-866.30	0.06
All Prey[h] + Distance to Roost	11	1,751.45	8.19	0.00	-864.43	0.06
Coquina Clam + Miscellaneous Prey[g] + Tide	15	1,752.34	9.09	0.00	-860.63	0.07
Coquina Clam + Crustacean + Miscellaneous Prey[g] + TDF Count[i]	15	1,752.41	9.16	0.00	-860.66	0.07
Blue Mussel + Crustacean + Tide	15	1,753.80	10.55	0.00	-861.36	0.07
Blue Mussel + Coquina Clam + Crustacean + Miscellaneous Prey[g] + TDF Count[i]	17	1,753.84	10.59	0.00	-859.22	0.07

All Prey[h] + TDF Count[i]	11	1,754.88	11.63	0.00	-866.14	0.06
Blue Mussel + Coquina Clam + Crustacean + Tide	17	1,757.20	13.94	0.00	-860.90	0.07
Crustacean + Tide	13	1,757.29	14.03	0.00	-865.23	0.06
Blue Mussel + Crustacean	11	1,759.82	16.57	0.00	-868.61	0.06
Blue Mussel + Crustacean + Distance to Roost	13	1,760.36	17.10	0.00	-866.77	0.06
Miscellaneous Prey[g]	9	1,760.37	17.11	0.00	-870.98	0.06
Blue Mussel + Miscellaneous Prey[g]	11	1,760.67	17.42	0.00	-869.04	0.06
Coquina Clam + Crustacean + Tide	15	1,760.90	17.64	0.00	-864.90	0.06
Blue Mussel + Miscellaneous Prey[g] + Distance to Roost	13	1,761.36	18.11	0.00	-867.27	0.06
Miscellaneous Prey[g] + Distance to Roost	11	1,762.35	19.10	0.00	-869.88	0.06
Blue Mussel + Coquina Clam + Crustacean	13	1,763.05	19.80	0.00	-868.11	0.06
Blue Mussel + Coquina Clam + Miscellaneous Prey[g]	13	1,763.34	20.09	0.00	-868.26	0.06
Coquina Clam + Miscellaneous Prey[g]	11	1,763.42	20.17	0.00	-870.41	0.06
Miscellaneous Prey[g] + TDF Count[i]	11	1,763.69	20.44	0.00	-870.55	0.06
Blue Mussel + Crustacean + TDF Count[i]	13	1,763.77	20.52	0.00	-868.48	0.06

Blue Mussel + Coquina Clam + Crustacean + Distance to Roost	15	1,763.88	20.63	0.00	-866.40	0.06
Blue Mussel + Miscellaneous Prey[g] + TDF Count[i]	13	1,764.30	21.05	0.00	-868.74	0.06
Coquina Clam + Miscellaneous Prey[g] + Distance to Roost	13	1,765.45	22.19	0.00	-869.31	0.06
Coquina Clam + Miscellaneous Prey[g] + TDF Count[i]	13	1,766.90	23.64	0.00	-870.04	0.06
Blue Mussel + Coquina Clam + Crustacean + TDF Count[i]	15	1,766.98	23.73	0.00	-867.95	0.06
Crustacean	9	1,770.50	27.25	0.00	-876.05	0.05
Blue Mussel + Tide	13	1,770.93	27.67	0.00	-872.05	0.06
Blue Mussel + Distance to Roost + Tide	15	1,771.96	28.71	0.00	-870.44	0.06
Crustacean + Distance to Roost	11	1,772.79	29.54	0.00	-875.10	0.05
Coquina Clam + Crustacean	11	1,773.46	30.21	0.00	-875.43	0.05
Blue Mussel + Coquina Clam + Tide	15	1,773.72	30.46	0.00	-871.31	0.06
Crustacean + TDF Count[i]	11	1,774.30	31.05	0.00	-875.85	0.05
Coquina Clam + Crustacean + Distance to Roost	13	1,775.83	32.57	0.00	-874.50	0.05
Coquina Clam + Crustacean + TDF Count[i]	13	1,777.10	33.85	0.00	-875.14	0.05
Tide	11	1,781.17	37.92	0.00	-879.29	0.05

	Coquina Clam + Tide	13	1,784.47	41.22	0.00	-878.82	0.05
	Blue Mussel + Distance to Roost	11	1,785.03	41.78	0.00	-881.22	0.05
	Blue Mussel	9	1,785.04	41.78	0.00	-883.32	0.04
	Blue Mussel + Coquina Clam	11	1,786.66	43.40	0.00	-882.03	0.05
	Blue Mussel + Coquina Clam + Distance to Roost	13	1,786.79	43.53	0.00	-879.98	0.05
	Blue Mussel + Distance to Roost + TDF Count[i]	13	1,788.76	45.51	0.00	-880.97	0.05
	Blue Mussel + TDF Count[i]	11	1,788.83	45.57	0.00	-883.12	0.04
	Blue Mussel + Coquina Clam + TDF Count[i]	13	1,790.60	47.34	0.00	-881.89	0.05
	Coquina Clam	9	1,809.39	66.13	0.00	-895.49	0.03
	TDF Count[i]	9	1,809.83	66.57	0.00	-895.71	0.03
	Distance to Roost	9	1,810.66	67.40	0.00	-896.13	0.03
	Coquina Clam + TDF Count[i]	11	1,812.85	69.60	0.00	-895.13	0.03
	Coquina Clam + Distance to Roost	11	1,813.56	70.31	0.00	-895.48	0.03
	Null	2	1,852.19	108.94	0.00	-924.08	0.00
Peak	Blue Mussel + Coquina Clam + Crustacean + TDF Count[i]	15	4,103.66	0.00	0.24	-2,036.65	0.07

Blue Mussel + Coquina Clam + Crustacean + Miscellaneous Prey[g] + Distance to Roost	25	4,104.14	0.48	0.19	-2,026.57	0.08
Blue Mussel + Coquina Clam + Crustacean + Miscellaneous Prey[g] + TDF Count[i]	17	4,104.21	0.55	0.18	-2,034.87	0.07
Blue Mussel + Coquina Clam + TDF Count[i]	13	4,104.88	1.23	0.13	-2,039.30	0.07
Blue Mussel + Coquina Clam + Distance to Roost	13	4,106.03	2.37	0.07	-2,039.88	0.07
Blue Mussel + Coquina Clam + Crustacean + Distance to Roost	15	4,106.20	2.54	0.07	-2,037.92	0.07
Blue Mussel + Coquina Clam + Crustacean + Miscellaneous Prey[g] + Distance to Roost	17	4,106.70	3.05	0.05	-2,036.12	0.07
Blue Mussel + Coquina Clam + Crustacean	13	4,109.37	5.71	0.01	-2,041.54	0.07
Blue Mussel + Coquina Clam	11	4,109.50	5.84	0.01	-2,043.65	0.07
Blue Mussel + Coquina Clam + Miscellaneous Prey[g]	13	4,110.38	6.72	0.01	-2,042.05	0.07
Blue Mussel + Coquina Clam + Crustacean + Miscellaneous Prey[g]	15	4,110.39	6.73	0.01	-2,040.01	0.07
Blue Mussel + Coquina Clam + Crustacean + Tide	19	4,111.02	7.36	0.01	-2,036.22	0.07

Blue Mussel + Coquina Clam + Crustacean + Miscellaneous Prey[g] + Tide	21	4,112.11	8.45	0.00	-2,034.70	0.07
Blue Mussel + Coquina Clam + Tide	17	4,113.15	9.49	0.00	-2,039.34	0.07
Coquina Clam + Crustacean + TDF Count[i]	13	4,126.01	22.35	0.00	-2,049.86	0.07
Coquina Clam + Crustacean + Miscellaneous Prey[g] + TDF Count[i]	15	4,126.70	23.04	0.00	-2,048.17	0.07
Coquina Clam + Crustacean + Distance to Roost	13	4,127.27	23.61	0.00	-2,050.50	0.07
Coquina Clam + Crustacean + Miscellaneous Prey[g] + Distance to Roost	15	4,127.79	24.13	0.00	-2,048.71	0.07
Coquina Clam + TDF Count[i]	11	4,130.15	26.49	0.00	-2,053.97	0.06
Coquina Clam + Distance to Roost	11	4,130.54	26.88	0.00	-2,054.17	0.06
Coquina Clam + Crustacean	11	4,130.58	26.92	0.00	-2,054.19	0.06
Coquina Clam + Miscellaneous Prey[g] + TDF Count[i]	13	4,130.73	27.07	0.00	-2,052.22	0.06
Coquina Clam + Miscellaneous Prey[g] + Distance to Roost	13	4,130.76	27.10	0.00	-2,052.24	0.06
Coquina Clam + Crustacean + Miscellaneous Prey[g]	13	4,131.62	27.96	0.00	-2,052.67	0.06
Coquina Clam + Crustacean + Tide	17	4,132.16	28.50	0.00	-2,048.84	0.07

Coquina Clam + Crustacean + Miscellaneous Prey[g] + Tide	19	4,133.34	29.68	0.00	-2,047.38	0.07
Coquina Clam	9	4,133.73	30.07	0.00	-2,057.80	0.06
Coquina Clam + Miscellaneous Prey[g]	11	4,134.52	30.86	0.00	-2,056.16	0.06
Coquina Clam + Tide	15	4,137.13	33.47	0.00	-2,053.38	0.06
Coquina Clam + Miscellaneous Prey[g] + Tide	17	4,138.06	34.40	0.00	-2,051.79	0.06
All Prey[h] + Distance to Roost + Tide + TDF Count[i]	19	4,161.36	57.70	0.00	-2,061.39	0.06
All Prey[h] + Tide + Distance to Roost	17	4,162.13	58.47	0.00	-2,063.83	0.06
All Prey[h] + Tide + TDF Count[i]	17	4,164.24	60.58	0.00	-2,064.88	0.06
All Prey[h] + Distance to Roost	11	4,164.59	60.93	0.00	-2,071.19	0.06
All Prey[h] + Tide	15	4,164.73	61.08	0.00	-2,067.18	0.06
All Prey[h] + TDF Count[i]	11	4,167.49	63.83	0.00	-2,072.64	0.06
All Prey[h]	9	4,168.76	65.10	0.00	-2,075.31	0.05
Blue Mussel + Distance to Roost + TDF Count[i]	13	4,177.36	73.70	0.00	-2,075.54	0.05
Blue Mussel + Crustacean + TDF Count[i]	13	4,178.73	75.07	0.00	-2,076.23	0.05
Blue Mussel + Miscellaneous Prey[g] + Distance to Roost	13	4,179.01	75.35	0.00	-2,076.36	0.05
Blue Mussel + Miscellaneous Prey[g] + TDF Count[i]	13	4,179.60	75.94	0.00	-2,076.66	0.05

Blue Mussel + Crustacean + Distance to Roost	13	4,179.64	75.99	0.00	-2,076.68	0.05
Blue Mussel + Distance to Roost	11	4,180.02	76.37	0.00	-2,078.91	0.05
Blue Mussel + TDF Count[i]	11	4,180.42	76.76	0.00	-2,079.11	0.05
Blue Mussel + Crustacean + Tide	17	4,180.73	77.08	0.00	-2,073.13	0.05
Blue Mussel + Crustacean + Miscellaneous Prey[g]	13	4,181.94	78.28	0.00	-2,077.83	0.05
Blue Mussel + Distance to Roost + Tide	17	4,182.21	78.56	0.00	-2,073.87	0.05
Blue Mussel + Crustacean	11	4,182.33	78.67	0.00	-2,080.06	0.05
Blue Mussel + Miscellaneous Prey[g]	11	4,182.58	78.92	0.00	-2,080.19	0.05
Blue Mussel	9	4,182.90	79.24	0.00	-2,082.38	0.05
Blue Mussel + Tide	15	4,183.75	80.10	0.00	-2,076.69	0.05
Blue Mussel + Miscellaneous Prey[g] + Tide	17	4,183.77	80.12	0.00	-2,074.65	0.05
Crustacean + Miscellaneous Prey[g] + Distance to Roost + TDF Count[i]	15	4,192.57	88.91	0.00	-2,081.10	0.05
Crustacean + Miscellaneous Prey[g] + Distance to Roost	13	4,195.90	92.24	0.00	-2,084.81	0.05
Crustacean + Miscellaneous Prey[g] + Distance to Roost + Tide	19	4,196.29	92.64	0.00	-2,078.86	0.05

Crustacean + Distance to Roost	11	4,196.94	93.28	0.00	-2,087.37	0.05
Crustacean + TDF Count[i]	11	4,197.72	94.06	0.00	-2,087.76	0.05
Crustacean + Tide	15	4,198.78	95.12	0.00	-2,084.21	0.05
Miscellaneous Prey[g] + Distance to Roost	11	4,198.82	95.16	0.00	-2,088.31	0.05
Crustacean + Miscellaneous Prey[g]	11	4,199.74	96.09	0.00	-2,088.77	0.05
Distance to Roost	9	4,200.01	96.35	0.00	-2,090.94	0.05
Crustacean	9	4,200.19	96.54	0.00	-2,091.03	0.05
Miscellaneous Prey[g] + TDF Count[i]	11	4,200.66	97.00	0.00	-2,089.23	0.05
TDF Count[i]	9	4,201.45	97.79	0.00	-2,091.66	0.05
Miscellaneous Prey[g]	9	4,202.38	98.72	0.00	-2,092.12	0.05
Miscellaneous Prey[g] + Tide	15	4,203.45	99.80	0.00	-2,086.54	0.05
Tide	13	4,203.62	99.97	0.00	-2,088.67	0.05
Null	2	4,390.95	287.29	0.00	-2,193.47	0.00

[*] Three models did not converge during early migration period and were eliminated from the model set: (1) Blue Mussel + Coquina Clam + Crustacean + Miscellaneous Prey + Distance to Roost + TDF Count + Tide; (2) Crustacean + Miscellaneous Prey + Distance to Roost + Tide; (3) Blue Mussel + Coquina Clam + Crustacean + Miscellaneous Prey + Distance to Roost.

[a] K = Number of parameters.

[b] AIC_c = Akaike's Information Criterion corrected for sample size.

[c] ΔAIC_c = Difference between a model's AIC and that of the best fitting model.

[d] w_i^d = Akaike model weight.

[e] LL = Log-Likelihood.

[f] GOF = Goodness of fit = [log-likelihood(null model) – log-likelihood(model)]/log-likelihood(null model).

[g] Miscellaneous Prey = Sum of horseshoe crab eggs (*Limulus polyphemus*), angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae, snails, worms).

[h] All Prey = Sum of coquina clams + blue mussels + crustaceans + miscellaneous prey.

[i] TDF Count = Tierra del Fuego Count = Counts of red knots using Tierra del Fuego wintering grounds by year (i.e., as an index for the total number of red knots in the flyway; Morrison and Ross 1989, Morrison et al. 2004, Morrison 2014, Dey et al. 2015, USFWS 2015, Western Hemisphere Shorebird Reserve Network 2017, 2018).

Appendix 5. Full model sets for generalized linear mixed-effects regression models predicting crustacean, coquina clam, and miscellaneous prey abundances (organisms/275 cm^3) captured in 10 cm diameter x 3.5 cm deep cores on peat banks early in red knot migration (May 14 – 20, 2008 – 2018; *n* = 457; 'early') and on sand and peat banks at the approximate peak of red knot migration (May 21 – 27, 2007 – 2018; *n* = 1,322; 'peak'), Virginia's barrier islands. All models contained "Island" and "Year" as random effects.

Period	**Prey**	**Model**	**K** [a]	**AIC_c** [b]	**ΔAIC_c** [c]	**w_i** [d]	**LL**[e]	**MR2**[f]	**CR2**[g]
Early	Crustacean	Tide + Water Temperature	6	110,491.00	0.00	0.61	-55,239.42	0.02	0.57
		Tide + Water Temperature + Island Type	7	110,491.90	0.88	0.39	-55,238.83	0.06	0.56
		Tide	5	113,026.10	2,535.07	0.00	-56,507.99	0.02	0.58
		Tide + Island Type	6	113,027.30	2,536.24	0.00	-56,507.55	0.05	0.57
		Water Temperature	4	119,599.50	9,108.47	0.00	-59,795.71	0.00	0.57
		Water Temperature + Island Type	5	119,600.70	9,109.62	0.00	-59,795.26	0.03	0.56
		Null	3	121,090.60	10,599.54	0.00	-60,542.26	0.00	0.57
		Island Type	4	121,091.90	10,600.87	0.00	-60,541.91	0.03	0.57

Coquina Clam	Tide + Water Temperature	6	1,952.17	0.00	0.71	-969.99	0.01	0.00
	Tide + Water Temperature + Island Type	7	1,954.23	2.06	0.25	-969.99	0.01	0.01
	Tide	5	1,958.56	6.39	0.03	-974.21	0.00	0.00
	Tide + Island Type	6	1,960.60	8.43	0.01	-974.21	0.00	0.01
	Water Temperature	4	1,991.88	39.71	0.00	-991.90	0.01	0.00
	Water Temperature + Island Type	5	1,993.91	41.74	0.00	-991.89	0.01	0.01
	Null	3	1,997.67	45.50	0.00	-995.81	0.00	0.01
	Island Type	4	1,999.70	47.53	0.00	-995.81	0.00	0.00
Misc. Prey[h]	Tide + Water Temperature	6	8,721.57	0.00	0.71	-4,354.69	0.04	0.69
	Tide + Water Temperature + Island Type	7	8,723.40	1.84	0.29	-4,354.58	0.07	0.66
	Water Temperature	4	8,797.22	75.65	0.00	-4,394.57	0.02	0.67
	Water Temperature + Island Type	5	8,799.07	77.51	0.00	-4,394.47	0.04	0.68

		Tide	5	8,816.72	95.15	0.00	-4,403.29	0.01	0.69
		Tide + Island Type	6	8,818.59	97.03	0.00	-4,403.20	0.03	0.66
		Null	3	8,868.55	146.99	0.00	-4,431.25	0.00	0.68
		Island Type	4	8,870.43	148.87	0.00	-4,431.17	0.02	0.67
Peak	Crustacean	Tide + Substrate + Water Temperature	8	61,060.87	0.00	0.56	-30,522.38	0.02	0.22
		Tide + Substrate + Water Temperature + Island Type	9	61,061.39	0.51	0.44	-30,521.62	0.05	0.23
		Tide + Substrate	7	61,173.29	112.41	0.00	-30,579.60	0.01	0.24
		Tide + Substrate + Island Type	8	61,173.48	112.61	0.00	-30,578.69	0.05	0.24
		Tide + Water Temperature	7	62,545.87	1,484.99	0.00	-31,265.89	0.01	0.23
		Tide + Water Temperature + Island Type	8	62,547.20	1,486.32	0.00	-31,265.55	0.05	0.23
		Tide	6	62,705.37	1,644.49	0.00	-31,346.65	0.01	0.23
		Tide + Island Type	7	62,706.46	1,645.58	0.00	-31,346.19	0.05	0.23

	Substrate + Water Temperature	5	62,790.91	1,730.03	0.00	-31,390.43	0.00	0.22
	Substrate + Water Temperature + Island Type	6	62,791.54	1,730.67	0.00	-31,389.74	0.04	0.22
	Substrate	4	62,852.65	1,791.78	0.00	-31,422.31	0.00	0.22
	Substrate + Island Type	5	62,853.09	1792.21	0.00	-31,421.52	0.04	0.22
	Water Temperature	4	64,072.87	3,011.99	0.00	-32,032.42	0.00	0.23
	Water Temperature + Island Type	5	64,074.20	3,013.32	0.00	-32,032.08	0.04	0.22
	Null	3	64,171.97	3,111.10	0.00	-32,082.98	0.00	0.22
	Island Type	4	64,173.14	3,112.26	0.00	-32,082.55	0.03	0.22
Coquina Clam	Tide + Substrate + Water Temperature	8	35,893.06	0.00	0.69	-17,938.47	0.04	0.26
	Tide + Substrate + Water Temperature + Island Type	9	35,894.64	1.58	0.31	-17,938.25	0.05	0.26
	Tide + Water Temperature	7	36,087.31	194.25	0.00	-18,036.61	0.02	0.27

Tide + Water Temperature + Island Type	8	36,088.72	195.66	0.00	-18,036.31	0.03	0.27
Tide + Substrate	7	36,271.95	378.89	0.00	-18,128.93	0.03	0.25
Tide + Substrate + Island Type	8	36,273.66	380.60	0.00	-18,128.78	0.03	0.25
Tide	6	36,496.63	603.57	0.00	-18,242.28	0.01	0.25
Tide + Island Type	7	36,498.21	605.15	0.00	-18,242.06	0.02	0.25
Substrate + Water Temperature	5	36,750.74	857.69	0.00	-18,370.35	0.03	0.25
Substrate + Water Temperature + Island Type	6	36,752.23	859.17	0.00	-18,370.08	0.03	0.25
Water Temperature	4	36,911.93	1,018.88	0.00	-18,451.95	0.01	0.25
Water Temperature + Island Type	5	36,913.27	1,020.21	0.00	-18,451.61	0.02	0.25
Substrate	4	37,159.65	1,266.59	0.00	-18,575.81	0.02	0.23
Substrate + Island Type	5	37,161.27	1,268.22	0.00	-18,575.61	0.02	0.23

	Null	3	37,348.14	1,455.08	0.00	-18,671.06	0.00	0.23
	Island Type	4	37,349.65	1,456.59	0.00	-18,670.81	0.01	0.23
Blue Mussel	Tide + Substrate + Water Temperature + Island Type	9	22,816.70	0.00	0.51	-11,399.28	0.06	0.07
	Tide + Substrate + Water Temperature	8	22,816.77	0.07	0.49	-11,400.33	.	.
	Tide + Substrate	7	22,898.73	82.03	0.00	-11,442.32	.	.
	Tide + Substrate + Island Type	8	22,899.02	82.32	0.00	-11,441.46	0.03	0.03
	Tide + Water Temperature + Island Type	8	26,026.80	3,210.10	0.00	-13,005.35	0.05	0.07
	Tide + Water Temperature	7	26,027.23	3,210.53	0.00	-13,006.57	0.00	0.00
	Tide	6	26,184.13	3,367.43	0.00	-13,086.04	0.00	0.00
	Tide + Island Type	7	26,184.23	3,367.53	0.00	-13,085.07	0.00	0.00
	Substrate + Water Temperature + Island Type	6	27,006.40	4,189.70	0.00	-13,497.17	0.04	.

Substrate + Water Temperature	5	27,006.97	4,190.27	0.00	-13,498.46	0.00	0.00
Substrate + Island Type	5	27,127.18	4,310.48	0.00	-13,558.57	.	.
Substrate	4	27,127.26	4,310.56	0.00	-13,559.62	0.03	0.03
Water Temperature + Island Type	5	33,593.99	10,777.29	0.00	-16,791.97	.	.
Water Temperature	4	33,595.12	10,778.42	0.00	-16,793.54	0.00	0.00
Island Type	4	33,774.91	10,958.21	0.00	-16,883.44	0.00	0.00
Null	2	46,762.83	23,946.13	0.00	-23,379.41	0.00	0.00

---

[a] K = Number of parameters.

[b] AIC_c = Akaike's Information Criterion corrected for sample size.

[c] ΔAIC_c = Difference between a model's AIC and that of the best fitting model.

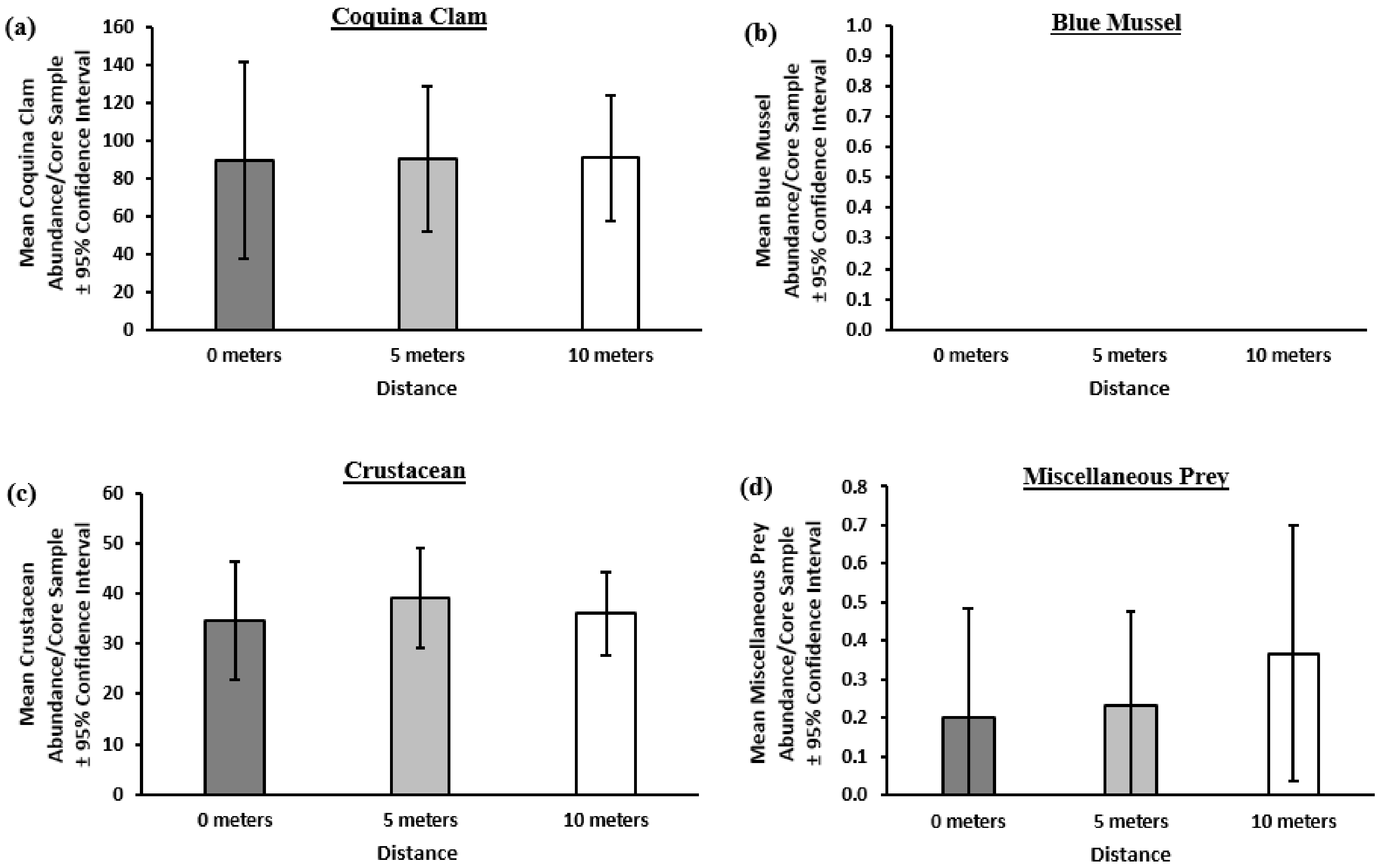
[d] w_i^{d} = Akaike model weight.

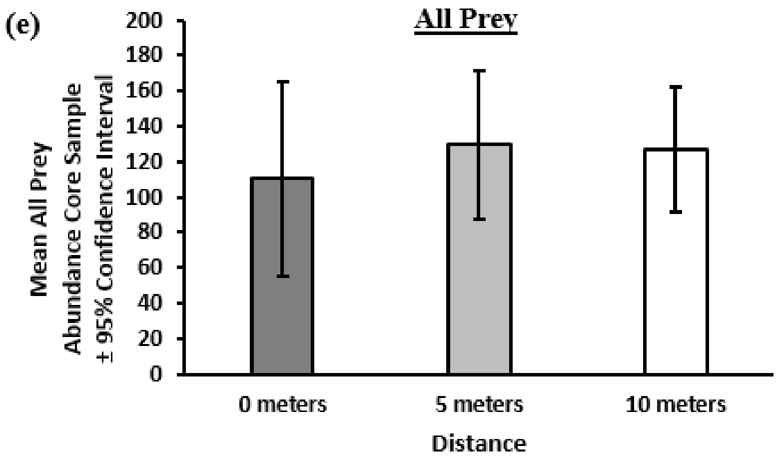
[e] LL = Log-Likelihood.

[f] MR2 = Marginal r-squared = Considers only the variance of the fixed effects (Nagelkerke 1991).

[g] CR2 = Conditional r-squared = Considers the variance of both fixed and random effects (Nagelkerke 1991).

[h] Misc. Prey = Miscellaneous Prey = Sum of horseshoe crab eggs (*Limulus polyphemus*), angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae, snails, worms).


(a)
Coquina Clam
Mean Coquina Clam Abundance/Core Sample ± 95% Confidence Interval
0
20
40
60
80
100
120
140
160
0 meters
5 meters
10 meters
Distance
(b)
Blue Mussel
Mean Blue Mussel Abundance/Core Sample ± 95% Confidence Interval
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0 meters
5 meters
10 meters
Distance
(c)
Crustacean
Mean Crustacean Abundance/Core Sample ± 95% Confidence Interval
0
10
20
30
40
50
60
0 meters
5 meters
10 meters
Distance
(d)
Miscellaneous Prey
Mean Miscellaneous Prey Abundance/Core Sample ± 95% Confidence Interval
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0 meters
5 meters
10 meters
Distance



Appendix 6. Mean (a) coquina clam, (b) blue mussel, (c) crustacean, (d) miscellaneous prey, and (e) all prey abundances (organisms/275 cm^3) captured in 10 cm diameter x 3.5 cm deep cores on sand during sand tidal spatial sampling, May 20, 2019, Hog Island, Virginia's barrier islands. Distance represents the distance from the center sampling point (0 meters). Kruskal-Wallis tests found no differences ($p < 0.05$) in prey abundances by distance.

ABUNDANCE AND ORIGIN OF BLUE MUSSELS (*MYTILUS EDULIS*) ON VIRGINIA'S PEAT BANKS WITH IMPLICATIONS FOR THE CONSERVATION OF THE RED KNOT (*CALIDRIS CANUTUS RUFA*)

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ABSTRACT

As climate change causes ocean temperatures to rise, some intertidal organisms, like blue mussels (*Mytilus edulis*), are retreating northward. Species that consume blue mussels may be negatively affected by this range contraction. For example, the red knot (*Calidris canutus rufa*), a federally threatened shorebird that specializes in consuming bivalves, including blue mussels, stops on Virginia's barrier islands each spring to regain the fat required to continue flights to Arctic breeding grounds. We investigated the variation in blue mussel abundance from 2010 – 2018 on Virginia's barrier islands by collecting core samples containing prey. We compared the oxygen isotopic composition of the calcite ($\delta^{18}O_c$) measured in blue mussel shells (*n* = 74) to

$\delta^{18}O_c$ calculated to be in equilibrium with regional ocean water to predict their origins. During peak red knot migration (May 14 – 27), we estimated that between 34 – 538 blue mussels were available/core sample, with blue mussel abundance decreasing over the duration of the study. Stable isotope analyses indicated that umbo (first precipitated shell) $\delta^{18}O_c$ ($\bar{x}$ = -0.23‰, SE = 0.12) were more ^{18}O enriched than shell edge (most recently precipitated shell) $\delta^{18}O_c$ ($\bar{x}$ = -0.53‰, SE = 0.10). Blue mussel umbo $\delta^{18}O_c$ were not different than the range of $\delta^{18}O_c$ calculated in equilibrium with regional ocean water off the Virginia and Delaware coasts from 2010 – 2018. Umbo $\delta^{18}O_c$ were more ^{18}O enriched than the expected estuarine signature at the mouth of the Delaware Bay, precluding an estuarine origin, and more ^{16}O enriched than the expected $\delta^{18}O_c$ off New Hampshire's coast, likely precluding an origin that far north. We concluded that juvenile blue mussels found in Virginia most likely originated somewhere in the regional ocean between the Delaware Bay and Cape Henry, VA, buoys; however, they could originate as far north as New Jersey in some years. We also concluded that $\delta^{18}O_c$ variations in Virginia's blue mussels likely do not result from changes in water temperature but more likely from changes in atmospheric precipitation and the subsequent flux of less saline waters out of estuaries into coastal waters. Continued regional sea surface temperature and/or precipitation variations may continue to affect the availability of blue mussels to foraging red knots in Virginia.

KEY WORDS *Calidris canutus rufa*, Delaware Bay, *Mytilus edulis*, stable isotopes, Virginia's barrier islands

INTRODUCTION

Aquatic organisms that live in the intertidal to shallow subtidal zones may be affected by rising ocean temperatures and changing salinities. For example, the boreo-temperate, cold-water obligate marine blue mussel (*Mytilus edulis*), which thrives in saline waters with temperatures between 5 and 20°C and dies with repeated exposure to temperatures above 30°C and in freshwater (Ansell 1983, Jones et al. 2009), is retreating northward. Historically, adult blue mussels lived in large intertidal and subtidal colonies from the Arctic through North Carolina (Dall 1889, Wells and Gray 1960). Due to increasing sea surface temperatures, Virginia's barrier islands currently are the southern edge of the blue mussels' intertidal juvenile range, while Delaware is the southern edge of their known adult, intertidal breeding range. Breeding adult blue mussel intertidal and subtidal populations extend northward into Canada (Bayne 1976, Jones et al. 2010, Wethey et al. 2011). Blue mussels spawn in the spring to early summer and rely on ocean currents to passively transport their larvae up to hundreds of kilometers (Bayne 1976, Strathmann 1985, Levin 1990, Gilg and Hilbish 2003, Lercari and Defeo 2006). Thus, juvenile blue mussels may not originate in the place they ultimately settle between 20 – 41 days after spawning (Chipperfield 1953, Le Gall 1970, Jørgensen 1981, Sprung 1984, De Vooys 1999).

Climate change causes distribution and range changes to occur among species at varying rates, potentially causing temporal and spatial mismatches between predators and their prey (Edwards and Richardson 2004, Post and Forchhammer 2008, Both et al. 2006, Parmesan 2006, Durant et al. 2007). The effects of climate change on predator-prey interactions is especially evident in migratory shorebirds and their prey. Spring migration occurs within a short period each year, as shorebirds have short windows of time to breed in the Arctic and thus cannot be extensively delayed at stopover grounds (Duijins et al. 2019). Migratory refueling may be further challenged as coastal and marine prey abundances may become more variable with changing ocean temperature and salinity (Schneider and Harrington 1981, Piersma et al. 1994, Durant et al. 2007). For example, every year during spring migration, thousands of shorebirds, including the federally threatened Western Atlantic red knot (*Calidris canutus rufa*; 'red knot') use Virginia's barrier islands as a migratory stopover to replenish the fat needed to continue migrating to their breeding grounds (Federal Register 2014, Lyons et al. 2018, USFWS 2019).

Much attention was historically given to red knots and their reliance on horseshoe crab eggs in their Delaware Bay stopover habitat (*Limulus polyphemus*; Karpanty et al. 2006, 2011, Smith et al. 2008, Cohen et al. 2009, Niles et al. 2009, Fraser et al. 2010, 2012, Watts 2013, Smith and Robinson 2015, Watts and Truitt 2015, Burger et al. 2018) because that site consistently supports between 50 – 70% of the annual red knot stopover population (Cohen et al. 2010a, 2011, Federal Register 2014, Watts and Truitt 2015). However, red knots select bivalves throughout much of their annual cycle (Piersma 1991, Zwarts and Blomert 1992, Dekinga and Piersma 1993, Tulp and Schekkerman 2008), as they have adaptively large gizzards and colons that enable them to crush and pass bivalve shells (Dekinga and Piersma 1993, Piersma et al. 1993, 1999, Battley and Piersma 2004, van Gils et al. 2003, Quaintenne et al. 2009, Onrust et al. 2013).

This bivalve specialization includes red knots using Virginia's barrier islands, where they forage upon numerous invertebrate prey, including bivalves like blue mussels and coquina clams (*Donax variabilis*; Cohen et al. 2009, 2010a, b, 2011, Watts and Truitt 2015, Heller et al. In Prep$_a$). Blue mussels and coquina clams have been important food for red knots using Virginia's barrier island stopover for at least 80 years (Sperry 1940). Using DNA metabarcoding, Heller et al. (In Prep$_a$) found that red knots continued to select blue mussels and coquina clams during their spring migratory stopover in Virginia between 2007 – 2018. The red knot's reliance on bivalves in Virginia could make them susceptible to changes in the abundance and ranges of these prey. Such changes are occurring in one of the bivalves upon which red knots depend, the blue mussel.

While juvenile blue mussels are found on Virginia's barrier island intertidal peat banks (partially decayed marsh found on the ocean-side of Virginia's barrier islands; Bachmann et al. 2002, Cohen et al. 2010b), it is unknown where these blue mussels originate. Virginia's intertidal waters currently are too warm to support breeding blue mussels throughout the summer, and juvenile blue mussels die by mid-late summer. Thus, the continued presence of blue mussels at this stopover site for red knots depends on reproductive populations located elsewhere. Two hypotheses regarding the origin of juvenile mussels on Virginia's peat banks exist: 1) juvenile blue mussels found in Virginia make their way from more estuarine (less saline) northern waters (Yund et al. 2015), such as near the mouth of the Delaware Bay or within the Bay itself; or 2) juvenile blue mussels originate in more saline and/or colder ocean water either off Virginia's coast or farther north. Understanding how blue mussel abundances have changed over time and where Virginia's juvenile blue mussels originate may help us predict whether blue mussels will continue to be a reliable food source for foraging red knots and other fish and wildlife in Virginia.

We conducted stable isotope analyses to assist in determining points of origin of juvenile blue mussels that live on Virginia's peat banks (Hobson 1999, Chauvaud et al. 2005, Yan et al. 2009). The oxygen isotope composition ($\delta^{18}O_c$) of the shells of many bivalve species, including blue mussels, are in isotopic equilibrium with the water in which the shells are formed (Wanamaker et al. 2007, Versteegh et al. 2012). This isotopic equilibrium is primarily controlled by water temperature and the isotopic composition of water, which relates to salinity and temperature ($\delta^{18}O_w$, see *Calcite-Water Equilibrium Expression* below). Climate change affects not only the range and distribution of species (Edwards and Richardson 2004, Post and Forchhammer 2008, Both et al. 2006, Parmesan 2006, Durant et al. 2007), but also the properties of coastal water, and thus $\delta^{18}O_w$ due to increased water temperature (Rayner et al. 2003, Figueira and Booth 2010, Gobler et al. 2017, Durack et al. 2018) and decreased salinity caused by increased atmospheric precipitation in some regions (Karl 1998, Milly et al. 2002, Trenberth 2011). More specifically, increased atmospheric precipitation increases the discharge of water from estuaries that outflow into the ocean, forming plumes of less saline water moving seaward (Arnell 1999, Kiely 1999, Struyf et al. 2004, Wong 1998, 1999, Dogramaci et al. 2012, Price et al. 2012). Plumes of water from estuaries can extend up to 1,000 km from where the estuary meets the ocean, resulting in areas of ocean water with more ^{16}O enriched $\delta^{18}O_w$ than water outside of these plumes (Münchow and Garvine 1993, Khim and Krantz 1996, Muller-Karger et al. 1995, Wong 1998, 1999, Isobe and Matsuno 2008). Changes in $\delta^{18}O_w$ resulting from changing sea surface temperature and/or salinity are ultimately expressed in the $\delta^{18}O_c$ of marine bivalve shells.

Blue mussels are good study organisms for recording sea surface temperature and salinity conditions during different stages of their growth because they begin to precipitate their shells within 24 hours of being spawned and continue growing their shells throughout their lives (Richardson 1989, Bayne 1976, Wanamaker et al. 2007). By comparing the $\delta^{18}O_c$ in shell umbos to that of shell edges, we can infer the conditions within which each portion of the shell was precipitated (Dettman et al. 1999, Chauvaud et al. 2005, Wanamaker et al. 2007, McConnaughey and Gillikin 2008). More ^{16}O enriched (more negative values) $\delta^{18}O$ values signify a more estuarine (less saline) and/or warmer environment, while ^{18}O enriched (more positive values) values signify a more saline and/or colder environment. We can also calculate what we would expect $\delta^{18}O_c$ of shells formed in different areas to be using known $\delta^{18}O_w$ and water temperatures and then compare these expected $\delta^{18}O_c$ to $\delta^{18}O_c$ measured in blue mussel shells (see *Calcite-Water Equilibrium Expression* below).

Differences between blue mussel edge and umbo $\delta^{18}O_c$ could point to a different environment for the origin of blue mussel larvae versus where they were collected (here, the intertidal peat banks of Virginia's barrier islands). If blue mussels' umbos contain more ^{16}O enriched compositions than their shells' edges, blue mussels originated in less saline and/or warmer water than where they settled and were collected. However, if blue mussels' umbos contain more ^{18}O enriched compositions than their shells' edges, they originated in more saline and/or colder water than those found in Virginia's intertidal zone (Khim and Krantz 1996, Schmidt et al. 1999).

Understanding if blue mussel abundance has changed over time and where the juvenile blue mussels found in Virginia may have originated may help us better predict if blue mussels will continue to be available to foraging shorebirds, like red knots, using Virginia's barrier island stopover in the future. Therefore, our objectives were to determine: 1) if blue mussel abundances on Virginia's peat banks varied across years (2010 – 2018); 2) if blue mussel umbos and edges exhibited any $\delta^{18}O_c$ trends over time; 3) if the $\delta^{18}O_c$ of blue mussels' umbos vs. edges differed from each other; and 4) if we can use the oxygen isotopic composition of the calcite of blue mussels' umbos to better infer their origin.

STUDY AREA

We conducted our study on three barrier islands that red knots used as spring migratory stopover habitat on Virginia's barrier islands off the coast of the Eastern Shore of Virginia (Cedar Island, Metompkin Island, and Myrtle Island; 37°23.7'N, 75°42.5'W; Cohen et al. 2010a, b, 2011, Watts and Truitt 2015; Fig. 1). These islands are part of the Virginia Coast Reserve Long Term Ecological Research Site, which consists of 11 barrier islands used by red knots, from Assawoman Island in the north to Fishermans Island in the south. Cedar Island, Metompkin Island, and Myrtle Island were sampled due to the relative abundance of peat banks they contained. These peat banks consistently supported juvenile blue mussel colonies during the course of the study (Cohen et al. 2009, 2010a, b, 2011, Heller et al. In Prep$_{a,b}$). Peat banks form over time when ocean-side beaches erode and ultimately transgress over bay-side tidal marshes (Truitt et al. 2001, Bachmann et al. 2002, Cohen et al. 2010, Watts and Truitt 2015, Watts and Truitt 2015, Kreuzburg et al. 2018). The vegetation under the sand during the island's transgression partially decomposes, creating peat, which is exposed when the island moves toward the mainland. The islands are predominantly undeveloped and are protected and monitored by The Nature Conservancy, the United States Fish and Wildlife Service, the Virginia Department of Game and Inland Fisheries, and the Commonwealth of Virginia's Department of Conservation and Recreation's Natural Heritage Program (Smith et al. 2008, Cohen et al. 2011, Watts and Truitt 2015). They thus provide relatively undisturbed habitat for both shorebirds and their prey, like blue mussels.

METHODS

Blue Mussel Collection

We collected live blue mussels on Atlantic Ocean intertidal peat habitat on Cedar Island, Metompkin Island, and Myrtle Island during two weeks corresponding to the peak red knot migratory stopover (May 14 – 27, 2010 – 2018; Watts and Truitt 2000, Cohen et al. 2009, Watts 2013; Fig. 1). We randomly generated sampling points on each island using the Hawth's Tools extension (Beyer 2004), the Geospatial Modelling Environment extension (Beyer 2009) in ArcGIS 10.1 and then 10.5 (ESRI 2012, 2017; Heller et al. In Prep$_b$), and orthophotography imagery from The United States Department of Agriculture (USDA) Farm Service Agency's National Agriculture Imagery Program (NAIP; USDA Farm Service Agency 2018). To determine the location of peat banks on which to generate random points, we visually examined orthophotography imagery before randomly generating points using methods described above (Heller et al. In Prep$_b$). Later, we measured the total length of available peat bank habitat to the closest 0.1 kilometer parallel to the shoreline in ArcGIS 10.5 using available imagery from 2013 and 2017 (ESRI 2017).

At each randomly generated point, we collected a core sample from peat banks at the water-line using a section of PVC pipe (10 cm diameter x 3.5 cm deep; core volume = 275 cm^3) cut to the approximate length of a red knot's bill (Tomkovich 1992). The PVC was pushed into the peat until the top was even with the peat's surface, then a trowel was slipped under the PVC to prevent the sample from falling out as the PVC was retrieved. Samples were placed in a zipper-lock plastic bag, returned to the lab, and frozen until processed. In the lab, we counted the number of blue mussels in each sample and separated blue mussels from other prey using a series of sieves (smallest mesh size #40; 0.32 mm holes) and a dissecting microscope. Later, we randomly selected blue mussels from these samples to use for stable isotope analyses.

Blue Mussel Preparation

We manually separated the valves of each blue mussel and removed the soft tissues within each shell using sterilized stainless steel forceps. Then we bleached the blue mussel shells in a standard bleach solution (7.86% available chlorine and 8.25% sodium hypochlorite) for 15 to 20 minutes, depending on shell size, to eliminate organic matter in the shells' periostracum (thin organic coating that covers the outermost layer of mollusk shells; Fig. 2). We rinsed bleached shells three times with deionized water (Yan et al. 2009) for 15 minutes each time, for a total rinsing period of 45 minutes. After rinsing, the shells air-dried for up to three hours and were measured length-wise to the nearest 0.25 millimeter (Fig. 2). We used one of the two shells from each individual for stable isotope analyses, while we retained the other for replicate analyses if needed. Using sterilized stainless steel dissecting probes, we separated approximately 0.5 mm of the umbo and 0.5 mm of the edge from each shell (Fig. 2). Then we crushed each umbo and edge separately using a sterilized mortar and pestle, creating a powder, of which we used 350 – 450 micrograms for stable isotope analyses.

Stable Isotope Analyses - Blue Mussel $\delta^{18}O_c$ - Umbo vs. Edge

We measured shell $\delta^{18}O_c$ using sample powders from each blue mussel's umbo and edge and placed the powder in sealed, helium-purged glass vials. These powders were acidified with 100% ortho-phosphoric acid (H_3PO_4) and were heated at 60°C for a minimum of 2 hours to ensure the dissolution of the calcite. The carbon dioxide (CO_2) evolved in the headspace of the vials was analyzed on a MultiFlowGeo headspace sampler connected to an Isoprime 100 continuous-flow, gas-source isotope ratio mass spectrometer (CF-GS- IRMS) in the Department of Geosciences at Virginia Tech. Three international isotopic standards (IAEA CO-1: $\delta^{18}O_c$ = -2.4‰; IAEA CO-9:

δ^{18}O-$BaCO_3$ = -15.6‰, and NBS-18: $\delta^{18}O_c$ = -23.2‰) were spaced among the samples to monitor precision and to normalize the instrumental data into delta values relative to the international Vienna Pee Dee Belemnite (VPDB) standard. The reproducibility of the standard and sample replicates was better than 0.2‰ for all analytical runs. The resulting $\delta^{18}O_c$ for each blue mussel umbo and edge sample were calculated using the following equation:

$$\delta^{18}O_c = \left(\frac{\left(\frac{18O}{16O}\right) sample - \left(\frac{18O}{16O}\right) standard}{\left(\frac{18O}{16O}\right) standard} - 1 \right) x\ 1000\ ‰$$

Blue mussel edge and umbo $\delta^{18}O_c$ were compared to each other to determine if there were differences in $\delta^{18}O_c$ between edges and umbos (2010 – 2018; Appendix 1; see *Significance Testing* below).

Data Analyses

Blue Mussel Expansion

To estimate how many blue mussels were found per m^2 of peat banks, we first took the sum of all blue mussels calculated in a given year and then divided that number by the number of core samples collected to determine the mean number of blue mussels/core sample each year (4-in diameter core = 81 cm^2/core). We then expanded each year's mean blue mussel abundance/core sample to m^2 by multiplying by 123 (1 m^2 = 123 cores; Table 1).

Determining the Origin of Blue Mussels Using Oxygen Isotopes – Calculated Calcite $\delta^{18}O_c$

To determine the environment where Virginia's blue mussels originated, we compared shell umbo $\delta^{18}O_c$ (measured from the blue mussels we collected in Virginia) with $\delta^{18}O_c$ calculated using 1) regional offshore sea surface water temperatures and 2) measured $\delta^{18}O_w$ within the potential blue mussel spawning range (i.e., ocean water between the Delaware Bay and Virginia; see *Calcite-Water Equilibrium Expressions* below; Khim and Krantz 1996, Schmidt et al. 1999). We used the upper $\delta^{18}O_w$ (-0.98‰) and lower $\delta^{18}O_w$ (-1.38‰) of the only reported ocean $\delta^{18}O_w$ dataset available within our study region (from Khim and Krantz 1996, archived in Schmidt et al. 1999). We used sea surface temperature data from buoy stations 44009 (Delaware Bay, DE; 38°27'24" N 74°42'6" W; sea temperature depth = 0.60 m; ~ 30.58 km offshore; NDBC 2020a) and 44099 (Cape Henry, Virginia; 36°54'50" N 75°43'13" W; sea temperature depth = 0.46 m; ~ 24.14 km offshore; NDBC 2020b; Fig. 1) to calculate the $\delta^{18}O_c$ that we would expect to be in equilibrium with this range of temperatures and ocean water $\delta^{18}O_w$ (Yan et al. 2009).

The buoys we used (Delaware Bay buoy and Cape Henry, VA, buoy; Fig. 1) represented the northern (Delaware Bay buoy) and southern (Cape Henry, VA, buoy) geographic limits to define the temperature limits for ocean water within the expected origin range (i.e., spawning locations) for blue mussels found in Virginia. For both the Delaware Bay and Cape Henry, VA, buoys (Fig. 1), we calculated the mean sea surface temperatures in April and May for each year (2010 – 2018). April and May represented the estimated spawning season of blue mussels in this region. We estimated this spawning season based on ecological data suggesting that blue mussels float passively near the ocean's surface in open water for 20 – 41 days after spawning (Chipperfield 1953, Le Gall 1970, Jørgensen 1981, Sprung 1984, De Vooys 1999). We used these mean sea surface temperatures and the measured $\delta^{18}O_w$ (-0.98‰ and -1.38‰) described above from Khim and Krantz (1996; archived in Schmidt et al. 1999) in the following calcite-water equilibrium expressions (Equations 1 and 2; Kim and O'Neil 1997; Fig. 3a, b) to calculate a range of $\delta^{18}O_c$ expected for this region.

Calcite-Water Equilibrium Expressions

$$(1)\ \alpha = e\hat{}[(18.03(10^3T^{-1}) - 32.42)/1000];$$

$$(2)\ \delta^{18}O_c = \alpha(1000 + \delta^{18}O_w) - 1000;$$

where, T = temperature (Kelvin);

α = fractionation factor between calcite and water = ratio of ^{18}O to ^{16}O;

$\delta^{18}O_w$ = oxygen isotope composition of the water;

$\delta^{18}O_c$ = oxygen isotope composition of the shell calcite

The resulting $\delta^{18}O_c$ (calculated $\delta^{18}O_c$) represented a comprehensive range of the $\delta^{18}O_c$ expected for shells that precipitated in equilibrium with ocean water between the Delaware Bay and Cape Henry, VA, buoys during the months and years of our study. Calculated $\delta^{18}O_c$ were relative to the Vienna Standard Mean Ocean Water (VSMOW) scale (Khim and Krantz 1996, Schmidt et al. 1999). This necessitated us to convert the calculated $\delta^{18}O_c$ from the VSMOW to the VPDB scale ($\delta^{18}O_w\text{VPDB} = \delta^{18}O_w\text{VSMOW} - \frac{30.92}{1.03092}$) to directly compare measured calcite $\delta^{18}O_c$ (from blue mussel umbos) to the calculated $\delta^{18}O_c$ range (see *Statistical Analyses* below).

If the $\delta^{18}O_c$ measured from blue mussel shells fell within the range of calculated $\delta^{18}O_c$, we concluded that Virginia's blue mussels could have originated in ocean waters between the Delaware Bay buoy and the Cape Henry, VA, buoy. If the $\delta^{18}O_c$ measured from blue mussel shells were more ^{18}O enriched than the upper range of calculated $\delta^{18}O_c$, we concluded that Virginia's blue mussels originated in water that was more saline and/or colder (likely farther north or farther from the coast) than those found between the Delaware Bay buoy and the Cape Henry, VA, buoy. Finally, if the $\delta^{18}O_c$ measured from blue mussel shells were more ^{16}O enriched than the lower calculated range $\delta^{18}O_c$, we concluded that Virginia's blue mussels originated in water that was less saline and/or warmer (i.e., in more estuarine or estuarine derived water near the coast) than those found in ocean water between the Delaware Bay buoy and the Cape Henry, VA, buoy.

To further pinpoint the origin of Virginia's blue mussels, we used the most ^{16}O enriched $\delta^{18}O_w$ (-3.05‰, taken at the mouth of the Delaware Bay estuary; depth = 2 m; ~ 5.87 km offshore) from Khim and Krantz (1996) and water temperature from the Delaware Bay buoy in the above expressions to calculate what the expected $\delta^{18}O_c$ would be for blue mussels spawned at the mouth of the Delaware Bay. By calculating the $\delta^{18}O_c$ expected for that area, we could conclude whether Virginia's blue mussels originated near the mouth of the Delaware Bay (i.e., in more estuarine water). While we initially planned to use the above expressions to obtain calculated $\delta^{18}O_c$ from waters north of Delaware to determine an upper origin boundary for Virginia's blue mussels, there was a lack of available $\delta^{18}O_w$ from this region (Schmidt et al. 1999). Therefore, to determine if we could ascertain a $\delta^{18}O_c$ for a northernmost origin for Virginia's blue mussels', we used the farthest north marine $\delta^{18}O_w$ (-0.98‰) from Khim and Krantz (1996) and water temperature data from buoys farther north in the above expressions. These more northern buoys were stations 44065 (New York Harbor Entrance, NY; 40°22'10" N 73°42'10" W; sea temperature depth = 1.5 m; ~22.85 km offshore; NDBC 2020c) and 44098 (Jeffrey's Ledge, NH; 42°47'53" N 70°10'5" W; sea temperature depth = 0.46 m; ~ 50.21 km offshore; Fig. 1; NDBC 2020d).

Statistical Analyses

Shapiro-Wilk normality tests indicated that data for blue mussel abundance/core sample ($W = 0.81$, $p < 0.001$), sea surface temperature ($W = 0.92$, $p < 0.001$), and atmospheric precipitation ($W = 0.46$, $p < 0.001$) were not normally-distributed. Therefore, we used a Spearman's correlation coefficient, which is less sensitive to non-normal data (Kowalski 1972, Chok 2010, Ahad et al. 2011), to determine the strength and association of the potential relationships 1) among blue mussel abundances/core samples over time (2010 – 2018), 2) between sea surface temperature in April and May over time (2010 – 2018), and 3) between atmospheric precipitation in April and May over time (2010 – 2018). We used atmospheric precipitation data from Norfolk, VA (U.S. Climate Data. 2020a), and Salisbury, MD (U.S. Climate Data. 2020b), as these sites represented two coastal sites within the watersheds of the two estuaries (Chesapeake Bay and Delaware Bay) that discharge estuarine runoff into the ocean within our study region.

We used Pearson's correlation to determine the strength and association in umbo $\delta^{18}O_c$ (comparing umbo $\delta^{18}O_c$ to umbo $\delta^{18}O_c$) and edge $\delta^{18}O_c$ (comparing edge $\delta^{18}O_c$ to edge $\delta^{18}O_c$) over time (2010 – 2018). Pearson's correlation were used, as both umbo and edge data were normally distributed (Shapiro-Wilk normality test: W = 0.98, p = 0.41 and W = 0.99, p = 0.78, respectively; Ahad et al. 2011). We used a paired t-test to determine if there were differences between measured blue mussel edge and umbo $\delta^{18}O_c$. We used Program R package "base" (R Core Team 2017; R Version 3.4.1, www.r–project.org, accessed 10 March – 17 April 2020) for all analyses.

RESULTS

We collected 277 peat core samples from Cedar Island (*n* = 121), Metompkin Island (*n* = 73), and Myrtle Island (*n* = 83), May 14 – 27, 2010 – 2018 (annual x̄ = 31, range = 23 – 40; Table 1). On average, 207 (SE = 22.41) blue mussels were counted per core sample (25,484 blue mussels/m^2). Blue mussel abundance/core sample was highest in 2010 (x̄ = 538/core sample, SE

= 85.85) and lowest in 2016 (x̄ = 34/core sample, SE = 6.96) and decreased over the study's duration (Spearman's correlation coefficient: ρ(rho) = -0.31, $p < 0.001$; Table 1; Fig. 4).

From these 277 peat core samples, we randomly selected 74 blue mussels (Cedar Island, *n* = 37; Metompkin Island, *n* = 19; and Myrtle Island; *n* = 18), with a mean of 8 blue mussels analyzed per year (range = 4 – 10; Table 1). Shell lengths ranged from 1.5 mm – 14 mm (x̄ shell length = 6 mm; Appendix 2). The total length of available peat bank habitat on the three islands we studied (Cedar Island, Metompkin Island, and Myrtle Island) was 7.22 km in 2013 and 7.40 km in 2017 (x̄ length = 7.31; SE = 0.09). When including peat banks on all of Virginia's barrier islands that contained peat in 2013 and 2017 (i.e., Assawoman Island, Cedar Island, Cobb Island, Metompkin Island, Myrtle Island, Parramore Island, Smith Island, and Wreck Island), the total length of available peat banks was 11.74 km in 2013 and 11.60 km in 2017 (x̄ length = 11.67; SE = 0.07).

Blue Mussel $\delta^{18}O_c$

Blue mussel umbo $\delta^{18}O_c$ became more ^{16}O enriched over the duration of the study (Pearson's correlation coefficient; t = -2.17, df = 72, p = 0.03). There was no linear trend in edge $\delta^{18}O_c$ over the duration of the study (Pearson's correlation coefficient; t = -1.62, df = 72, p = 0.11). Blue mussels' umbos had more ^{18}O enriched $\delta^{18}O_c$ than shell edges (paired t-test; t = -3.80, df = 73, $p < 0.001$; Fig. 5; Appendices 1, 2).

Blue Mussel Origin – Blue Mussel Umbo $\delta^{18}O_c$ - Calculated $\delta^{18}O_c$ Comparison

Blue mussel umbo $\delta^{18}O_c$ ± 95% confidence intervals fell within or overlapped the range of calculated $\delta^{18}O_c$ for the duration of this study (2010 – 2018), suggesting that Virginia's blue mussels likely originated in ocean waters between the Delaware Bay buoy and the Cape Henry, VA, buoy during this study's period. However, in 2018, the mean umbo $\delta^{18}O_c$ itself (not including 95% confidence intervals) was more ^{16}O enriched than the calculated range (Fig. 5; Appendix 3). Blue mussel umbo $\delta^{18}O_c$ ± 95% confidence intervals were consistently more ^{18}O enriched than the expected $\delta^{18}O_c$ for shells forming at the mouth of the Delaware Bay and more ^{16}O enriched than the expected $\delta^{18}O_c$ for shells forming near the Jeffrey's Lodge, NH, buoy. However, blue mussel umbo $\delta^{18}O_c$ ± 95% confidence intervals fell within or overlapped the range of expected $\delta^{18}O_c$ for shells forming near the New York Harbor Entrance buoy in 2012, 2014, 2015, and 2016 (Fig. 5).

Sea Surface Temperature and Atmospheric Precipitation

Mean sea surface temperature in April and May showed no linear trend between 2010 – 2018 for data collected at both the Delaware Bay buoy (Spearman's correlation coefficient: ρ(rho) = -0.25, p = 0.52) and the Cape Henry, VA, buoy (Spearman's correlation coefficient: ρ(rho) = -0.28, p = 0.46; Fig. 3a, b). Mean atmospheric precipitation in April and May showed a strong increasing linear trend between 2010 – 2018 in both Norfolk, VA (Spearman's correlation coefficient: ρ(rho) = 0.73, p = 0.03) and Salisbury, MD (Spearman's correlation coefficient: ρ(rho) = 0.82, p = 0.01; Fig. 6a, b).

DISCUSSION

Blue mussel abundance/core sample declined on Virginia's peat banks between 2010 – 2018. This finding is consistent with other studies documenting that the range of adult breeding blue mussels has contracted northward due to increasing sea surface temperatures and/or decreased salinity (Jones et al. 2010, Wethey et al. 2011, Versteegh et al. 2012, Hale et al. 2017). Yet, this decline only signifies the trend in blue mussel abundances within peat banks, rather than signifying a negative trend in blue mussels in Virginia overall. Food availability is not only a function of prey abundance but also of the availability of suitable habitat for prey. Thus, if blue mussel abundances within available habitat decreased, but the exposed area of peat banks increased, the overall abundance of blue mussels may not have declined. However, available peat bank length varied little between 2013 and 2017 on our sampled islands; thus, the blue mussel abundance decrease observed here represented an overall decline in blue mussels in our study area.

Blue mussels' umbo $\delta^{18}O_c$ became more ^{16}O enriched over time, suggesting that the waters within which Virginia's blue mussels first precipitated their shells became less saline and/or warmed over the duration of this study. While sea surface temperature data showed no linear trend in sea surface temperature between 2010 – 2018, our measured shell umbo and edge $\delta^{18}O_c$ findings, for 2018 especially, suggested that both the blue mussel's place of origin and settlement were less saline and/or warmer than in previous years. Because we did not find an increase in sea surface temperatures between the Delaware Bay buoy and the Cape Henry, VA, buoy between 2010 – 2018, we suspect that signatures becoming more ^{16}O enriched was caused by a change in the amount of estuarine water outflowing into the ocean during these years. Our atmospheric precipitation data support this supposition, as we found that atmospheric precipitation in April and May showed an increasing trend over time. Thus, we conclude that sea surface temperature alone was not the driving force affecting $\delta^{18}O_c$ in our study, but that $\delta^{18}O_w$ (which is affected by temperature and estuarine discharge) was also a driver, with precipitation changes of key importance during the most recent years of our study. Additionally, the more ^{16}O

enriched values observed in both umbos and edges in 2018 suggest that the water conditions within which blue mussels originated and settled may be changing. Future studies should consider using other isotopic tracers such as lead or neodymium isotopes, which can trace anthropogenic pollution, to delineate the location of spawning populations (Alves 2019, Cong et al. 2019, Zhao et al. 2019, Poitevin et al. 2020).

Blue mussels' umbo $\delta^{18}O_c$ were more ^{18}O enriched than shell edges, suggesting that Virginia's blue mussels originated in more saline and/or colder water than that in which they precipitated shell edges (here, intertidal zones on Virginia's barrier islands). These results were further supported by the more ^{16}O enriched expected $\delta^{18}O_c$ we calculated for blue mussels if they had formed at the estuarine mouth of the Delaware Bay. Similarly, studies conducted on *Mytilus californianus* (Keith et al. 1964) and *Pinna nobilis* (Kennedy et al. 2001), two marine bivalves that live in both intertidal and subtidal ocean waters, also found that $\delta^{18}O_c$ became more ^{16}O enriched with increasing distance from the shells' umbo. While these data alone do not distinguish whether blue mussels originated in unknown subtidal populations in Virginia or in subtidal populations farther north, they do suggest that juvenile blue mussels in Virginia did not originate in estuarine waters (e.g., near the mouth of or within the Delaware Bay) or farther south than Cape Henry, VA. We found that the $\delta^{18}O_c$ we would expect blue mussels to have if they originated near the New York Harbor Entrance, NY, buoy overlapped with our blue mussel umbo signatures in some years, potentially suggesting that blue mussels travelled from ocean waters as far as New York in those years. Calculations of the expected $\delta^{18}O_c$ near the Jeffrey's Point, NH, buoy, however, were consistently too ^{18}O enriched to suggest that Virginia's blue mussels originated that far north. Thus, while we cannot definitely delineate the exact northern origin boundary for Virginia's blue mussels, we can conclude that it stretches as far as the New York buoy, but not as far north as the Jeffrey's Point, NH, buoy.

We conclude that the blue mussels we collected in Virginia originated either directly offshore of Virginia or north of Virginia and likely used the Delaware Coastal Current to travel to Virginia's intertidal peat banks (Khim and Krantz 1996). Blue mussels may have originated within ocean waters near the Delaware Bay buoy but potentially as far north as the New York Harbor Entrance buoy in some years. The more northern potential origin location may decrease the risk of blue mussels being extirpated from Virginia in the near future, as water in New York will likely stay colder for longer than more southern waters. In all years but 2018, the mean signatures for blue mussel edge and umbo indicated that blue mussels precipitated their shells in more saline and/or colder water than water near the Cape Henry, VA, buoy to the south of our study area. Further precluding a more southern origin, it is unlikely that the Gulf Stream, which flows from south to north, would transport blue mussels to Virginia's barrier islands as 1) blue mussels do not currently breed south of Virginia, 2) the Gulf Stream would push larvae away from the mainland, and 3) umbo $\delta^{18}O_c$ would have indicated a warmer origin than it did, due to the warmer water in this current (Haas et al. 2017, Schoonover et al. 2017). It is more likely that blue mussels travelled to Virginia using the Delaware Coastal Current, which flows from north to south and moves water along the coast.

We acknowledge three limitations of our calculated $\delta 18O_c$, all of which relate to uncertainty of the calculated ranges for $\delta^{18}O_c$. First, the $\delta^{18}O_w$ data we used from Khim and Krantz (1996) to calculate expected $\delta^{18}O_c$ in shells were collected in 1993 (Schmidt et al. 1999). While we could not locate more recently collected $\delta^{18}O_w$ data within our study's region (Schmidt et al. 1999), if Khim and Krantz's (1996) study were replicated now, different ranges of $\delta^{18}O_w$

may exist due to changes in sea surface temperatures and/or salinities causing variations in calculated $\delta^{18}O_c$. However, our calculated $\delta^{18}O_c$ values still provide a good estimate of the $\delta^{18}O_c$ we would expect to be in equilibrium within ocean waters between the Delaware Bay buoy to Cape Henry, VA, despite the 17+ year time difference as ocean $\delta^{18}O_w$ is less variable over time than those in the intertidal-zone (Daniel and Boyden 1975, Drouin et al. 1985).

Second, we used sea surface temperature values from April and May, under the assumption that these months coincided with when the juvenile blue mussels in Virginia spawned. While we cannot be certain this is the case, literature concerning the reproductive time-frames of adult blue mussels suggests that they typically spawn for the first time in a given season in the late spring through early summer (Bayne 1976). Therefore, it is possible that the juvenile blue mussels found in Virginia were spawned earlier than April. However, given the estimated time that blue mussels are pelagic after spawning (Chipperfield 1953, Le Gall 1970, Jørgensen 1981, Sprung 1984, Strathmann 1985, De Vooys 1999, Lercari and Defeo 2006), we suspect that most of Virginia's blue mussels spawned in April – early May. Additionally, March sea surface temperatures were typically colder than either April and May in this region (NDBC 2020a, b); thus, calculated $\delta^{18}O_c$ would be more ^{18}O enriched than those calculated for April and May.

Third, when determining an upper origin boundary for Virginia's blue mussels, we used the farthest north marine $\delta^{18}O_w$ from Khim and Krantz (1996) to inform the *Calcite-Water Equilibrium Expressions*, rather than measured $\delta^{18}O_w$ near the New York Harbor Entrance buoy and the Jeffrey's Ledge, NH, buoy, due to data limitations (Schmidt et al. 1999). While we used sea surface temperature data from these buoys in our calculations, we acknowledge that by not also using $\delta^{18}O_w$ from those regions, our accuracy of the upper origin boundary was poorer than if we had both sea surface temperature and $\delta^{18}O_w$ data from that region.

Because red knots forage on different substrates at different times during the tidal cycle (Evans et al. 1980, Evans 1981, Alerstam 1990, Piersma et al. 1994, Gonzalez et al. 1996, Heller et al. In Prep$_b$), appear to track prey over space and time, and are dependent on consistent prey resources along migration routes (Charnov 1986, Krebs et al. 1978, Cowie and Krebs 1979, Martin 1985, Martin and Karr 1986), any changes to the abundance of blue mussels and other prey along migration routes may negatively affect them (Cohen et al. 2009, 2010a, b, Watts and Truitt 2015, Heller et al. In Prep$_a$). If the range of adult breeding blue mussels continues to contract northward (Jones et al. 2010, Wethey et al. 2011, Versteegh et al. 2012, Hale et al. 2017) and/or if blue mussels continue to be affected by ocean acidification (Kurihara 2008, Doney et al. 2009, De Moel et al. 2009), it is possible that juvenile blue mussels will become unavailable to red knots in Virginia in the future (Watts and Truitt 2015). Additionally, if intertidal waters in Virginia warm, any juvenile blue mussels that settle on Virginia's peat banks may not survive through the red knot stopover period. Because blue mussels have limited thermal tolerance, water temperatures above 30°C cause mass mortality (Jones et al. 2009, Watts and Truitt 2015, Seuront et al. 2019). If blue mussels are extirpated from Virginia, it could become more challenging for red knots to meet daily energy demands and gain the fat needed to complete their migration to breeding grounds in the Arctic.

The dynamic nature of barrier islands, being influenced by wind, waves, and extreme weather events, further suggests that rapid intertidal habitat alterations that affect invertebrate organisms may occur (Leatherman 1982; Pekala 1996). Specifically, weather events that cause shoreline erosion can alter peat banks and beach slopes (Bindoff et al. 2007), which in turn could affect where blue mussels settle. These changes could ultimately affect red knot habitat use, distribution, and survival, even in the absence of further blue mussel range contraction (Steimle and Zetlin 2000). Natural processes such as island transgression and shoreline erosion are required for the formation of peat banks. Because peat banks support blue mussels and other invertebrate prey in large abundances, ensuring that peat banks continue to be available on Virginia's barrier islands is imperative. Thus, managers should continue their ongoing protection of peat bank habitat by encouraging natural barrier island processes. When possible, avoidance of beach nourishment projects is encouraged as renourishment not only is known to bury and potentially kill intertidal prey but also impedes the processes that form peat banks (Bishop et al. 2006, Rosov et al. 2016). Other barrier beaches along the U.S. Atlantic Coast should also consider restoring natural barrier island processes, at least one some islands, that allow for peat formation, which may benefit blue mussels and other invertebrate prey that live in peat, and the imperiled red knot that feeds upon them.

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FIGURES

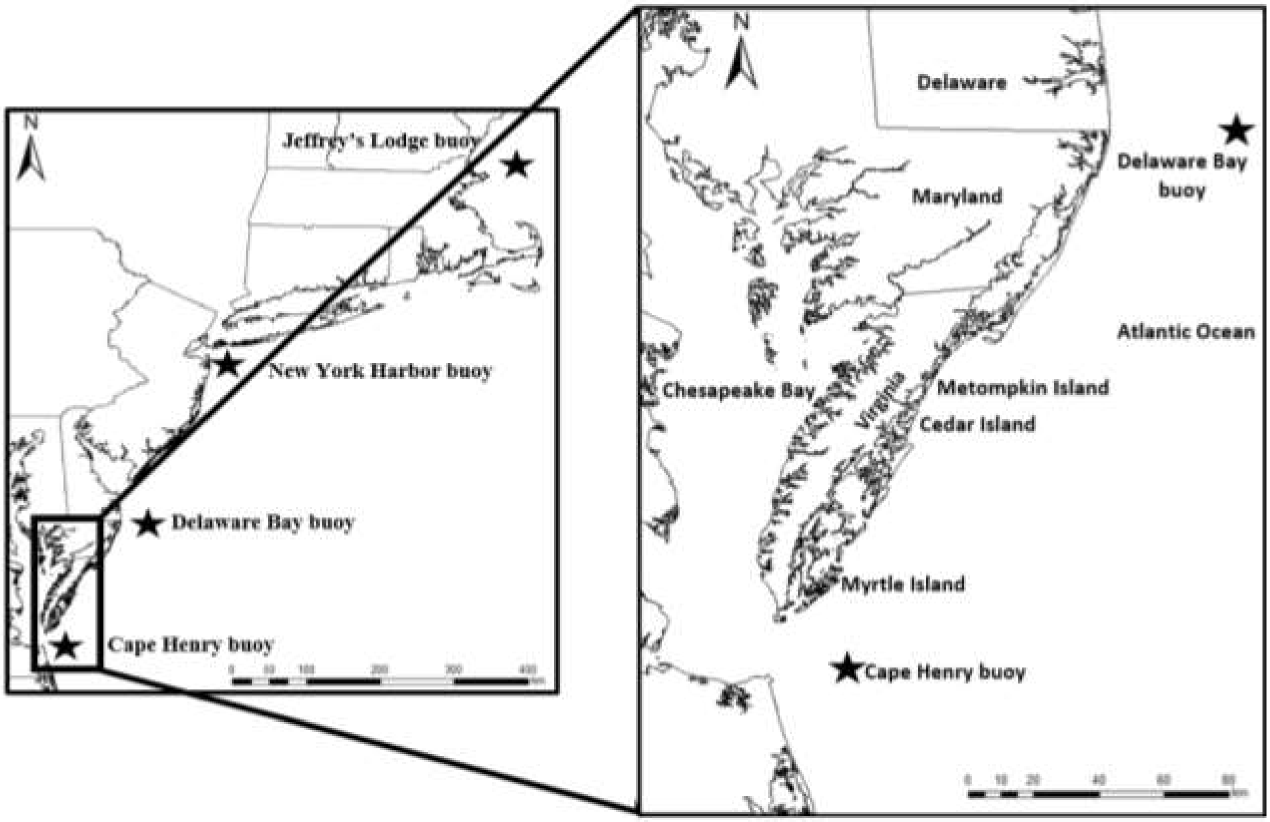

Figure 1. Study area on the Virginia Coast Reserve Long Term Ecological Research Site. Blue mussels were collected from Atlantic Ocean intertidal zone peat banks on Cedar Island (*n* = 37), Metompkin Island (*n* = 19), and Myrtle Island (*n* = 18), Virginia's barrier islands, May 14 – 27, 2010 – 2018. Locations for buoy stations 44009 (Delaware Bay, DE; 38°27'24" N 74°42'6" W; sea temperature depth = 0.60 m; ~ 30.58 km offshore; NDBC 2020a), 44099 (Cape Henry, Virginia; 36°54'50" N 75°43'13" W; sea temperature depth = 0.46 m; ~ 24.14 km offshore; NDBC 2020b), 44065 (New York Harbor Entrance, NY; 40°22'10" N 73°42'10" W; sea temperature depth = 1.5 m; ~22.85 km offshore; NDBC 2020c), and 44098 (Jeffrey's Ledge, NH; 42°47'53" N 70°10'5" W; sea temperature depth = 0.46 m; ~ 50.21 km offshore; Fig. 1; NDBC 2020d) are denoted by black stars.

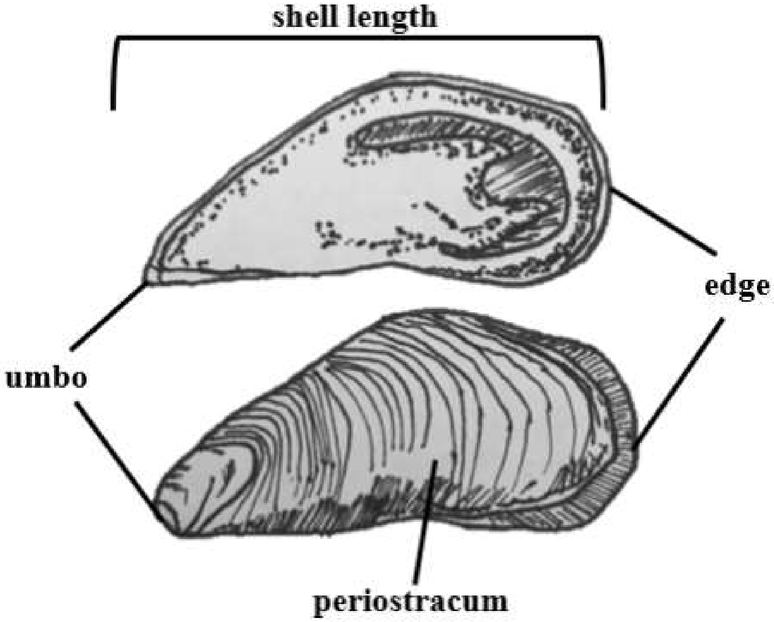

Figure 2. Blue mussel shell with labels of the umbo (first part of the shell precipitated), edge (last part of the shell precipitated), shell length, and periostracum (thin organic coating that covers the outermost layer of mollusk shells).

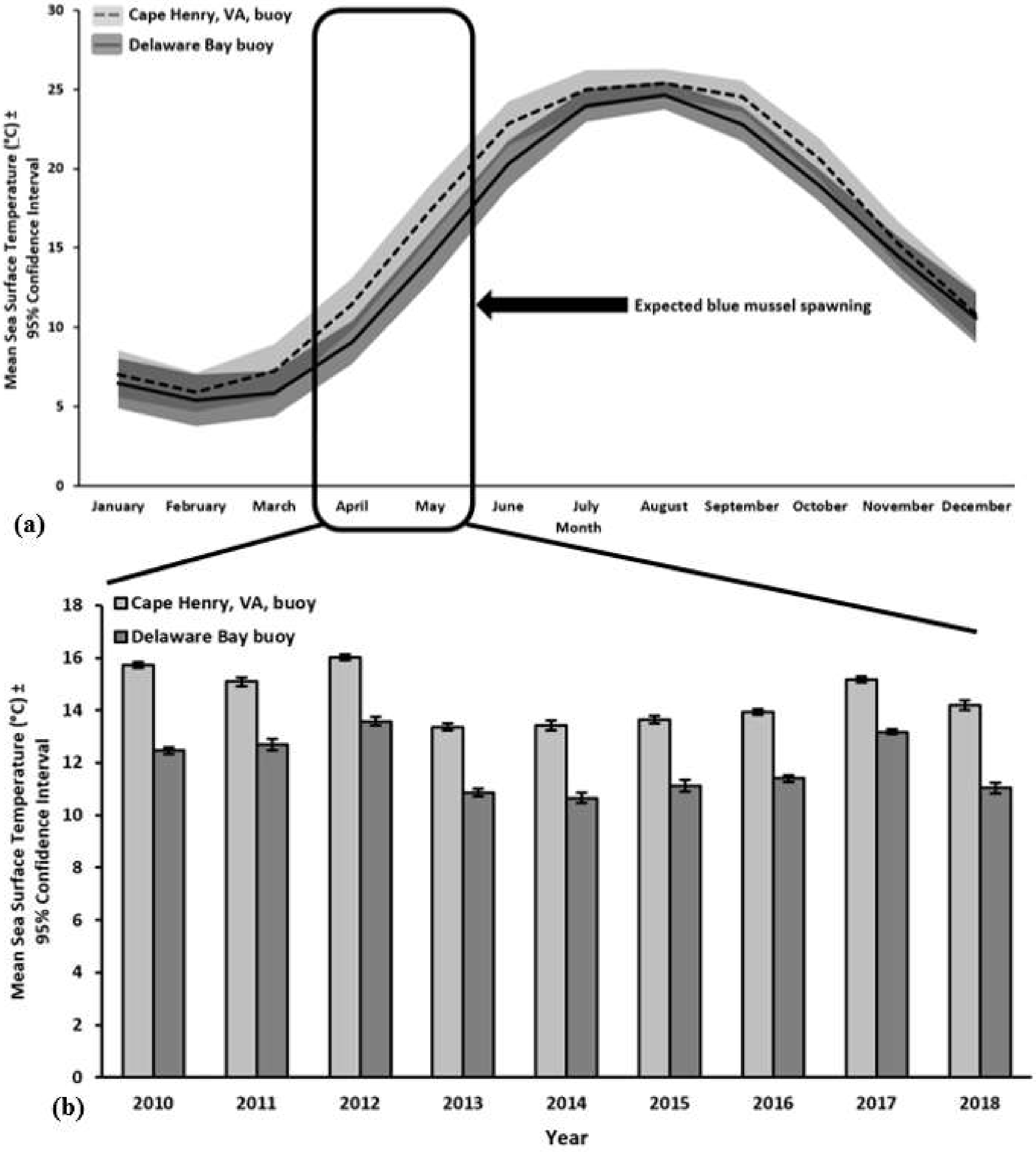

Figure 3. Mean sea surface temperatures (°C ± 95% CI) for buoy stations 44009 (Delaware Bay, DE; 38°27'24" N 74°42'6" W; sea temperature depth = 0.60 m; ~ 30.58 km offshore; NDBC 2020a) and 44099 (Cape Henry, Virginia; 36°54'50" N 75°43'13" W; sea temperature depth =

0.46 m; ~ 24.14 km offshore; NDBC 2020b) by (a) month between 2010 – 2018 and (b) year (mean for April and May ± 95% CI, 2010 – 2018). Buoy 44009 did not have data for January 2013, August – December 2016, January – March 2017, and October – December 2018. Outline around April and May (a) signifies the time period during which we expected adult blue mussels spawned the juvenile blue mussels found on Virginia's barrier islands.

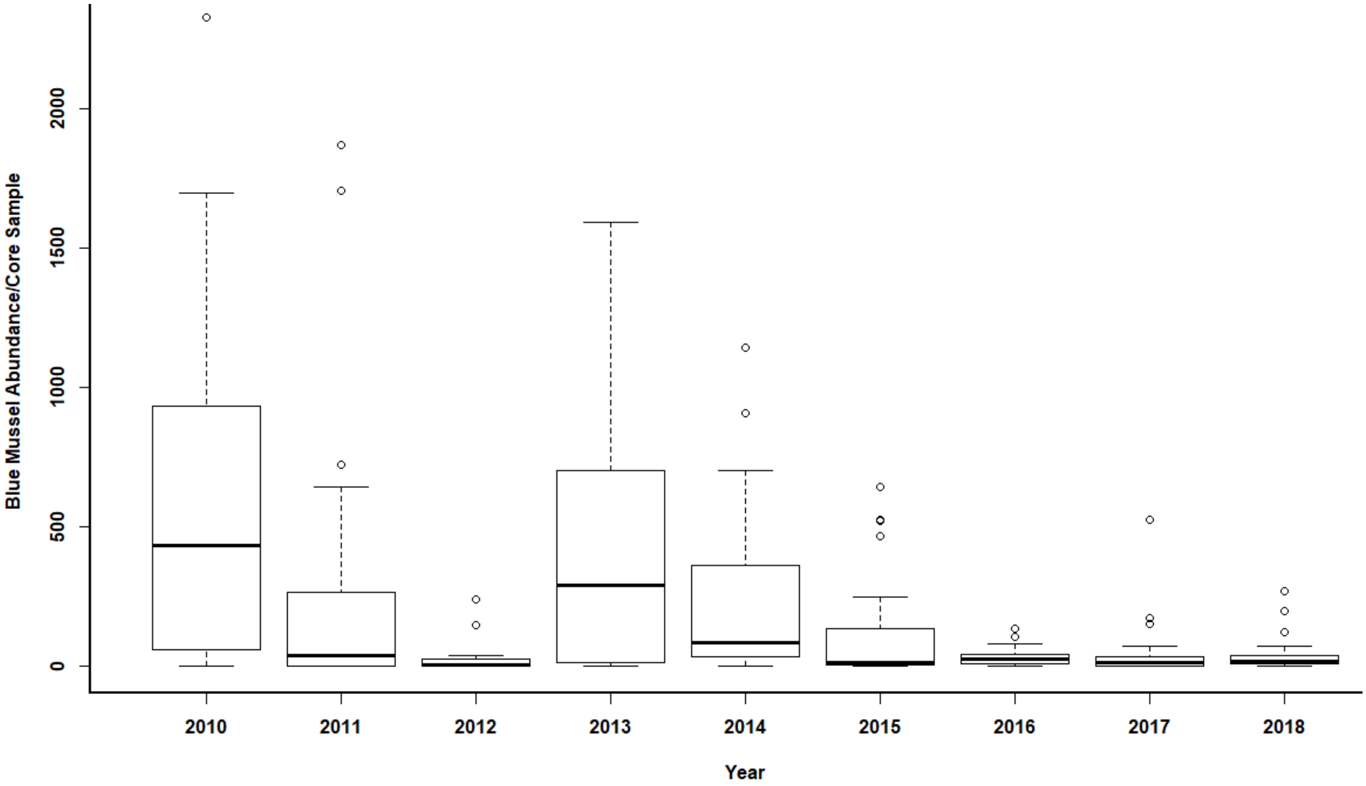

Figure 4. Box-and-whisker plot of mean blue mussel abundances/core sample by year found in peat banks on Cedar Island (*n* = 121), Metompkin Island (*n* = 73), and Myrtle Island (*n* = 83), Virginia's barrier islands, May 14 – 27, 2010 – 2018. Bolded horizontal black line = median; top and bottom of box plot = upper and lower quartiles; whiskers = standard deviation; open circles = outliers.

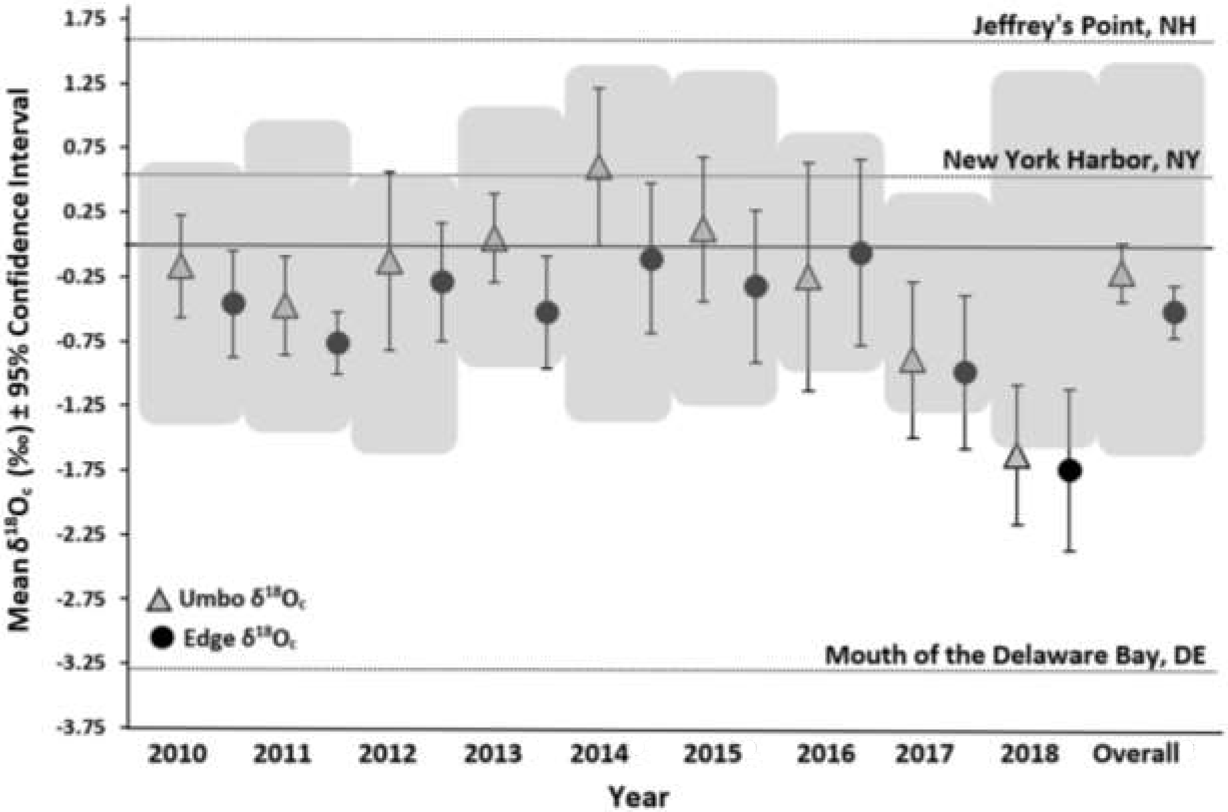

Figure 5. Mean oxygen isotopic composition of shell calcite ($\delta^{18}O_c$; ‰) obtained from blue mussel shell umbo (first portion of the shell precipitated) and edge (most recently precipitated shell) collected on peat banks on Cedar Island (*n* = 37), Metompkin Island (*n* = 19), and Myrtle Island (*n* = 18), Virginia's barrier islands, May 14 – 27, 2010 – 2018. Shaded grey bars indicate the lower and upper $\delta^{18}O_c$ we would expect for shell calcites precipitated in April and May in ocean water between the Delaware Bay buoy and the Cape Henry, VA, buoy. Calculated $\delta^{18}O_c$ values (gray bars) were estimated using mean April and May sea surface temperatures (°C) for buoy stations 44009 (Delaware Bay, DE; 38°27'24" N 74°42'6" W; sea temperature depth = 0.60 m; ~ 30.58 km offshore; NDBC 2020a) and 44099 (Cape Henry, Virginia; 36°54'50" N 75°43'13" W; sea temperature depth = 0.46 m; ~ 24.14 km offshore; NDBC 2020b), 2010 – 2018, $\delta^{18}O_w$ from Khim and Krantz (1996), and *Calcite-Water Equilibrium Expressions* (see

text) from Kim and O'Neil (1997). Dotted horizontal lines indicate the expected $\delta^{18}O_c$ for blue mussels spawned near the mouth of the Delaware Bay and near buoy stations 44065 (New York Harbor Entrance, NY; 40°22'10" N 73°42'10" W; sea temperature depth = 1.5 m; ~22.85 km offshore; NDBC 2020c) and 44098 (Jeffrey's Ledge, NH; 42°47'53" N 70°10'5" W; sea temperature depth = 0.46 m; ~ 50.21 km offshore; Fig. 1; NDBC 2020d) in April.

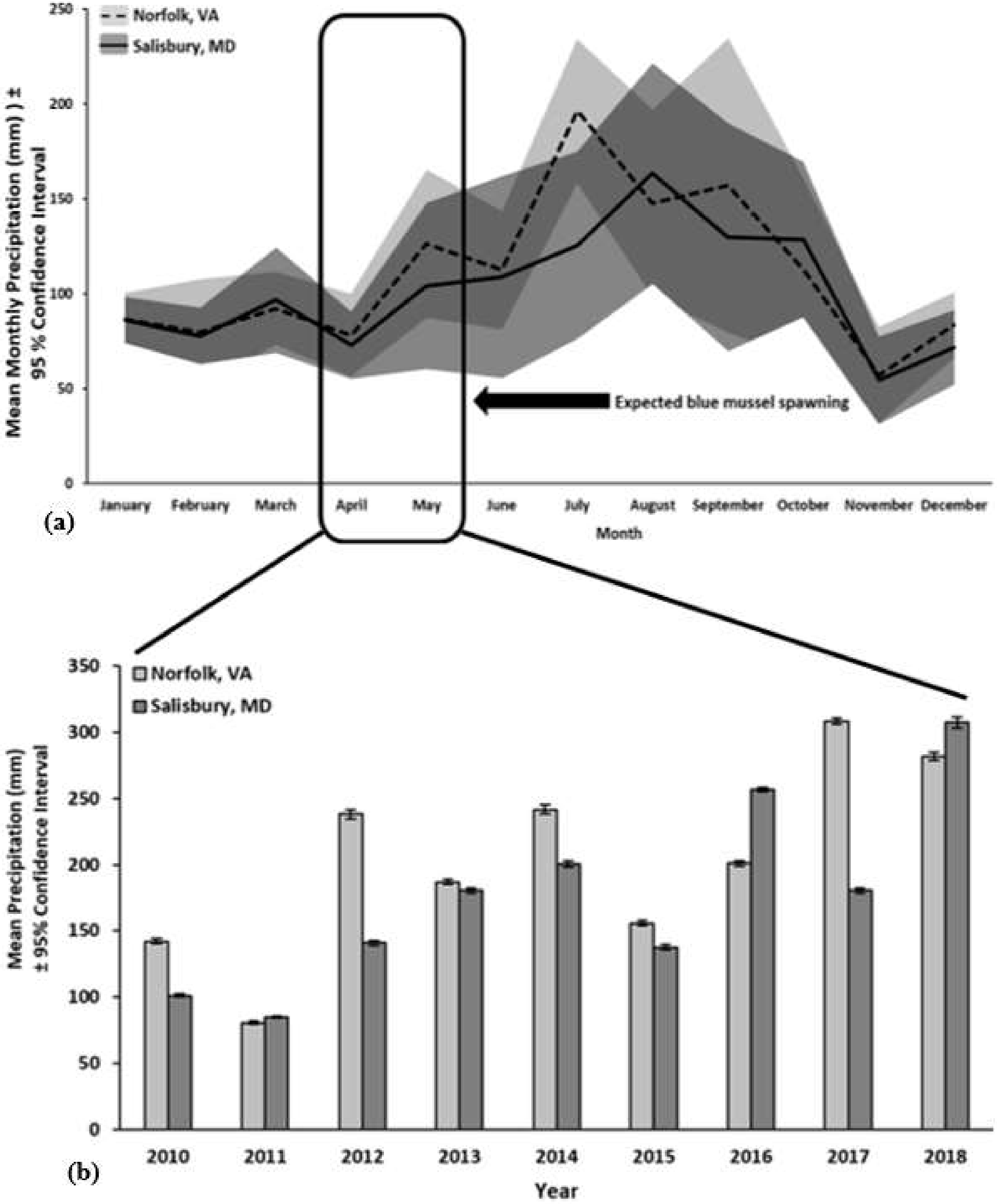

Figure 6. Mean precipitation (mm ± 95% CI) for a site in coastal Virginia near the Cape Henry, VA, buoy (Norfolk; 36° 51' 0" N 76° 17' 24" W; U. S. Climate Data 2020a) and in Maryland

near the Delaware Bay buoy (Salisbury, Maryland; 38° 20' 24" N - 75° 30' 36" W; U. S. Climate Data 2020b), by (a) month (2010 – 2018) and (b) year (April and May averaged, 2010 – 2018). These weather stations were selected based on consistent availability of historic data during the study period and proximity to the buoys used to calculate the approximate south and north range for blue mussels collected on Virginia's barrier islands. Outline around April and May signifies the time period during which we expected adult blue mussels spawned the juvenile blue mussels found on Virginia's barrier islands.

TABLE

Table 1. Number of peat core samples collected, mean blue mussel abundance/core sample with standard error, the estimated abundance/m^2 of blue mussels found in peat banks, and the number of blue mussels analyzed using stable isotope analyses by year. Blue mussels were collected on Cedar Island (*n* = 37), Metompkin Island (*n* = 19), and Myrtle Island (*n* = 19), Virginia's barrier islands, May 14 – 27, 2010 – 2018.

Year	N^{1a}	Mean Blue Mussel Abundance/Core Sample (SE)^b	Estimated Blue Mussel Abundance/m^2	N^{2a}
2010	40	537.88 (85.85)	66,158.63	9
2011	30	232.40 (84.73)	28,585.20	9
2012	23	36.36 (14.94)	4,508.22	8
2013	36	422.33 (75.13)	51,947.00	10
2014	26	233.73 (60.03)	28,748.88	10
2015	32	104.16 (31.91)	12,811.22	8
2016	24	34.08 (6.96)	4,192.25	9
2017	34	41.09 (16.16)	5,053.85	4
2018	32	38.50 (10.42)	4,735.50	7

Overall	277	207.19 (22.41)	25,484.09	8.22

[1a] *N* = Number of peat core samples collected.

[b] Mean Abundance Blue Mussels/Core Sample (SE) = Mean abundance of blue mussels collected/core sample (SE).

[2a] *N* = Number of blue mussels analyzed.

APPENDICES

Appendix 1. Mean oxygen isotopic composition of shell calcite ($\delta^{18}O_c$; ‰) obtained from blue mussel shells collected on peat banks on Cedar Island (*n* = 37), Metompkin Island (*n* = 19), and Myrtle Island (*n* = 18), Virginia's barrier islands, May 14 – 27, 2010 – 2018.

Year	**N**[a]	**Shell**[b]	**Mean $\delta^{18}O_c$**[c]	**SE**[d]	**LCI**[e]	**UCI**[e]	**Range**[f]
2010	9	edge	-0.46	0.21	-0.87	-0.05	-1.57, 0.37
	9	umbo	-0.17	0.20	-0.56	0.22	-1.37, 0.54
2011	9	edge	-0.77	0.12	-1.01	-0.53	-1.28, -0.15
	9	umbo	-0.48	0.19	-0.86	-0.09	-1.24, 0.35
2012	8	edge	-0.29	0.23	-0.75	0.17	-1.33, 0.71
	8	umbo	-0.13	0.35	-0.83	0.56	-1.31, 1.42
2013	10	edge	-0.53	0.22	-0.96	-0.10	-1.94, 0.42
	10	umbo	0.05	0.18	-0.30	0.39	-0.60, 1.05
2014	10	edge	-0.11	0.30	-0.69	0.47	-1.68, 1.26
	10	umbo	0.60	0.31	-0.02	1.21	-0.65, 1.85

2015	8	edge	-0.33	0.30	-0.92	0.26	-1.37, 0.91
	8	umbo	0.11	0.29	-0.45	0.67	-0.72, 1.70
2016	9	edge	-0.07	0.37	-0.79	0.65	-1.89, 1.59
	9	umbo	-0.25	0.45	-1.14	0.63	-2.34, 1.87
2017	4	edge	-1.00	0.30	-1.59	-0.40	-1.67, -0.27
	4	umbo	-0.90	0.31	-1.51	-0.30	-1.61, -0.11
2018	7	edge	-1.76	0.32	-2.39	-1.13	-2.95, -0.56
	7	umbo	-1.64	0.28	-2.19	-1.10	-2.54, -0.28
Overall	74	edge	-0.53	0.10	-0.73	-0.33	-2.95, 1.59
	74	umbo	-0.23	0.12	-0.46	0.00	-2.54, 1.87

[a] *N* = Number of blue mussels analyzed.

[b] Shell = Shell portion sampled (edge = most recently precipitated shell; umbo = shell precipitated first).

[c] SE = Standard error.

[d] LCI = Lower 95% confidence interval.

[e] UCI = Upper 95% confidence interval.

[f] Range = Range of calculated $\delta^{18}O_c$.

Appendix 2. Location, collection date, shell length (mm), shell portion (edge or umbo), and mean oxygen isotopic composition of shell calcite ($\delta^{18}O_c$; ‰) obtained from blue mussel shells collected on peat banks on Cedar Island (*n* = 37), Metompkin Island (*n* = 19), and Myrtle Island (*n* = 18), Virginia's barrier islands, May 14 – 27, 2010 – 2018.

Location	**Collection Date**	**Shell Length (mm)**	**Shell Portion Sampled**	**Mean $\delta^{18}O_c$[a]**
Cedar	5/14/2010	8.00	edge	-0.04
			umbo	0.54
Cedar	5/14/2010	6.00	edge	0.18
			umbo	0.38
Cedar	5/14/2010	10.00	edge	0.37
			umbo	0.36
Cedar	5/14/2010	6.75	edge	-0.44
			umbo	-0.07
Cedar	5/14/2010	4.00	edge	-0.56
			umbo	-0.40
Metompkin	5/15/2010	7.00	edge	-0.42
			umbo	-1.37

Metompkin	5/15/2010	8.00	edge	-0.37
			umbo	-0.03
Myrtle	5/14/2010	7.75	edge	-1.57
			umbo	-0.24
Myrtle	5/14/2010	9.00	edge	-1.29
			umbo	-0.71
Cedar	5/20/2011	11.00	edge	-0.86
			umbo	0.24
Cedar	5/17/2011	8.00	edge	-0.54
			umbo	0.35
Cedar	5/17/2011	10.00	edge	-0.15
			umbo	0.00
Cedar	5/17/2011	8.25	edge	-1.09
			umbo	-0.48
Cedar	5/17/2011	9.50	edge	-0.65
			umbo	-0.93

Cedar	5/20/2011	10.25	edge	-0.36
			umbo	-1.19
Metompkin	5/17/2011	4.75	edge	-1.00
			umbo	-0.45
Metompkin	5/17/2011	4.00	edge	-0.98
			umbo	-0.59
Myrtle	5/16/2011	9.00	edge	-1.28
			umbo	-1.24
Cedar	5/16/2012	7.50	edge	0.24
			umbo	0.76
Cedar	5/16/2012	10.00	edge	0.12
			umbo	1.42
Cedar	5/16/2012	6.00	edge	0.71
			umbo	0.60
Cedar	5/16/2012	4.50	edge	-0.55
			umbo	-0.52

Cedar	5/16/2012	3.50	edge	-0.68
			umbo	-1.01
Metompkin	5/17/2012	2.50	edge	-0.81
			umbo	0.07
Myrtle	5/24/2012	4.00	edge	-0.05
			umbo	-1.31
Myrtle	5/17/2012	5.00	edge	-1.33
			umbo	-1.08
Cedar	5/22/2013	14.00	edge	0.36
			umbo	1.05
Cedar	5/19/2013	11.00	edge	0.42
			umbo	0.71
Cedar	5/19/2013	8.50	edge	-0.64
			umbo	0.01
Cedar	5/22/2013	7.50	edge	-1.17

			umbo	0.48
Cedar	5/19/2013	9.00	edge	-0.49
			umbo	-0.09
Cedar	5/22/2013	10.00	edge	-1.94
			umbo	-0.60
Metompkin	5/23/2013	11.00	edge	-0.34
			umbo	0.13
Metompkin	5/23/2013	10.50	edge	-0.21
			umbo	-0.46
Myrtle	5/19/2013	7.00	edge	-0.89
			umbo	-0.23
Myrtle	5/16/2013	7.75	edge	-0.39
			umbo	-0.54
Metompkin	5/18/2014	4.50	edge	0.68
			umbo	1.85
Cedar	5/18/2014	5.50	edge	1.26

			umbo	0.72
Cedar	5/18/2014	4.00	edge	0.81
			umbo	1.72
Metompkin	5/18/2014	7.75	edge	0.65
			umbo	1.84
Cedar	5/18/2014	4.75	edge	-0.37
			umbo	0.17
Cedar	5/18/2014	5.00	edge	-0.47
			umbo	0.77
Metompkin	5/18/2014	3.50	edge	-0.22
			umbo	-0.65
Metompkin	5/18/2014	6.00	edge	-0.73
			umbo	0.66
Myrtle	5/17/2014	3.25	edge	-1.04
			umbo	-0.45
Myrtle	5/17/2014	4.50	edge	-1.68

			umbo	-0.65
Cedar	5/17/2015	4.00	edge	0.91
			umbo	1.70
Cedar	5/17/2015	6.00	edge	0.89
			umbo	0.60
Cedar	5/17/2015	5.50	edge	-0.05
			umbo	0.44
Cedar	5/17/2015	8.50	edge	-1.37
			umbo	-0.64
Metompkin	5/19/2015	4.50	edge	-0.35
			umbo	0.22
Metompkin	5/19/2015	5.75	edge	-0.93
			umbo	-0.27
Myrtle	5/19/2015	4.25	edge	-0.87
			umbo	-0.72
Myrtle	5/19/2015	7.00	edge	-0.86

			umbo	-0.43
Cedar	5/16/2016	.	edge	1.59
			umbo	0.82
Cedar	5/16/2016	2.00	edge	1.11
			umbo	1.87
Myrtle	5/19/2016	4.50	edge	0.82
			umbo	1.29
Cedar	5/16/2016	6.25	edge	0.21
			umbo	-1.14
Cedar	5/16/2016	2.75	edge	-1.89
			umbo	-2.34
Cedar	5/16/2016	5.75	edge	-0.67
			umbo	-1.09
Myrtle	5/19/2016	4.50	edge	-0.99
			umbo	-0.22
Myrtle	5/19/2016	5.25	edge	-0.47

			umbo	-1.21
Myrtle	5/19/2016	4.75	edge	-0.33
			umbo	-0.27
Cedar	5/19/2017	7.25	edge	-0.78
			umbo	-1.00
Metompkin	5/16/2017	6.50	edge	-0.27
			umbo	-0.11
Metompkin	5/16/2017	4.75	edge	-1.27
			umbo	-0.89
Metompkin	5/16/2017	5.75	edge	-1.67
			umbo	-1.61
Cedar	5/15/2018	4.00	edge	-2.10
			umbo	-1.70
Metompkin	5/22/2018	5.25	edge	-0.89
			umbo	-0.28
Metompkin	5/16/2018	4.50	edge	-1.51

			umbo	-1.52
Metompkin	5/16/2018	3.00	edge	-2.49
			umbo	-2.33
Myrtle	5/21/2018	4.50	edge	-1.80
			umbo	-1.37
Myrtle	5/21/2018	3.50	edge	-2.95
			umbo	-1.75
Myrtle	5/15/2018	2.00	edge	-0.56
			umbo	-2.54

[a] $\delta^{18}O_c$ = Mean ^{18}oxygen stable isotope signatures for blue mussels analyzed using mass spectrometry.

Appendix 3. Mean oxygen isotopic composition of shell calcite ($\delta^{18}O_c$; ‰) values obtained from blue mussel umbos collected on peat banks on Metompkin Island (*n* = 19), Cedar Island (*n* = 37), and Myrtle Island (*n* = 18), Virginia's barrier islands, May 14 – 27, 2010 – 2018, and the calculated $\delta^{18}O_c$ across the expected origin range for blue mussels spawned in April and May.

Year	**Mean $\delta^{18}O_c$ Blue Mussel Umbo (LCI, UCI)**[a]	**Calculated $\delta^{18}O_c$ Range (Lower, Upper)**[b]
2010	-0.17 (-0.56, 0.22)	-1.36, 0.66
2011	-0.48 (-0.86, -0.09)	-1.47, 0.95
2012	-0.23 (-0.83, 0.56)	-1.60, 0.49
2013	0.05 (-0.30, 0.39)	-0.98, 1.07
2014	0.49 (-0.02, 1.21)	-1.39, 1.36
2015	0.11 (-0.45, 0.67)	-1.25, 1.32
2016	-0.25 (-1.14, 0.63)	-1.03, 0.80
2017	-0.82 (-1.51, -0.30)	-1.27, 0.34
2018	-1.71 (-2.19, -1.10)	-1.54, 1.30
Overall	-0.33 (-0.46, 0.00)	-1.60, 1.36

[a] Mean $\delta^{18}O_c$ Blue Mussel Umbo (LCI, UCI) = Mean measured $\delta^{18}O_c$ for blue mussel umbos with lower and upper 95% confidence intervals.

[b] Calculated $\delta^{18}O_c$ Range (Lower, Upper) = Range of $\delta^{18}O_c$ calculated using sea surface temperature data from buoy stations 1) lower - 44099 (Cape Henry, Virginia; 36°54'50" N 75°43'13" W; sea temperature depth = 0.46 m; ~ 24.14 km offshore; NDBC 2020b) and 2) upper - 44009 (Delaware Bay, DE; 38°27'24" N 74°42'6" W; sea temperature depth = 0.60 m; ~ 30.58 km offshore; NDBC 2020a), 2010 – 2018.

CONCLUSION

INTRODUCTION

The Western Atlantic red knot (*Calidris canutus rufa*) is a federally threatened long-distance migratory shorebird that relies on stopover sites throughout North and South America as it migrates from predominately South American wintering grounds to breeding grounds in the Canadian Arctic (Gonzalez et al. 1996, D'Amico et al. 2014, Federal Registrar 2014, Watts and Truitt 2015). Virginia's barrier islands serve as one of the red knot's most used North American spring stopover grounds, second only to the Delaware Bay region (MacKay 1893, United States Commission on Ocean Policy 2004, Thomas et al. 2006, Cohen et al. 2010a). During their relatively brief (~ 2 week) stopover in Virginia, red knots must quickly replenish the fat stores needed to successfully complete their migration to breeding grounds by foraging upon numerous invertebrate prey along ocean intertidal peat banks and sand (Watts and Truitt 2000, 2015, Truitt et al. 2001, Atkinson et al. 2007, Cohen et al. 2010a, b, 2011).

Any alterations in prey availability and distribution on stopover grounds, including Virginia's barrier islands, may make regaining the weight required for continued flight more challenging for red knots (Morrison et al. 2006; Hua et al. 2013). Weight gain challenges in red knots may be especially evident in the face of climate change, as climate-related factors contribute to range changes of numerous species (Parmesan and Yohe 2003, Parmesan 2006, Bindoff et al. 2007, Perovich et al. 2008, Jonzen et al. 2011). Relevant to Virginia's migrant shorebirds, warming ocean temperatures have caused the blue mussel's (*Mytilus edulis*) range to contract northward (Hansen et al. 2006, Jones et al. 2010), with Virginia now being the southernmost portion of their known intertidal range until mid-summer. By late-June to early

July, water temperatures become too warm for blue mussel survival, causing mass juvenile blue mussel mortality. Because red knots are bivalve specialists (Tulp and Schekkerman 2008), if the blue mussel's range continues to contract, red knots may be unable to adapt to subsequent variations in prey availability. Changes to prey availability may make it more challenging for red knots to gain the weight needed to complete their migration to the Arctic and quickly breed upon arrival (Guglielmo et al. 2005), which ultimately could lead to population declines, increasing the extinction vulnerability of an already threatened bird. Thus, understanding the factors that affect red knots in Virginia is paramount to implementing successful management practices.

Despite the contributions of numerous studies (e.g., Watts and Truitt 2000, 2015, Truitt et al. 2001, Cohen et al. 2009, 2010a, b, 2011), significant knowledge gaps remained at the onset of this study (USFWS 2015, 2019), particularly with regards to matters surrounding the red knot's diet and prey availability. Although continued research is required, as I discuss below (see *Future Research Needs*), my dissertation research contributed much information to help fill in these gaps. My three chapters, as described in the preceding sections, generally aimed to determine:

- Chapter 1: what red knots in Virginia consume and how prey consumption varies by foraging substrate and the relative availability of prey,
- Chapter 2: how many red knots use Virginia's barrier islands during peak migration and what factors affect red knot site selection and flock size and what factors affect red knot prey abundances and distributions in Virginia,
- Chapter 3: the abundance and origin of blue mussels using Virginia's peat banks and how blue mussel origin may affect red knot conservation.

In Chapter 1, we collected substrate core samples on intertidal peat and sand substrates to determine what prey were available to foraging red knots in May 2017 – 2019. We also collected red knot feces during this time and used fecal DNA metabarcoding analyses to determine which of the prey items available to red knots were actually consumed and selected by red knots during their migratory stopover on Virginia's barrier islands.

In Chapter 2, we counted red knots and available prey from substrate core samples at randomly determined points on Virginia's barrier islands in May 2007 – 2018. We analyzed the effects of prey, tide, distance to closest known roost sites, and counts of red knots on Tierra del Fuego wintering grounds on red knot site selection and flock size in Virginia using zero-inflated negative binomial mixed-effects regression models. We also analyzed the effects of tide, mean daily water temperature, substrate, and island geomorphology type (i.e., high vs. low elevation) on prey abundances using generalized linear mixed-effects regression models. Finally, we addressed how tide and substrate affected the temporal and spatial distribution and abundance of prey.

In Chapter 3, we collected core samples on Virginia's peat banks in May 2010 – 2018. From these core samples, we investigated the variation in blue mussel densities over time and randomly selected 74 blue mussels on which we conducted oxygen stable isotope analyses. Stable isotope analyses enabled us to estimate the origin of the juvenile blue mussels found on Virginia's intertidal peat banks by comparing the oxygen isotopic composition of the calcite ($\delta^{18}O_c$) in shell umbos (first portion of the shell precipitated) to shell edges (most recently precipitated portion of the shell) and to expected $\delta^{18}O_c$, calculated using sea surface temperature and $\delta^{18}O_w$ data from ocean water between New Hampshire and Virginia.

The data we collected and analyzed in this dissertation will inform effective recovery and management plans for red knots using Virginia and other mid-Atlantic stopover locations. Below, I summarize the key research findings, management implications, and future research needs from the research described in this dissertation.

KEY RESEARCH FINDINGS

Chapter 1: “Red knot (*Calidris canutus rufa*) diet reconstruction using DNA metabarcoding analyses of feces collected in Virginia”

- Red knots on spring stopover in Virginia consumed prey from Orders Veneroida (clams), Mytiloida (mussels), Diptera (flies), and Amphipoda/Calanoida (crustaceans).
- Prey availability varied by foraging substrate on Virginia’s barrier islands.
    - Crustaceans were the most available prey on both sand and peat, comprising 71% and 75% of all available prey, respectively.
    - Crustaceans and blue mussels were more available on peat than sand.
    - Coquina clams (*Donax variabilis*) were more available on sand than peat.
- The prey red knots consumed (used) varied by foraging substrate.
    - Coquina clams were the most used prey on sand.
    - Blue mussels were the most used prey on peat.
- Red knots selected bivalves (coquina clams and blue mussels) more than expected given their availability, supporting their status as bivalve specialists in Virginia.
    - Crustaceans and miscellaneous prey (horseshoe crab eggs (*Limulus polyphemus*), angel wing clams (*Cyrtopleura costata*), and other organisms (e.g., insect larvae,

snails, worms)) were used less than expected given their availability on peat and sand.

Chapter 2: "Factors that affect migratory Western Atlantic red knot (*Calidris canutus rufa*) and their prey during spring stopover on Virginia's barrier islands"

- The modelled predicted number of red knots during peak migration on Virginia's barrier islands were highest in 2012 (11,644) and lowest in 2014 (2,792), with an average of 7,055 (SD = 2,841) red knots using Virginia's barrier islands during peak migration each year. We minimized potential biases associated with the assumptions used in these modelled predictions by sampling red knots during peak migration (May 21 – 27) after most have arrived on the islands and when they tend to stay for at least 2 weeks (Clark et al. 1993, Watts and Truitt 2000, Cohen et al. 2009, Watts 2013), by having only highly trained observers identify and count red knots, and by staying at least 100 meters away from flocks to prevent flushing and double-counting.
- Red knot flock size was highest ($\bar{x}$ = 17 red knots/point, SE = 0.46) during peak migration (May 21 – 27), as compared to ($\bar{x}$ = 7 red knots/point, SE = 0.33) during early migration (May 14 – 20).
- Prey abundance varied by sampling timeframe.
    - <u>Early Migration</u>: Crustaceans were the most abundantly available prey/core sample ($\bar{x}$ = 262, SE = 45.04), followed by blue mussels ($\bar{x}$ = 231, SE = 57.51). Only peat banks, within 2 hours of low tide, were sampled during this early migration period.
    - <u>Peak Migration</u>: Crustaceans were the most abundantly available prey/core sample ($\bar{x}$ = 25, SE = 2.98), followed by coquina clams ($\bar{x}$ = 24, SE = 2.56). Both

sand and peat banks were sampled during this peak migration period, although samples were more common on sand, as sampling occurred regardless of tide state.

- Red knots selected foraging locations with more prey.
    - Early Migration: Red knots chose foraging sites with more blue mussels, miscellaneous prey (horseshoe crab eggs, angel wing clams, and other organisms (e.g., insect larvae, snails, worms)), and all prey.
    - Peak Migration: Red knots chose foraging sites with more crustaceans, coquina clams, miscellaneous prey (horseshoe crab eggs, angel wing clams, and other organisms (e.g., insect larvae, snails, worms)), and all prey.
- Red knot presence was influenced by prey abundance and Tierra del Fuego wintering red knot counts.
    - Early Migration: Miscellaneous prey (horseshoe crab eggs, angel wing clams, and other organisms (e.g., insect larvae, snails, worms)) positively influenced red knot presence.
    - Peak Migration: Blue mussels, coquina clams, and Tierra del Fuego counts positively influenced red knot presence.
- Red knot flock size did not consistently relate to prey abundance.
    - Early Migration: Crustaceans positively influenced red knot flock size.
    - Peak Migration: Blue mussels, crustaceans, and Tierra del Fuego counts negatively influenced red knot flock size.
- Tide, substrate, and water temperature affected prey abundances, demonstrating that red knots track prey resources over space and time while in Virginia.

    - Prey abundances per core sample were orders of magnitude higher on peat than on sand. However, peat banks comprise only ~ 6% of the intertidal zone each year, while sand comprises the remainder of the intertidal zone (Truitt et al. 2001, Bachmann et al. 2002, Cohen et al. 2010a, b, Watts and Truitt 2015). Thus, the abundance of prey in sand is likely much greater than the abundance of prey in peat on the entire barrier island chain.
    - Water temperature had varying effects on prey.
        - Crustacean, blue mussel, and miscellaneous prey (horseshoe crab eggs, angel wing clams, and other organisms (e.g., insect larvae, snails, worms)) abundances increased with warming water, while coquina clam abundance decreased with warming water.
- Only all prey combined varied within 10 meters of a central sampling point during peat spatial tidal sampling, suggesting that larger scale covariates (e.g., water temperature) may affect prey abundance and distribution in a given area or that prey vary at different spatial levels than those we measured.

Chapter 3: "Abundance and origin of blue mussels (*Mytilus edulis*) on Virginia peat banks with implications for the conservation of the red knot (*Calidris canutus rufa*)"

- Blue mussel abundance/core sample decreased over the duration of the study, with the highest abundance/core sample in 2010 ($\bar{x}$ = 538, SE = 85.85) and the lowest abundance/core sample in 2016 ($\bar{x}$ = 34, SE = 6.96).
- Total peat bank length (substrate that supports blue mussel communities) on Cedar Island, Metompkin Island, and Myrtle Island did not vary dramatically between 2013 and

2017 (7.22 km in 2013 and 7.40 km in 2017), suggesting that the decline in blue mussel abundance/core was not biased by available peat bank length.

- Blue mussel umbo $\delta^{18}O_c$ became more ^{16}O enriched (more negative) between 2010 – 2018.
  - The water in which blue mussels started precipitating their shells warmed and/or was influenced by an increase in estuarine water runoff into the ocean over this study's duration.
- Blue mussel umbo $\delta^{18}O_c$ ($\bar{x}$ = -0.23‰, SE = 0.12) were more ^{18}O enriched (more positive) than shell edges ($\bar{x}$ = -0.53‰, SE = 0.20).
  - Blue mussels found in Virginia likely originated in offshore ocean waters between Delaware and Virginia, though they may have originated in ocean water as far north as New York in some years, potentially decreasing the risk of blue mussels being extirpated from Virginia in the near future.

MANAGEMENT IMPLICATIONS

As mentioned in this dissertation's *Introduction*, the United States Fish and Wildlife Service (USFWS) has released research priorities for the red knot, several of which we addressed in this study. Priorities we addressed included: (1) "better characterize the nonbreeding diet and document baseline conditions regarding food resources;" (2) "support ongoing efforts to obtain regional population size estimates where needed to inform management decisions at major migration stopovers (e.g., Delaware Bay, Virginia, South Carolina, Georgia) and to expand such efforts to additional areas as important new stopovers are identified;" and (3) "communicate scientific findings to partners and the public, emphasizing the red knot's linkages to climate change, other shorebirds, the Arctic, and other imperiled coastal species" (USFWS 2015, 2019a, b). Addressing these priorities will help us better predict how red knots will fare in the face of global climate change, and to adjust management plans accordingly. Our research described here, combined with previous studies of red knots in Virginia and the Delaware Bay (e.g., Tsipoura and Burger 1999, Watts and Truitt 2000, 2015, Truitt et al. 2001, Baker et al. 2004, Karpanty et al. 2006, 2011, Gillings et al. 2007, Cohen et al. 2009, 2010a, b, 2011, Fraser et al. 2010, 2012, Smith and Robinson 2015, Lyons et al. 2018), supports the following management recommendations, based on the three aforementioned USFWS priorities.

USFWS Priority 1: "Better characterize the nonbreeding diet and document baseline conditions regarding food resources" (USFWS 2019).

- We demonstrated that non-bivalve prey (e.g., crustaceans) were more available to red knots than selected bivalves, but that they were not selected specifically. This relationship could be important if warming ocean temperatures (Hansen et al. 2006, Jones et al. 2010) and ocean acidification (Kurihara 2008, Doney et al. 2009, De Moel et al. 2009) negatively affect bivalve prey in the future, as red knots in Virginia may be able to rely more heavily on abundantly available non-bivalve prey, like crustaceans, which generally are less sensitive to ocean acidification than bivalves. However, while some crustaceans may be better able to combat the deleterious effects of ocean acidification because they have thinner calcified structures and metabolic mechanisms that more effectively regulate pH than bivalves, crustaceans are likely not immune to the effects ocean acidification and may also experience shell thinning (Whiteley 2011, Wittman and Pörtner 2013). Managers should continue to work with policy-makers on implementing laws and regulations to reduce emissions that contribute to ocean acidification and global climate change.

- We demonstrated that blue mussel abundance declined over the duration of this study. If ocean temperatures continue to increase (Rayner et al. 2003, Figueira and Booth 2010), the blue mussel's range may contract farther northward (Jones et al. 2010, Wethey et al. 2011, Hale et al. 2017), making them less available to red knots using Virginia stopover habitat in the future. This could make replenishing the fat stores needed for migration and breeding more challenging for red knots stopping over in Virginia if red knots are unable to rely more heavily on other prey items.
- Because Virginia's intertidal peat banks support an abundance of red knot prey, managers should continue ongoing protection of peat bank habitat by allowing natural island migration and turnover processes. Other coastal locations should also consider management actions that encourage natural barrier island processes, as peat bank formation could benefit numerous shorebird species.

USFWS Priority 2: "Support ongoing efforts to obtain regional population size estimates where needed to inform management decisions at major migration stopovers (e.g., Delaware Bay, Virginia, South Carolina, Georgia) and to expand such efforts to additional areas as important new stopovers are identified" (USFWS 2019).

- Long-term studies, such as this, are required to illustrate potential interannual variations in the factors that affect long-distance migrants, like the red knot. We found that the predicted number of red knots using Virginia during peak migration, while variable, showed no increasing or decreasing linear trend. Additionally, our 12-year average predicted peak red knot count (x̄ = 7,193, SE = 735.19) was similar to peak counts conducted by Watts and Truitt between 2007 – 2014 (x̄ = 6,787; B. Watts personal communication November 15, 2012 and August 19, 2014, Watts 2013, USFWS 2014).

- The continued protection of over 90% of Virginia's barrier islands by The Nature Conservancy, USFWS, the Virginia Department of Game and Inland Fisheries, and the Virginia Department of Conservation and Recreation's Natural Heritage Program (Watts and Truitt 2015), as well as of other stopover sites, is important to red knot conservation in Virginia. Managers in Virginia should continue to discourage beach stabilization and nourishment projects on the 11 barrier islands we studied, as these processes may negatively affect prey availability for red knots and other shorebirds by preventing island transgression and the formation of peat banks (Saloman 1974, Oliver et al. 1977, NRC 1995, Rakocinski et al. 1996, USACE 2001, Menn et al. 2003, Bishop et al. 2006, Rosov et al. 2016). Managers in Virginia and on other Atlantic Coast stopover grounds (e.g., Florida, Georgia, South Carolina, North Carolina, and the Delaware Bay; USFWS 2014) should also continue the limitation of anthropogenic activity, which may disrupt foraging and roosting birds' behaviors (Lilleyman et al. 2016, van der Kolk et al. 2019), particularly during migration and breeding periods.

USFWS Priority 3: "Communicate scientific findings to partners and the public, emphasizing the red knot's linkages to climate change, other shorebirds, the Arctic, and other imperiled coastal species" (USFWS 2019).

- Throughout the duration of this dissertation, we have presented our research at:
    - 8 national and international professional meetings
    - 14 regional and local professional meetings

    - 8 public outreach events

FUTURE RESEARCH NEEDS

While the research described in this dissertation has helped fill many knowledge gaps, we acknowledge that additional research is necessary to further enhance our understanding of the factors that affect red knots not only in Virginia, but also elsewhere in their wintering, breeding, and other stopover sites. Here, I divide future research needs into 2 broad categories, studies pertaining to red knots 1) during their spring stopover in Virginia specifically and 2) year-round.

Red knots during their spring stopover in Virginia:

- We found that red knots in Virginia selected bivalves over non-bivalve prey, despite the greater availability of non-bivalve prey. Future study should combine fecal DNA metabarcoding analyses with calorimetry analyses to ascertain if prey selection relates to the caloric content of prey and to predict if and/or how red knots alter their foraging behaviors to adapt to changing prey availability in Virginia.
- We counted red knots and sampled prey on peat banks exclusively during the early migration period. During peak migration, we counted red knots and sampled prey on both sand and peat substrates, depending upon which substrate was at each sampling point. Peat banks comprise only about 6% of the shoreline in a given year (Truitt et al. 2001, Cohen et al. 2010a, b, Watts and Truitt 2015), thus, we predominantly sampled sand during the peak migration period. However, both early migration and peak migration demonstrated the importance of peat bank habitat to prey that red knots consumed. Future study should consider more heavily sampling peat banks during peak migration

specifically, as the approach we used may have biased some of our results due to the relatively low sampling conducted on peat banks during the peak migration period.

- While red knots are generally considered long-distance migrants, birds stopping in Virginia travel from numerous wintering grounds throughout North and South America (Gonzalez et al. 1996, D'Amico et al. 2014, Federal Registrar 2014, Watts and Truitt 2015). Our study did not address how the distance between wintering and stopover grounds may affect prey selection, nor did it address any potential relationships between bird age or sex and foraging strategies. Less-experienced juveniles may not exhibit the same foraging behaviors as adults; additionally, female red knots have slightly longer bills than males (Tomkovich 1992, Baker et al. 1999) and thus may be able to access additional prey (Page and Fearis 1971, Carter 1984, Stein et al. 2008). Future research should consider using resight methodology combined with fecal DNA metabarcoding analyses to help determine if longer-distance migrants (i.e., those that winter farther south) select different prey than shorter-distance migrants (Baker et al. 2013, Red Knot *Calidris canutus*, In Neotropical Birds Online 2019) and if bird age and/or sex affects foraging strategies in Virginia (Elner and Seaman 2003, Placyk and Harrington 2004, Nebel 2005, Mathot et al. 2007, Jardine et al. 2015).
- Uncertainty existed around the oxygen isotopic signature ($\delta^{18}O_c$) ranges we calculated for blue mussel umbos, as we used water signatures ($\delta^{18}O_w$) collected in 1993 from Khim and Krantz (1996) to perform the required calculations. Future studies should collect and analyze $\delta^{18}O_w$ of water during their study period to reduce potential biases of using historic data to make current predictions. Future studies could also use more modern lead and/or neodymium isotopes to assist in tracing blue mussel spawning locations using

anthropogenic pollution tracers (Alves 2019, Cong et al. 2019, Zhao et al. 2019, Poitevin et al. 2020). We were unable to use these analyses due to cost.

- We used offshore subtidal buoys to provide the mean daily water temperature data used in our prey analyses, as we could not locate reliable intertidal water temperatures over the course of our study. Intertidal water temperatures are more variable over time and fluctuate more throughout the day than subtidal water temperatures (Lathlean et al. 2011, Nguyen et al. 2011, Diederich et al. 2013). Thus, future studies should measure intertidal water temperatures to get a more accurate representation of the temperature variations prey experience in Virginia, and how these variations may affect prey abundance and distribution across the landscape.

Red knots year-round:

- We were constrained by a lack of genetic information in the reference database (SILVA) that we used for fecal DNA metabarcoding analyses, a restriction that will inherently become less limiting over time, as more researchers contribute to reference databases. Thus, we were only able to identify some red knot prey in Virginia to the Order level; however, we were able to confirm that red knots consumed blue mussels (Order Mytiloida) and coquina clams (Order Veneroida). Future research in Virginia and beyond should obtain species-level data from fecal DNA metabarcoding analyses, as reference information becomes more available.
- While the predicted number of red knots during peak migration in Virginia were variable over this study's duration, we found no significant positive or negative linear trend. Future studies should test for potential cyclic patterns in red knot numbers across their

wintering, stopover, and breeding grounds, as red knots have varied cyclically in the past (Sutherland 1988, Blomqvist et al. 2002, Fraser et al. 2013).

- We found that peat bank habitat is important to invertebrate prey and consequently to red knots in Virginia. In the future, ecologists and geologists across the Atlantic Coast should collaborate to map the distribution and prevalence of historic and present intertidal peat and sand habitats (e.g., using orthophotography imagery of the entire Atlantic Coast and ground truthing from research studies). Emphasis on mapping coastal and peat substrates between Florida and Delaware Bay coasts is recommended, as red knots currently use coastal beaches in these areas, several of which (e.g., Delaware Bay, Virginia, South Carolina, and Georgia) appear to be final stopover grounds for red knots before they continue migrating to Artic breeding grounds (Harrington 2005a, b, SCDNR 2013, USFWS 2014). Further understanding larger-scale availability of both peat bank and sand habitat for foraging shorebirds could enhance our understanding of the importance of both substrates and may provide additional support of natural process management in coastal environments.
- Studying the diet of red knots in Virginia, while informative, only provided information during ~ 2 weeks of the red knot’s annual cycle. Future studies should collect red knot feces across wintering, breeding, and stopover grounds (including, but not limited to Virginia) to gain a more complete understanding of what red knots eat year-round. We also recommend that future research link other potentially relevant factors (e.g., habitat availability and suitability) across wintering, breeding, and stopover grounds.

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