Common Loon Eggshell Porosity & Thickness in North America

A Major Qualifying Project Report

Submitted to the Faculty

of the

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

By

Mary-Lee Barboza, Lusan DellaGrotte, Danielle Karcher, Courtney McGinness

Date: April 30, 2015

Approved:

_____

Professor Jill Rulfs

_____

Professor Mike Buckholt

_____

Professor Mark Pokras

Abstract

The goal of the project was to examine differences between eggshell characteristics of Common Loon (*Gavia Immer*) populations throughout the United States. We measured thickness, water vapor conductance, and pore density of eggshells collected from the Northeast, Midwest, and Northwest regions. Measurements were compared to determine whether eggshell characteristics vary between genetically distinct populations of the Common Loon. The data collected suggests that there is no significant difference between water vapor conductance of eggshells from the three regions studied. In addition, no significant difference in thickness was observed between eggshells from each of the three regions. Although the data indicated a statistically significant difference between the pore densities of eggshells collected from three different regions across North America, many limitations were encountered during this study including limited sample size, time constraints, and lack of relevant literature outlining appropriate methods. Based on our study, we recommend further research and experimentation to validate our findings.

Acknowledgements

We would like to thank Dr. Andrew Vitz, Massachusetts State Ornithologist, for his advice in the beginning stages of our project. Also, thank you to Professor Peter Hefti and the Center for Holographic Studies and Laser Micro-MechaTronics at Worcester Polytechnic Institute for allowing us to use the measure microscope to explore different thickness measurement methods. Thank you to Scott Robinson and L.S. Starrett Company for designing a custom micrometer for our eggshell thickness measurements. Further thank you to Abbie White and JoAnn Whitefleet-Smith for providing us with laboratory space and supplies. We would also like to thank many individuals for providing us eggshell samples and for continuous advice throughout our project: John Cooley and Harry Vogel from the Loon Preservation Committee, Ginger Stout, Dave Evers and Michael Chickering from the Biodiversity Research Institute, and Kevin Kenow from the United States Geological Survey. Also, thank you to Professor Elizabeth Ryder for statistical analysis support and guidance. Lastly, we would especially like to thank our three advisors, Dr. Mark Pokras from the Cummings School of Veterinary Medicine at Tufts University, Dr. Jill Rulfs, and Dr. Mike Buckholt. Thank you for consistently providing us with advice, guidance, and support throughout our research endeavors.

Table of Contents

Table of Tables

Table of Figures

Table of Equations

1.0 Introduction

The common loon is the most prevalent species of loon with a range that spans the entirety of the northern United States and extends throughout Canada and Alaska. Studies have shown that the southernmost range of the species is receding. It is predicted by the Audubon Society that in 50 years common loons will no longer inhabit Massachusetts and that the southernmost range will no longer be in the United States. There are many conservation groups within North America that have made it their goal to preserve the habitat and well-being of this beloved, iconic species. Elucidating the reason for such rapid decline in the common loon habitat range was the original motivation for this study. Specifically, the goal of this project was to examine the effects of climate change on their habitat range.

2.0 Background

2.1 Climate Change

Global climate change is a measurable variation in the statistical properties of a climate system regardless of cause. Global warming refers to an increase in the Earth's surface temperature as a result of anthropogenic actions. Both concepts are often misused interchangeably but have measurable impacts on the adaptive capacity of Earth's ecosystems across the globe.

Natural variations in flora and fauna and in events such as solar radiation, ocean currents, and volcanic eruptions are capable of altering the Earth's climate. The sun, whose cyclical nature is still not fully understood, went into a prolonged sunspot minimum period, commonly referred to as Maunder Minimum, from 1645 through 1715 (Bard et al, 2000). This reduction in sunspot activity contributed to a period of cooling referred to as the Little Ice Age that saw significant glacial expansion (Bard et al, 2000).

Ocean currents play a major role in Earth's climate by redistributing heat deep beneath the sea surface. Short term fluctuations in ocean currents have been observed and recorded such as El Niño Southern Oscillation, La Niña, Pacific Decadal Oscillation, North Atlantic Oscillation, and Arctic Oscillation (Trenberth et al, 2007). These oscillations result in increases or decreases in sea surface temperatures that generate extreme weather such as floods and droughts on a global scale. Despite the short term fluctuations at the surface, the oceans have a

very high thermal inertia and the depths have been recorded to lag in temperature adjustment from the Little Ice Age (Bard et al, 2000).

Fauna and flora, or the lack thereof, also play a major role in the Earth's system by regulating oxygen and carbon dioxide levels in the air. The largest measurable effect occurred during the *Azolla* event 49 million years ago, a period where the temperatures were warm enough for the existence of turtles and palm trees at the poles (Brinkhuis et al, 2006). Over an 800,000 year period, large volumes of *Azolla* ferns bloomed, died, and sank to the bottom of the Arctic ocean effectively removing carbon dioxide on such a scale that the average surface temperature dropped from 22 degrees Celsius, allowing the formation of the Arctic ice caps (Brinkhuis et al, 2006).

Volcanic eruptions also have the potential to alter Earth's climate through the introduction of sulfur dioxide into the stratosphere at concentrations high enough to absorb or scatter solar radiation (Miles et al, 2004). In 1991, the eruption of Mount Pinatubo was recorded as the second largest terrestrial eruption of the 20th century and caused global temperatures to decrease by 0.5 degrees Celsius for up to 3 years in certain regions (Diggles, 2005).

Anthropogenic influences such as burning of fossil fuels, increased animal agriculture, and deforestation have resulted in global warming, which contributes to global climate change. Concentrations of carbon dioxide and methane, two greenhouse gases responsible for warmer temperatures, have increased 36% and 148% respectively since 1750 (EPA, 2007).

2.1.1 Gauging the Impact of Climate Change on Ecological Systems and Species

Scientists are able to measure the impacts of global climate change through observations, testing, and examination of animal remains. The most notable observation indicative of global climate change is the reduction of the Arctic sea ice. Unlike the Antarctic sea ice that melts and reforms each year, the Arctic ice caps remain year after year. Satellite data compiled since 1979 shows a rate of decline of 11.5 percent per decade in the thickness and area of Arctic sea ice (NSIDC, 2014). As a result, the global sea level has risen four inches during that same time period (Nicholls et al, 2010). While scientists are unable to agree on the precise amount sea levels will rise in the future, there is a general consensus that they will; projections in the last seven years have ranged from seven inches to 79 inches by the year 2100. The latest projections included in the Third National Climate Assessment, released on May 6, 2014, indicate an

increase in sea levels of one to four feet by the year 2100 (NCA, 2014). Increases in sea levels are destroying coastal ecosystems and threaten major cities such as Miami, which is listed as the "number one most vulnerable city worldwide," with a potential for 416 billion dollars in damages (Goodell, 2013).

Another notable observation that shows the effects of global climate change is the earlier recorded flowering and fruiting times of various plants and the increased growth of invasive plants. In particular, the Great Lakes have been warming, resulting in increased toxic algae growth, evaporation levels, and frequency of extreme weather events (Bachelet et al, 2001). In 2011, the toxic algae bloom was rated a ten, on a scale of one to ten with ten being the worst, and was followed by years of blooms rated eight or higher. As a result, the Environmental Protection Agency (EPA) has provided 12 million dollars to research and address this problem (Eaton, 2014).

Global land precipitation measurements and dendroclimatology, the examination of tree rings, indicate periods of unfavorable conditions such as drought and how frequently they occur. These techniques have given scientists additional tools to chart climate trends (Hughes et al, 2010). The trend established during the 20th century was an overall increase of precipitation by two percent, with significant redistributions in location and intensity of storms (New et al, 2001). For example, the redistributions of precipitation have caused coastal ecosystems throughout the United States to suffer drought and flash flooding. These changes affect large areas and impact migratory species that seasonally rely on the coastal ecosystems (Hughes et al, 2010).

Different species thrive under varying climate conditions, which impact food webs, a strong metric of ecosystem health (Brown et al, 2010). Autotrophs, which are susceptible to rapid global climate changes, are the producers in the food chain and are necessary to sustain an ecosystem. Precipitation changes and storm intensity changes impact autotrophs on land by reducing life-sustaining water and removing vital nutrients in storm runoff. Autotrophs in water, such as algae, thrive in warmer temperatures with increased nutrients from storm runoff. Algal blooms lead to oxygen depletion, which indirectly kills other organisms in the ecosystem. In addition, algal blooms can produce toxins and kill off subsequent trophic levels (Brown et al, 2010).

Monitoring migration patterns is another strong tool to gauge the impact of global climate change. Scientists have been studying numerous migratory bird species that engage in latitudinal

or altitudinal migrations. American robins (*Turdus migratorius*) engage in altitudinal migration in the Rocky Mountains when the snow melts in the spring. Since the 1980's, American robins have been observed arriving at their spring location approximately one month prior to the availability of adequate food (Francis et al, 2004). Many North American wood warblers engage in latitudinal migration from the tropics. The timing of their migration is largely based on day length, which remains constant despite global warming. As spring temperatures in the United States and Canada increase, one of their primary food supplies, the eastern spruce budworm, hatches earlier than the North American wood warbler arrives resulting in shorter supplies and greater competition (Strode, 2009).

2.2 The Common Loon

Loons are members of the family *Gaviidae*. There are five species of Loons, all of which breed or have bred in North America. The smallest of the five species is the Pacific loon, *Gavia pacifica,* which is on average 24 inches long. The Pacific loon's most identifying characteristic during the breeding season is its black throat with purple reflections on its neck. The species' primary North American breeding area is from Alaska stretching to the Hudson Bay in northern Canada, but during the winter they spend their time along the Pacific coast. Pacific loons are rarely seen in the northeastern United States.

Up until 1985, the Arctic loon, *Gavia arctica*, and the Pacific loon were considered to be a single species, due to their close resemblance. The most visible difference between the two species is that the Arctic loon has a large white patch before the tail. The most unique characteristic of the Arctic loon is the color of their throat, which is either iridescent green or purple. Similarly to the Arctic loon's colorful throat patch, the red-throated loon, *Gavia stellata,* is known for its rusty colored throat patch during breeding season. They breed on small tundra ponds and potholes in Alaska, the Aleutian Islands, and the Canadian Arctic. During the winter they can be seen along both coasts of the United States. In contrast, the Arctic loon's breeding range consists of isolated areas in western Alaska.

One of the larger species of loons is the yellow-billed loon, *Gavia adamsii,* which can grow between 33-38 inches in length. The breeding and winter plumage of the yellow-billed resembles that of the common loon, however, its bill is an ivory-yellow color. The yellow-billed

loon is said to be the least abundant loon to nest in North America; the species breeds primarily in northern Alaska and Canada.

Known for its eerie call, the common loon, *Gavia immer*, is the most commonly identified loon species due to its wide range of habitat. An adult common loon and its chick is shown in Figure 1. The common loons' breeding range spans from the Aleutian Islands of Alaska to northern Canada in addition to California, Montana, and New England. They spend their winter on the Great Lakes, and along both coasts of the United States. Due to their vast habitat range, our team has decided to focus on the common loon as our research species.

Figure 1: Adult common loon and chick (Holland, 2012).

In a species profile, Harry Vogel and Kate Taylor of the Loon Preservation Committee (LPC) researched the habitat and the distribution of the common loon across the state of New Hampshire to develop a plan to conserve this species. Vogel and Taylor's findings indicate that the common loon prefers to nest on fresh-water lakes that are between 16-60 acres and contain either small islands or coves. Typically the nest is built one meter or less from the shoreline due to loons' difficulty walking on land, and can contain steep drop-offs for entrance and exit from the water. The presence of shoreline vegetation is known to be beneficial for young chicks to protect themselves from predators. Therefore, undisturbed island shorelines can provide a wide range of visibility and protection from predators (Vogel & Taylor, n.d.).

Not only has the LPC surveyed the distribution, but they have also been recording the reproductive success of the common loon in New Hampshire for many years to examine trends. Their results, which were recorded from 1978 to 2000, revealed a significant decline ($P < 0.05$) in loon reproduction success starting in 1982. Their study showed sign of declining adult New Hampshire loon population in a succession of five years. (Vogel & Taylor, n.d.). As a result, the LPC identified major problems that inhibit the population growth of the common loon and developed strategies to help protect them. Their main findings of habitat loss were due to shoreline development, motorized and non-motorized water crafts, lead fishing gear, and mercury. The LPC took the initiative to develop artificial nesting islands (rafts), which protected the nest from both fluctuating water levels and predators, such as raccoons which account for 80% or more of depredated nests. In their short-term monitoring, the rafts had a success rate of 1.2 or higher clutch size, which is the expected clutch size of a healthy common loon pair. (Vogel & Taylor, n.d.).

2.2.1 Changing Loon Habitat

The population of the common loon in areas such as New England has decreased over the last century. Over 500 common loons were found dead in this region due to both natural and anthropogenic factors. Some of these factors include trauma for both chicks and adult loons, as well as infection and lead ingestion in adult loons (Sidor, et. al, 2003).

The common loon population in the western United States also has experienced a contraction in its breeding range over the past few decades. Loons in this area are threatened from factors such as direct human disturbances at the shoreline and water level fluctuations as a result of climate change. Wyoming's common loon population is at a high risk of extinction from these factors, as well as the small, isolated nature of these populations. Loon pairs in Yellowstone Park are especially at risk, decreasing from 18 pairs around 2005 to only nine pairs in 2013. Since Wyoming's loon population is separated from other populations by over 220 miles, recolonization from other areas is extremely difficult (Biodiversity Research Institute, 2013).

The Audubon Society predicts that the habitat range of the common loon will continue to decrease as a result of climate change. Recent studies predict that by the year 2080, 56% of its' current summer range and 75% of its' winter range will decrease. Figure 2 depicts this

prediction, with the solid black line as the current loon range and the red area as the future range (Audubon Society, 2014).

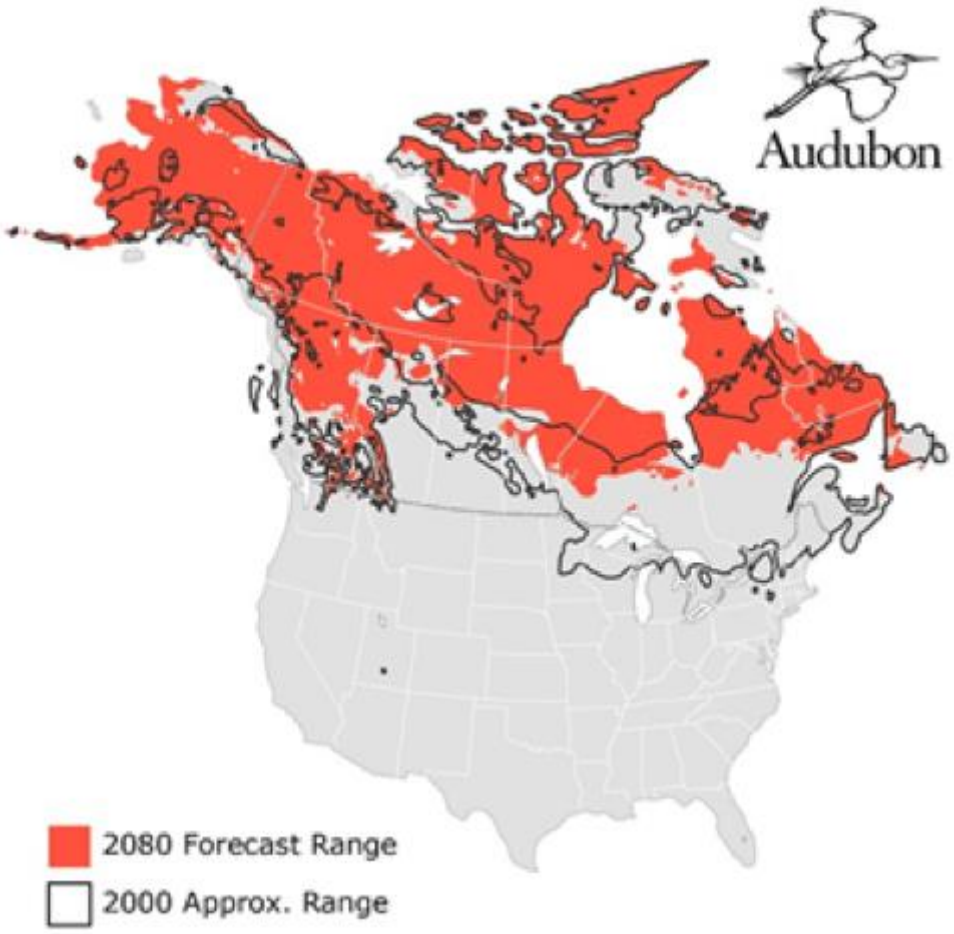

Figure 2. Loon habitat range shift.

As seen in Figure 2, the common loon habitat range is predicted to decrease significantly as a result of climate change, especially in the eastern side of the continent.

On the other hand, it is also hypothesized that the common loon may take time to adapt to the changing environment and eventually be able to live in more southern regions once again. It is possible that the common loons are moving north for the time being, but may migrate back to their original range once they have adapted to the changing climate. John Fitzpatrick, Director of the Cornell Lab of Ornithology, explains that many other factors in addition to climate change can affect birds and their movements. Both the plant and animal communities experience delayed

responses and undergo species-interactions that could delay or mitigate the temperature based results of the Audubon Society study (Dawes, 2014). Furthermore, some papers indicate that loon breeding range has actually expanded in the past few decades. A 2011 paper states that the breeding range of the common loon has expanded along the southern periphery of the habitat range in Michigan (Kaplan *et al*, 2010). According to the Wisconsin Department of Natural Resources, total loon number as well as the number of breeding pairs in Wisconsin is increasing. Wildlife toxicologist Michael Mayer describes the expanding loon population in Wisconsin, "We are seeing higher densities of nesting pairs on lakes and we are seeing loons using lakes that they haven't used for decades, plus there is a suggestion they are expanding southward" (Booth, 2013). Despite severe national declines in the common loon population, the population is now believed to be stable or increasing. Although populations are not expected to reach historic levels, the common loon population in New England appears to be increasing (United States Department of Agriculture, n.d.). Other surveys suggest that although the loon habitat range is declining, the rate of decline is not significant enough for the common loon to be classified as "vulnerable" on the IUCN Red List (BirdLife International, 2015). The common loon is classified as a population under "least concern" because of the species' wide habitat range (BirdLife International, 2012).

In 1961, the common loon became Minnesota's state bird as it was one of the predominant figures in the traditional artwork of the Native American people who resided there (Svingen & Herzel, 2000). As populations of the common loon began to decline, several research studies were conducted to redefine the expanse of the common loon's habitat. In a survey of the common loon in central Minnesota, they found that common loons were living in small lakes (less than 50 acres), which had previously been considered too small to support the needs of the common loon. This was presumably due to higher susceptibility of nest washout as a results of large waves and stronger wind. In 1987, the survey recorded five lakes between 13-20 acres that each hosted a pair of common loons. It was found that each pair was successful in reproducing offspring. With this knowledge, a two-year survey was conducted to determine what proportion of the lakes less than 50 acres were being used, and if the results were significant, to include them in a statewide population survey. The results showed an average 35% of at least one loon residing on a lake between 10-24 acres over two years. Comparatively, they found on average 36% of at least one loon present on a lake between 25-49 acres in the same time span. Although

they stated each lake may have their own individual characteristics that can have effect on the presence of the common loon, their results opened up speculation on the ability of loon pairs to nest in smaller habitat territories (Perry & Woizeschke, 2000).

Considering the results of various studies performed by different conservation groups, the current status of the common loon habitat range and population depends on the scope and location of individual studies.

2.3 Studying the Avian Eggshell

The microstructure of avian eggshells can be studied using standard microscopy techniques to analyze thin layers of eggshell. Additional techniques such as electron scanning microscopy and X-ray microtomography can be employed to create three-dimensional renderings of eggshells (Riley *et al*, 2014). Avian species typically incubate their eggs in an insulated nest to keep the eggs warm while the chick develops within an egg (Ar & Rahn, 1985). The hard avian eggshell further influences embryo development, as it creates a warm and protective "embryonic chamber" that promotes rapid growth and development (Riley *et al*, 2014).

The avian eggshell is composed of six layers. The two innermost layers are the uncalcified inner shell membrane and the uncalcified outer shell membrane, which surround the egg's albumin. After the two uncalcified layers, the innermost layer of the calcified shell is the mammillary knob layer, which is followed by the palisade layer, the vertical crystal layer, and finally the cuticle (Nys *et al*, 2004). The calcified protective material that forms an avian eggshell is impervious to gases and water, but microscopic pores that extend through the eggshell allow gases and water vapor to diffuse across the eggshell (Ar & Rahn, 1985). Figure 3 is a diagram of the general structure of an avian eggshell.

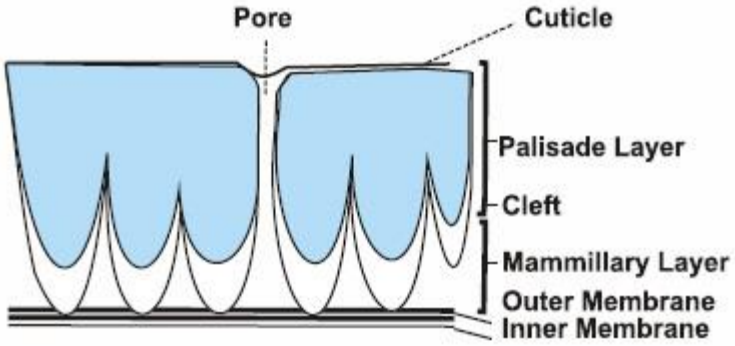

Figure 3. Structure of an avian eggshell (Maintaining eggshell quality, 2008).

As seen in Figure 3, the pore forms a canal through the eggshell, allowing for exchanges between the inner egg and the environment.

Pores are very important to the respiration and growth of the developing chick inside an egg. Before the chick is able to use its own lungs to conduct gas exchange, all gas exchange for the embryo occurs by diffusion through pores in the eggshell. As the embryo's metabolic needs change during different stages of development, so do the partial pressures of gases within the egg's airspace (Ar & Rahn, 1985). There are many parameters of avian eggshell pores that can be measured including number of pores, pore length, pore density, and pore cross-sectional area. Several studies have elucidated the effects these pore parameters have on the growth and development of developing embryos.

The hatchability of avian eggs depends on various ecological, geographical, and social factors including nest type, avian diet, temperature, humidity, and latitude. A comparative analysis of 155 studies examining 113 avian species posits that hatchability increases as populations are farther from the equator. Still, some of the causes of decreased hatchability are not clear (Koenig, 1982). Characteristics of avian eggshells such as size, thickness, number of pores, length, weight, and volume have been widely studied to elucidate the importance of these measurements in relation to avian reproduction, development, and survival (Boersma & Rebstock, 2009)(Anderson *et al.*, 1970). The importance of eggshell porosity has been studied in many species.

For example, a study examining the eggshells of 161 species found that eggshells with high pore density and large pore diameter have high gas exchange rates, while eggshells with large pore length have low gas exchange rates (Boersma & Rebstock, 2009). Gas exchange through an eggshell allows oxygen and carbon dioxide to pass through pores in the shell, which, without pores, is impermeable to gases. A gas space adjacent to capillaries lies between the outer and inner shell membranes. Gases diffuse from the capillaries into the air and from the air into the capillaries (Rahn and Paganelli, 1982). Water vapor is also lost through eggshell pores. Dehydration is prevented by the creation of metabolic water by the developing embryo and the existence of water inside the egg at oviposition (Ar & Rahn, 1980). Water loss from the egg depends on the number of pores in the shell, shell thickness, temperature, time, and pressure (Board, 1982).

Studies have also compared eggshell porosity between species. In a 2012 study, the eggshells of brown-headed cowbirds (*Molothrus ater*) were compared to the eggshells of their hosts, the red-winged blackbird (Age*laius phoeniceus*), and the dickcissel (*Spiza Americana*). In this study, eggshell thickness was measured, the number of pores were counted, and the gas conductance was calculated. Cowbird eggshells were thicker than those of the red-winged blackbird and the dickcissel. Of the three species, the eggshells of the cowbird had the greatest total pore area and had the highest a greater rate of gas exchange. In this study, the results supported the hypothesis that the rapid development of cowbirds is associated with increased eggshell porosity (Jaeckle *et al.*, 2012).

In addition, environmental factors affecting eggshell porosity and affecting the development of eggshells have been studied. In a 2012 study, eggshell traits including mass, thickness, pore density, and pigmentation were compared across 15 populations of pied flycatchers (*Ficedula hypoleuca*). The populations sampled cover the majority of the species' breeding range in Europe, ranging in latitude from 41°N to 69°N and in longitude from 24°E to 60°E. The study found that between populations, there was variation in all eggshell traits except pore density, but this variation was not dependent on latitude or longitude. In addition, eggshells were found to be thicker in populations where oviposition occurs at high ambient temperatures (Morales *et al.*, 2012).

2.3.1 Loon Eggshell Characteristics

Loon eggs have an average size of about 8.7cm in length and 5.5cm in width. Adult loons typically lay two eggs that are olive green in color with dark spots. Due to the large size of the egg and the amount of energy required to produce them, only two eggs are normally hatched. Nest sizes with three or four eggs have been documented, but are extremely rare. The specific egg coloring allows for better protection from predators, as it blends in with the surrounding nest area. Common loon nests are located on the shore with no covering, which makes them extremely vulnerable to predators such as eagles, gulls, egg eating mammals like raccoons, and snapping turtles (Journey North, 2014).

A loon eggshell is covered by a layer called the "inorganic spheres layer" that prevents the pores from closing and suffocating the embryonic chick (Yahya, 2001). The thickness of a loon eggshell varies with location, but can range from about 0.55-0.66mm, depending where on

the shell measurements are taken. This measurement includes the associated membranes of the egg. Studies have shown that the size and thickness of an egg decrease when exposed to lower than normal pH levels, which may affect the viability of the loon chick (Pollentier, et. al, 2007).

The pH of lakes has been shown to greatly affect the reproductive success of piscivorous birds such as loons. For example, a decrease in pH increases water clarity which gives visual predators a higher foraging efficiency, decreasing the amount of prey available for loons. Furthermore, certain loon prey are only tolerant at pH levels greater than 5. An increase in lake acidity has been shown to cause a high brood mortality. Also, fledging success has been found to be highly unlikely at a pH of 4.0 to 4.3. An increase in lake acidity also can result in altered availability of toxic metals such as mercury and aluminum. This has shown lower chick survival rates and was linked with reproductive risk in many observed lakes (Evers, 2004).

Lake pH has also been examined in relation to eggshell thickness and volume. A study performed on loon eggshells collected from Wisconsin lakes found that eggshells were three to four percent thinner on lakes with a pH less than or equal to 6.3 than on neutral-pH lakes. Furthermore, the egg volume of eggs collected from neutral-pH lakes tended to larger than those from acidic lakes. The study suggests that low lake pH may be associated with thinner eggshells and reduced egg volume in loons (Pollentier, et.al., 2007).

Pore number in bird eggshells has been studied based on a ratio between the observed number of pores and the predicted number of pores. Loon eggshells have a ratio higher than one, indicating that there was a higher number of observed pores than predicted. This is because loons live in wet environments where transpiration of water is higher. Loons can have a high number of pores because living in lakeshore areas, they are not often at risk of losing a large amount of water through the pores (Donaire, et.al, 2009).

3.0 Methods

3.1 Sample Acquisition

With the help of the United States Geological Survey, the Biodiversity Research Institute, and the Loon Preservation Committee, Common Loon eggshells were collected from various regions throughout the United States. Specifically, 132 samples from the Northeast region (MA, VT, NH, ME, NY), 16 samples from the Midwest region (WI), and 8 samples from the Northwest region (WA and MT) were collected and studied. Sample collection dates ranged from 1987 to 2014. Sample collection locations ranged in latitude from 42^{o}N to 61^{o}N and ranged in longitude from -150^{o}W to -68^{o}W. The samples were received either in the form of nearly whole eggshells or eggshell fragments from individual eggs. All samples were shipped to the WPI Project Laboratory in Goddard Hall by the United States Postal Service, with nearly whole eggshell samples housed within appropriately sized cylindrical plastic containers and fragments of eggshells packaged in separately labeled plastic snack and sandwich sized bags.

3.2 Eggshell Preparation

A procedure for cutting the eggshells was adapted from the procedures of Tharapoom, K. (2006) and Portugal, *et al.* (2010). Using a Dremel rotary tool on low power with a 545 Diamond Wheel attachment, six fragments were cut from the equatorial region of each nearly whole eggshell sample received. During this process, masks and safety eyewear were worn to prevent eggshell dust inhalation and to protect eyes from eggshell particles. For fragmented eggshell samples, six fragments were selected based on size and curvature. Fragments larger than 1 cm^2 with little curvature were selected, as these qualities suggested that the fragments originated from the equatorial region of the eggshell. Large fragments were trimmed to an appropriate size using the Dremel rotary tool with 545 Diamond Wheel attachment. A depiction of such methods can be seen in Figure 4.

Figure 4: Using a Dremel tool to cut equatorial pieces from a sample received.

The six fragments from each egg were stored in compartmented plastic bead organizers that were labeled according to a coding system developed to keep track of each eggshell sample. Some eggshell samples received were already catalogued using a pre-determined coding system. If samples obtained were not organized in such a manner, a coding system was created to identify the fragments based on location and date of collection. For each sample, three of the six selected fragments were set aside for thickness measurements and water vapor conductance tests while the remaining three fragments were set aside for pore counting.

For each eggshell characteristic explored in this study, three fragments per sample were tested for several reasons. Specifically, reviewing the methods used by past studies looking at eggshell characteristics of various bird species revealed varying sample sizes yielded results of statistical significance. Furthermore, there was no previously collected data of the three variables being tested in this study for the Common Loon and the physical states of the samples received for this study were variable. Due to these circumstances, it was decided for statistical purposes to call n=1 one whole eggshell, with n=1 being represented by three fragments of one individual sample.

Once fragments were selected and organized for testing, a procedure adapted from Stein, L. (2009) was used to treat all samples to remove their inner membranes and any organic material remaining. For four minutes, each eggshell fragment was submerged in a dish of 5%

Sodium Hydroxide (NaOH) (80-90^{o}C) on a hot plate. The equipment used to remove membranes from the eggshell fragments can be seen in Figure 5.

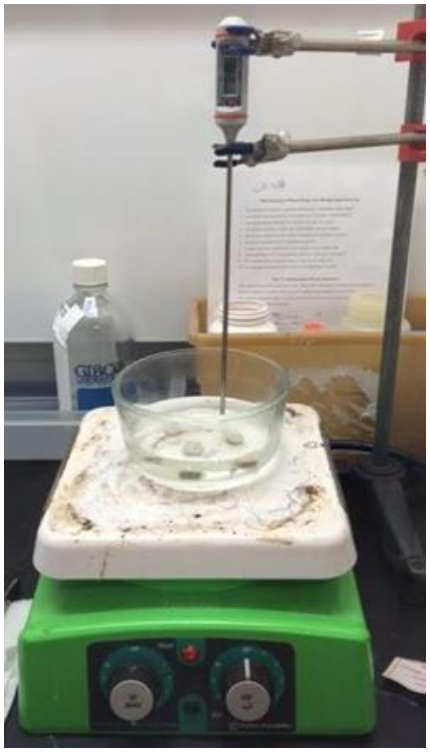

Figure 5: Apparatus for removing membranes from COLO eggshell fragments.

A 470 mL Pyrex bowl, filled approximately halfway with 5% NaOH, was placed on a hot plate positioned inside a fume hood. A thermometer was placed inside the base to ensure the temperature was raised to and stayed within the desired range. An example of the appearance of the samples towards the end of the four minute increments is depicted in Figure 6.

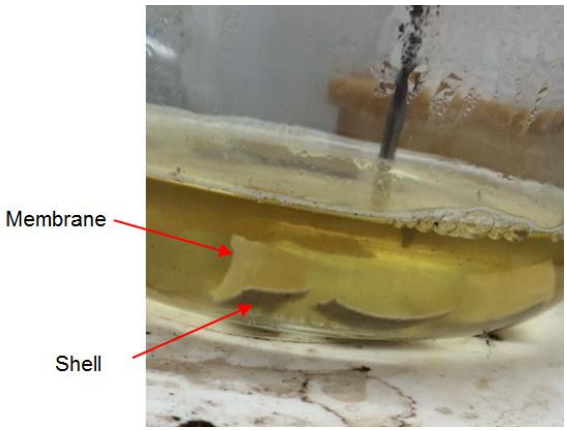

Figure 6: Treatment with 5% NaOH for membrane removal.

The reaction was stopped by immersing the fragments in deionized water and the eggshell fragments were allowed to dry. To enlarge the pores of the eggshell fragments used for pore counting, these fragments were additionally submerged in a 5% Nitric Acid (HNO_3) solution (room temperature) for seven seconds, then dipped in deionized water to stop the reaction and, again, allowed to dry.

3.3 Water Vapor Conductance

The protocol followed for measuring the water vapor conductance of each egg was adapted from Portugal, *et al.* (2010). For each egg, the water vapor conductance of three equatorial fragments, labeled A, B, and C, were measured.

The caps of 0.25 mL microfuge tubes were removed and the tubes were filled with 200 uL of deionized water and labeled according to a coding system created to identify each eggshell fragment. Using the provided applicator, Loctite Ultra Gel Control super glue was applied to the top of each 0.25 mL microfuge tube and the equatorial eggshell fragment corresponding to the code on the tube was pressed to the top of each tube for about 30 seconds until an adequate bond had formed. After being left to dry for about 30 minutes, Loctite UltraGel Control super glue was applied at the junction of the underside of each eggshell fragment and the circumference of each microfuge tube, as seen in Figure 7.

Figure 7: Eggshell super-glued to top of a 0.25 mL microfuge tube.

The microfuge tubes with attached eggshell fragments were stored in 0.25 mL polymerase chain reaction trays. An initial weight (g) to four decimal places was obtained for each tube , then each

tray of tubes was placed in a sealed desiccator filled with newly dried desiccant; two (10” x 10” x 8”) and one (5.5” x 5.5” x 6”) desiccators were used. The desiccators were stored in an incubator set to 25^{o}C, as seen in Figure 8, and were removed for weighing at the same time each day in 24 hour increments.

Figure 8: Desiccator placed in incubator set to 25^{o} C.

Measurements (g) were obtained 24 hours, 48 hours, 72 hours, and 96 hours after the start of each water vapor conductance trial.

To measure water vapor conductance, total water loss for each fragment was calculated by subtracting the weight of each tube at 96 hours from the initial weight of that tube. For most eggshell samples, measurements were obtained for three fragments. For statistical analysis, the median water loss value was selected for each egg. Occasionally, a fragment was discounted if it broke before measurements were completed, resulting in only two fragments for that sample. In this case, an average of the two total water loss values was calculated. Median water loss values were used to calculate average water loss (water vapor conductance) for eggshells from each of three regions--the Northeast, Midwest, and Northwest. An Analysis of Variance (ANOVA) test

was performed on the median water loss values of each region to further analyze the data and to determine if the data had statistical significance.

3.4 Pore Density

The average pore density of each egg was estimated based on the procedures of Ar & Rahn (1985), Morales, *et al.* (2013), and Stein, L. (2009). Using a 1 cm^2 mat of known area, constructed with masking tape, and an ultra-fine point Sharpie permanent marker, a 1 cm^2 area was traced onto the inner surface of each eggshell fragment treated with hot NaOH and HNO_3. An example of a mat of known area can be seen in Figure 9 below.

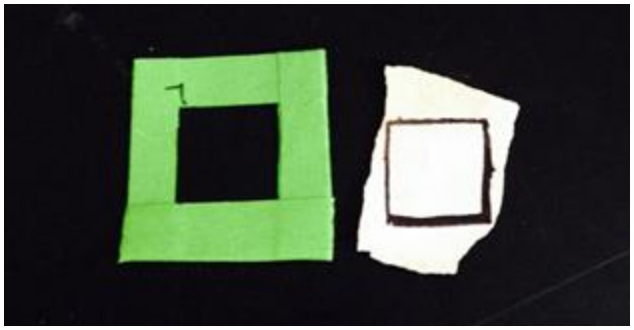

Figure 9: 1 cm^2 mat used to trace known area on inner surface of eggshell.

For ease of counting, lines were drawn within the 1 cm^2 area to separate the area into 9 squares of about equal size.

Fragments were viewed convex side up, using a Zeiss Axio.Vert A1 inverted light microscope at 50X magnification. Pores were visible as points of light coming through the eggshell and strict pore counting guidelines were determined. All distinct, circular points of light were counted regardless of size and a fragment was discounted if it had a crack running through it large enough to disturb the integrity of the pore count. The variation of pore size, and pore size distinction can be viewed in Figure 10.

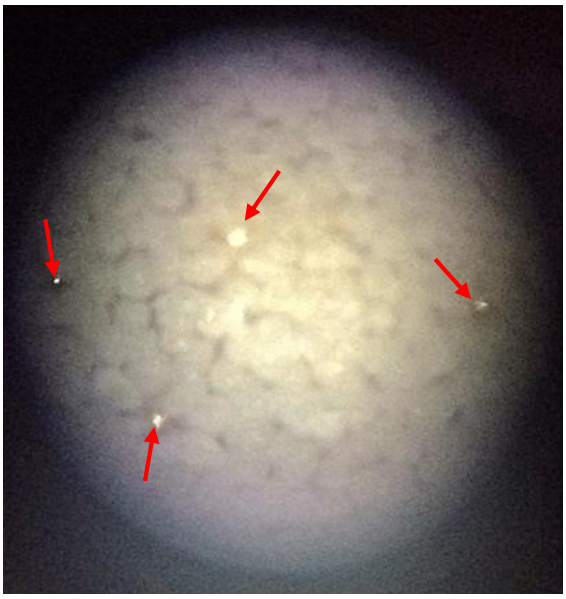

Figure 10: Pores observed under a light microscope (50X magnification).

Hand tally counters were used to keep track of the number of pores counted. For each eggshell sample, pores were counted on two to three different equatorial fragments. To ensure consistency, two different group members performed a pore count for each fragment of each egg. For each fragment, the mean of the two group members' counts was determined, then the mean of the averages for each fragment were calculated, resulting in an average pore density for each egg. An ANOVA test was performed on the final pore density values of each region to further analyze the data and to determine if the data had statistical significance. Because the ANOVA results indicated that there was a statistically significant difference in pore densities between regions, F-tests and t-tests were performed to compare regions and determine which regions had statistically significant differences in pore density.

3.5 Thickness

Based on the procedures for measuring eggshell thickness of Pollentier *et al.* (2007) and Portugal *et al.* (2010), thickness measurements were obtained using a custom 1010M Starrett micrometer with round anvils, precision of 0.001 mm, and standard pressure of 75 grams. The custom micrometer can be seen in Figure 11.

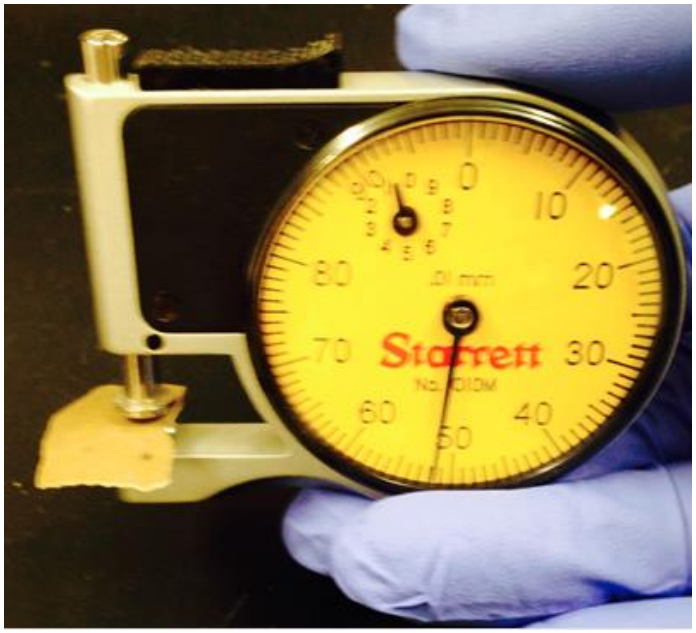

Figure 11: Obtaining thickness measurements with a custom 1010M Starrett micrometer.

Adapted from the procedure outlined in Morales *et al.* (2013), three thickness measurements were taken on at least three different equatorial fragments of each eggshell by two different group members, totaling at least 18 measurements per eggshell. Using these measurements, an average was calculated for each egg sample. An ANOVA test was performed on the final thickness values of each region to further analyze the data and to determine if the data had statistical significance.

4.0 Results

Throughout this study, we examined the water vapor conductance, pore density, and thickness of common loon eggshells from the Northeast, Midwest, and Northwest regions of the United States. The final averages and medians that were analyzed to statistically evaluate the data can be found compiled in Appendices 8.1-8.3.

4.1 Water Vapor Conductance

The average percent water loss for common loon eggshells in the three regions studied was calculated by performing water vapor conductance experiments over the course of four days. Three fragments from one eggshell were examined and the median percent water loss value was taken to be the percent water loss for that single eggshell. The median percent water loss of the three fragments represented n = one eggshell. The median percent water loss values for all the eggshells in one region were averaged to determine the average percent water loss for that specific region (Table 1). Table 1 also shows the standard deviation and standard error for each region. The standard error was calculated by using Equation 1:

Equation 1: Standard Error for Medians

$$\text{SE} = \frac{Standard\ Deviation}{\sqrt{n}}\ X\ 1.253$$

Table 1: Average percent water loss by region.

Region	Sample Size (n)	Average % Water Loss	Standard Deviation	Standard Error
Northeast	115	2.67	1.96	0.228
Midwest	16	2.73	1.93	0.606
Northwest	8	2.56	2.11	0.934

As seen in Table 1, the average percent water loss across regions were extremely similar to each other with a range of only 0.17. Figure 12 displays the average percent water loss graphically, with the standard error values of Table 1 used to create the error bars for each region. As seen in Figure 12, the error bars of the three regions overlap each other.

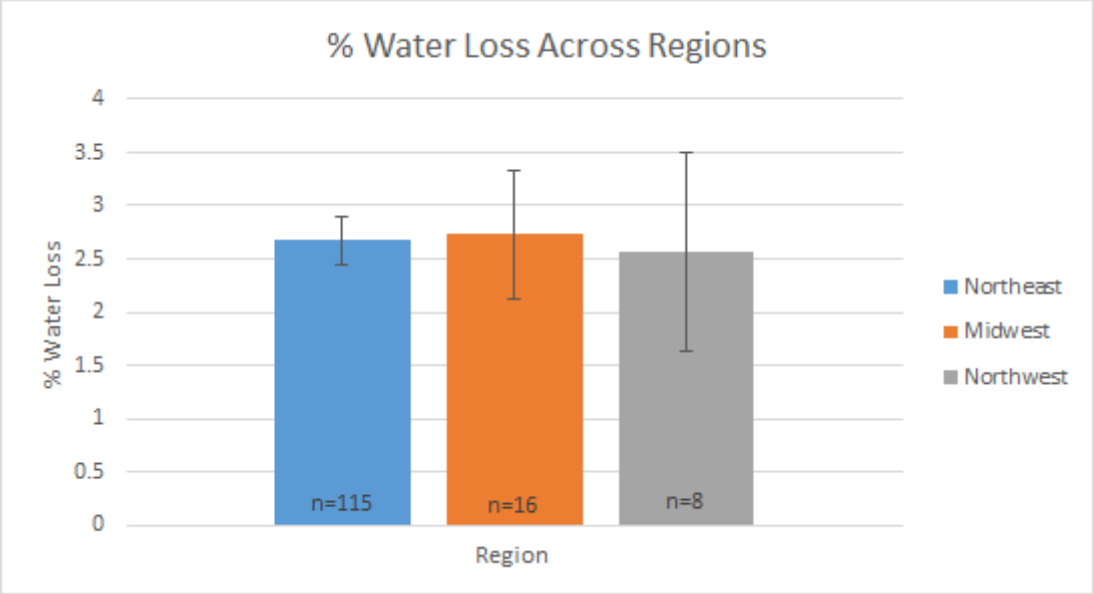

Figure 12: Average percent water loss across regions.

An Analysis of Variance (ANOVA) test was performed on the percent water loss data to determine if the values from each region were statistically different from each other. As seen in Table 2, the P-value for the percent water loss across the three regions is 0.979.

Table 2: Percent water loss ANOVA.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Northeast	115	307.1539	2.670903	3.821667
Midwest	16	43.7283	2.733019	3.736371
Northwest	8	20.46558	2.558197	4.439318

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.163294	2	0.081647	0.02124	0.978987	3.0627
Within Groups	522.7909	136	3.84405			
Total	522.9542	138				

4.2 Pore Density

The average number of pores per one square centimeter were found for each eggshell by examining eggshell fragments convex side up, using a Zeiss Axio.Vert A1 inverted light microscope at 50X magnification. For one eggshell, the number of pores in the square centimeter were counted from two to three fragments by two group members. The average for each fragment was calculated and the average pore number for one eggshell was calculated by averaging the means of each fragment. This final average for a single eggshell represented n = one eggshell. Table 3 shows the average number of pores per square centimeter for each of the three regions studied, as well as the standard deviation and standard error. The standard error was calculated by using Equation 2.

Equation 2: Standard Error for Averages

$$SE = \frac{Standard\ Deviation}{\sqrt{n}}$$

Table 3: Average pore count per one square centimeter across regions.

Region	Sample Size (n)	Average Number Pores per cm^2	Standard Deviation	Standard Error
Northeast	34	246.7	120.2	20.6
Midwest	11	162.6	66.54	20.1
Northwest	7	305.4	147.9	51.7

Table 3 displays the average number of pores per cm^2 across the three regions, with a range of 142.8 pores/cm^2. The Northeast n value varied compared to the previous experiments because of discounted fragments. Fragments were discounted because they did not have a flat one centimeter square surface. Additionally, if a sample did not have at least two fragments to count, then that entire eggshell sample was discounted. Figure 13 displays the average pore numbers from Table 3 graphically, using the standard error values for the error bars of each region. As seen in the figure, the error bars of the Northeast and Northwest overlap each other, yet these two error bars do not overlap the error bar of the Midwest region.

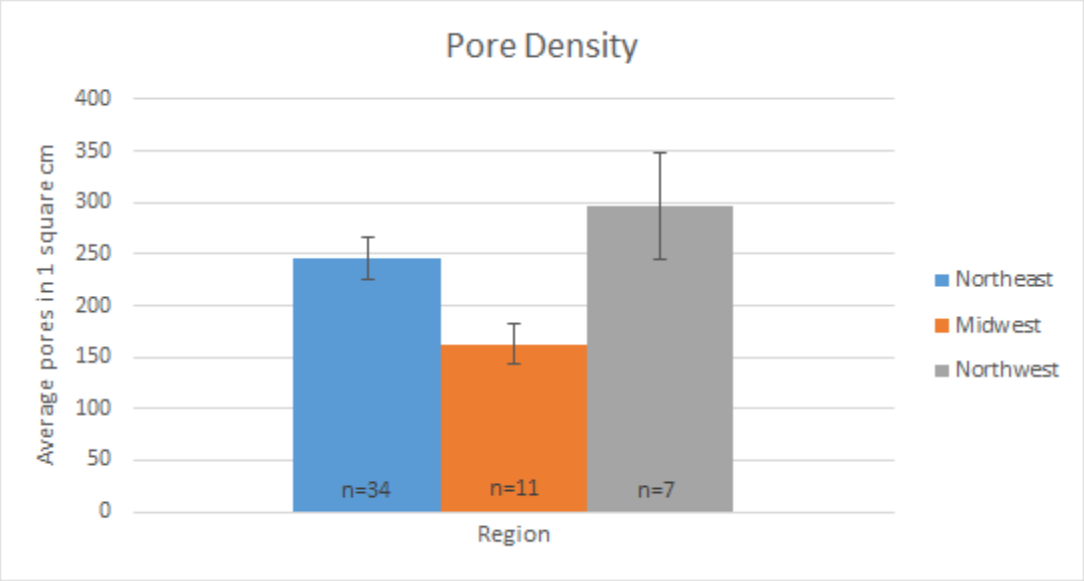

Figure 13: Average pore density across regions.

An ANOVA test was performed on the average pore density data to determine if there was any statistical difference between the regions tested. As seen in Table 4, the P-value for the average pore number in one square centimeter across the three regions is 0.0393.

Table 4: Pore density ANOVA.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Northeast	34	8386.833	246.6716	14459.82
Midwest	11	1788.333	162.5758	4427.941
Northwest	7	2080.917	297.2738	18684.84

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	89450.8	2	44725.4	3.459082	0.039333	3.186582
Within Groups	633562.5	49	12929.85			
Total	723013.3	51				

Because the P-value shown in Table 4 is less than 0.05, our results indicated that there is a statistically significant difference between pore densities in the three regions. First, F-tests were performed to determine whether the groups had unequal or equal variance, then t-tests were performed to determine which of the three regions had pore densities statistically different from one another. The F-tests performed comparing the Northeast and the Midwest, the Northeast and the Northwest, and the Northwest and the Midwest revealed that the groups had unequal, equal, and unequal variances, respectively. For all the t-Tests, the null hypothesis stated that there is no significant difference between the pore densities of the eggshells from the two regions being compared. The first t-Test, comparing the Northeast and Midwest pore density values, resulted in a P two-tail value of 0.00632. Since this value is less than 0.05, the null hypothesis can be rejected, suggesting that there is a significant difference between the pore densities of the Northeast and Midwest regions. The P-value of the t-Test comparing the Northeast and the Northwest was 0.327, which means that the null hypothesis is accepted and that there is no significant difference between the pore densities of eggshells from the Northwest and Northeast. Lastly, the P-value from the t-Test comparing the Midwest and the Northwest was 0.0412. As such, the null hypothesis can be rejected, suggesting a significant difference between the pore densities of eggshells from the Midwest and Northwest.

4.3 Percent Water Loss vs. Pore Density

To further examine the previous results, the percent water loss and pore density data of eggshells from each of the three regions were compared. Figure 14 displays the percent water loss data (Table 1) and the pore density data (Table 3), separated by each region. The error bars in the figure were created from the standard error values.

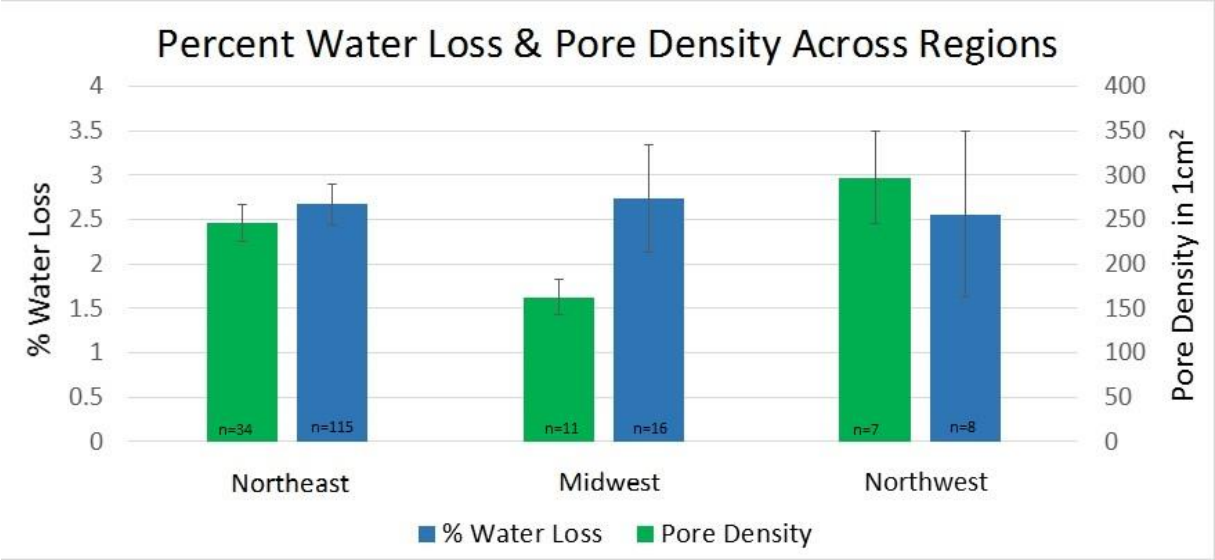

Figure 14: Percent water loss vs. pore density.

As seen in Figure 14, there is no pattern between the percent water loss and pore density data bars. A high pore density did not lead to a high percent water loss and vice versa.

4.4 Thickness

Thickness measurements were obtained for each eggshell by using a custom 1010M Starrett micrometer with round anvils, precision of 0.001 mm, and standard pressure of 75 grams. Three thickness measurements were taken on at least three different equatorial fragments of one eggshell by two group members, totaling at least 18 measurements per eggshell. Using these measurements, an average was calculated for each egg, which represented n = one eggshell. Table 5 displays the average thickness for the eggshells in each region, along with the standard deviation and standard error. The standard error for the thickness measurements were calculated by using Equation 2.

Table 5: Average eggshell thickness across regions.

Region	Sample Size (n)	Average Thickness (mm)	Standard Deviation	Standard Error
Northeast	132	0.500	0.0463	0.004
Midwest	16	0.483	0.0532	0.013
Northwest	8	0.504	0.0673	0.024

As seen in Table 5, the average eggshell thickness measurements for each of the three regions were within a small range of 0.021mm. Figure 15 displays the average thickness for each region graphically, using the standard error values of Table 5 to create the error bars. As seen in the figure, the error bars for each region overlap each other.

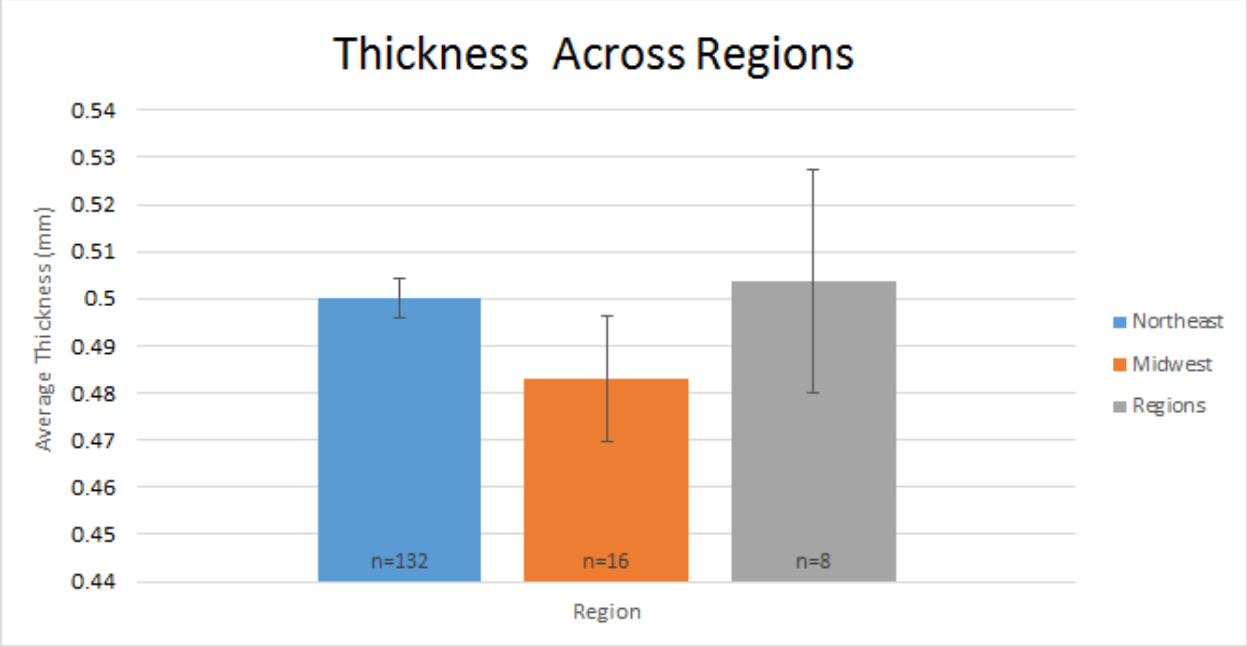

Figure 15: Average thickness measurements across regions.

An ANOVA test was performed on the average thickness measurements of each region for further statistical analysis. As seen in Table 6, the P-value for the average eggshell thickness across the three regions is 0.391.

Table 6: Thickness measurements ANOVA.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Northeast	132	66.01511	0.500114	0.002146
Midwest	16	7.72853	0.483033	0.002836
Northwest	8	4.030333	0.503792	0.004532

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.004395	2	0.002198	0.946122	0.390503	3.055162
Within Groups	0.355385	153	0.002323			
Total	0.35978	155				

5.0 Discussion/Further Recommendations

In light of recent literature indicating that the Southernmost breeding range of the common loon has receded over the past several decades, the original motivation for this project was to explore possible factors causing the decline of the southernmost common loon breeding populations. Integral to the breeding success of avian populations, eggshells were collected from regions throughout the breeding range of the common loon. The goal was to obtain eggshells representing all latitudes within the species' breeding range, which extends from the Northern United States to Northern Canada. Such comprehensive eggshell collection would have allowed us to compare eggshell characteristics of samples collected from the Southernmost and Northernmost regions of breeding range of the common loon to determine whether eggshell structure varied with latitude. Further factors could have been studied, such as different latitudes' average temperatures at breeding time and the effect of temperature at oviposition on eggshell structure, possibly indicating a link between the receding common loon habitat and climate change.

Plotting the GPS coordinates of the common loon eggshells received from various conservation groups throughout the United States, however, revealed that the collected samples represented three distinct groups within the same latitude of the species' breeding range, rather than representing many latitudes of the range. The geographic distribution of the acquired samples limited the study, as it was not possible to compare common loon eggshells collected from nesting sites located within various latitudes of the breeding range. Instead, the study shifted focus; eggshell characteristics of samples from the Northeast, Midwest, and Northwest United States were compared. The eggshell characteristics of samples from each of the three regions were compared considering the unpublished findings of Dr. Alec Lindsay on common loon population genetics. Dr. Lindsay's studies indicate that there are no genetically distinct subpopulations of the common loon across North America. The species' population does, however, follow an isolation by distance model where more geographically distant populations are more genetically diverse than populations located closer to one another (Lindsay, 2015). It was hypothesized that if common loon eggshell structure is influenced by genotype, greatest

variation would be observed between the Northeast and the Northwest regions, the most geographically distant regions considered.

After performing our experiments, we found that the pore density values between the three regions studied was the only eggshell characteristic tested that significantly differed across regions. The p-value for pore density was 0.039, suggesting that there is a statistically significant difference between the number of pores in each region. After further analysis with t-Tests, it was also found that the eggshell pore densities of the Northeast and Midwest, as well as the Northwest and Midwest, statistically differ from each other. Yet there is no statistical difference of the eggshell pore densities between the Northeast and the Northwest.

Since the average number of pores in the Northeast and the Northwest were similar, our original hypothesis that the eggshell characteristics would differ the most between the most geographically distant regions was not supported by our results. In addition, the Midwest pore values differed the most out of the three regions, which further contradicts our initial hypothesis.

The eggshell characteristics of thickness and water vapor conductance did not differ between the regions studied. The p-values for both thickness (0.391) and water vapor conductance (0.979) were above 0.05, indicating that there is no statistically significant difference between these characteristics across the Northeast, Midwest, and Northwest. Perhaps there was no statistical difference between the regions because there may be a physiological limit to these eggshell characteristics.

5.1 Limitations

Throughout our study, several limitations including small sample size, varying sample size across regions, and time constraints affected the overall outcome of our data. Furthermore, there was a lack of relevant literature that outlined concrete methods to use for our experiments and the literature found for methodology did not provide consistent results. It is recommended that in future experiments these particular limitations are points of focus to increase the validity of our findings. The most significant limitation was the sample size, which was also the cause for changing the focus of this study from analyzing samples from different latitudes, to comparing samples from three regions all within the same latitude. This shift in direction still allowed for a feasible study, yet, the number of samples that were donated for experimentation varied greatly in original location, age, physical condition, and prior history. Several hundred samples were

collected, but the number of samples received was not equally divided between the Northeast, Midwest, and Northwest regions. Only eight samples were collected from the Northwest region and 16 samples were collected from the Midwest. With the majority of the samples collected from the Northeast, particularly New Hampshire and Maine, we capped our selection of samples from this region to 132 eggshells. Additionally, the eggshell samples varied in year collected, with some dating back to the 1980's and some collected as recently as 2014. Based on how the eggshells were preserved, the physical condition in which they were received varied from whole eggshells to small fragments that seemed more fragile and worn than other pieces. Furthermore, we were unable to deduce what part of the eggshell the fragments originated from when we did not have whole eggshells. As a result we chose fragments with less curvature and assumed them to be from areas around the equator. Additionally, there is no scientific data which supports nor disapproves examining the characteristics of this study on only equatorial fragments of the eggshell.

As many samples were donated from scientific-based loon organizations, several of the eggshells were previously tested for elements such as mercury, for which we did not have the procedural records of how they were treated and the possible effects of the treatment on our experiments. There was also no consistent information across samples providing details such as whether an eggshell was from a successful hatch or post mortem. If such information had been known, samples could have been chosen with a greater consistency, avoiding samples whose eggshell characteristics may have been compromised from oviposition abnormalities.

Due to the lack of relevant and recent literature, it took significant time to determine the best methodologies for the purpose of our study. Membrane removal through the use of sodium hydroxide (NaOH) was on average 80% successful, however, if the method was unsuccessful, we were reluctant to re-treat the sample to control the consistency of methods. Samples for which this treatment did not completely remove the inner membrane were discarded from our data because of our inability to properly count pores and measure thickness, and the eggshell's inability to effectively conduct water in the water vapor conductance trials.

The methods outlining the appropriate procedures for pore counting were unclear and inconsistent, which led to large experimental error. Adapted from Ar & Rahn (1985), Morales, *et al.* (2013), and Stein, L. (2009) pore counting was determined from a sample fragment of 1 cm^2, which is not an accurate representation of the entire eggshell, however was done for the purpose

of time and efficiency. With the equipment available for our experimentation, we found significant error if fragments had slight curvature, which did not allow the light source from fully shining through. Additionally, there was no literature explicitly defining what was considered a pore from the objective of a light microscope. Based on this limitation, we determined a set of standards to reduce the amount of ambiguity of what would be counted as a pore. Two of the four team members took turns counting the amount of pores, with instructions of only counting points of light that were explicitly round. Any points of light that resided within a crack in the surface of the eggshell were not included. Once both members counted the same fragment, the number of pores was averaged. Even with these criteria, many samples had points of light of varying size, which resulted in substantial variability of pore counting between samples as well as within the fragments of the same sample.

Another limitation to our study was the inconsistency in water vapor conductance methodology. Although these methods were adapted from Portugal, *et al.* (2010), there was inconsistency with the application of the glue. At first, the glue selected was not conducive for our study as it dripped down the inside of the microfuge tube and created a seal that prevented water vapor loss. The author, Steven J. Portugal, directed our attention to Loctite UltraGel Control which was viscous enough to inhibit the glue from trickling. However, after conducting our 92 hour measurements, we removed the fragments off the tubes to observe any abnormalities and discovered that a thin film-like residue covered the surface of the distilled water. We do not know how this thin film affects the water vapor conductance, and we did not find a way to correct this problem. This caused a wide variability of data and per statistician recommendation, the median of the three water loss values per sample was used in our data, while the extremes were discounted.

Lastly, the time constraints of this project inhibited our capabilities to solve for these limitations. A significant amount of time was used for communication with our eggshell donors and for gathering our eggshell samples. Once the samples were received and we began to perform our experiments, a large amount of time was spent to perfect the methods found in the literature and to create standards for our methodology.

5.2 Future experiments

We have several recommendations to further expand our research and experimentation for a more comprehensive analysis of potential variation of common loon eggshell characteristics across Northern America.

Due to the previously mentioned limitations of the pore counting and membrane removal methods used in this study, we recommend to further explore techniques that would aid in more definitive pore counting and consistent membrane removal. In the literature, dyes, such as methylene blue and safranin, applied to one side of an eggshell evaporate through the pores to the other side. This forms large dye spots on the other side of the eggshell, allowing for ease of counting (second to the treatment of nitric acid to increase the pore size). Though dyes were tested with the common loon eggshell samples, they did not provide accurate pore counting due to the samples' high pore density (Tharapoom, 2006).

Overall, improved methods would allow for increased accuracy of each pore count. For example, a scanning laser microscope that can detect and count pores of samples regardless of concavity differences can increase the accuracy and efficiency of the overall pore count. This type of microscope negates the effects of eggshell concavity that are seen with the light microscope method. Regardless of the method chosen for pore counting, further explorative studies should be done to determine what defines a pore in terms of amount of light that shines through, and depth of hole in the eggshell. General literature currently exists on the various types of pores but there is a gap in information in regards to what pores look like in practice and the best methods to count them accurately.

Additionally, we recommend to further explore the membrane removal method. As previously mentioned, when all samples were treated with 5% NaOH for four minutes, on average, for one out of every eight to ten samples, the membranes were not fully removed by the treatment. Due to these results, a more consistent method should be determined in order to ensure complete membrane removal for all fragments. An ideal method would maintain the integrity of the sample while allowing for easy and complete membrane removal. During this study, we found that after treating the fragments with NaOH, not all of the membrane was removed. Detaching this membrane involved lightly rubbing the samples in between our fingers while submerging the samples in water. In attempt to remove the remaining inner membrane, eggshell fragments we broken.

Since one of the greatest limitations of this study was small sample size within the regions tested, we advise a more comprehensive sample collection from all latitudes and longitudes within the common loon habitat range. Ideally, hundreds of samples should be collected from each region. Samples studied in this project were collected from MA, NH, VT, NY, ME, WI, MT, and WA, but common loons also inhabit several other northern U.S. states such as MI, MN, and other northeast states such as CT. Furthermore, Canada and Alaska comprise the northernmost range of the common loon habitat. During the span of this project, we were unable to collect and test samples from Canada and Alaska. A comprehensive sample collection would allow for comparison of eggshells from the northernmost habitat range, where common loon populations are stable, to eggshells from the southernmost range, where populations are receding.

The original motivation for this project stemmed from curiosity about why the common loon's southernmost habitat range was receding and whether that had any correlation to climate change. To explore this concept, samples could be collected from the same region, for example the Northeast, over several decades. Eggshell characteristic data could be examined in relation to data on the environmental temperature and fluctuations from that region during the corresponding decades. Since climate change is a slow process, pertinent issues facing such a study could include time consuming sample collection and adequate sample storage and preservation. As such, comparing data related to possible slight changes in temperature may take up to several decades.

Looking beyond climate change as the reason for the receding southernmost range of the common loon, we recommend considering point source factors influencing the habitat patterns of the species. Current literature exists on both the effects of mercury and varying acidity levels of lakes on eggshell viability. Local pesticide use and instances of pollution in certain areas may correlate to different effects on eggshell characteristics as well. Examining these relationships could reveal patterns between high pollution areas and common loons no longer inhabiting those areas.

6.0 Conclusions

Eggshell characteristics including pore density, thickness, and percent water loss were compared between eggshell samples from three distinct regions across the United States. It was hypothesized that eggshell thickness, pore density, and water vapor conductance would vary between common loon eggshells collected from three geographically distinct regions including the Northeast, Midwest, and Northwest. It was predicted that the most variation in eggshell characteristics would be seen between samples collected from the Northeast and the Northwest, which were the most geographically distant populations studied. The data collected did not appear to support the hypothesis.

Based on the results shown in Figures 1 and 2, this study indicates that there are no significant differences in eggshell thickness or in percent water loss across regions. Furthermore, there appears to be no correlation between the pore density and the percent water loss of common loon eggshells, as seen in Figure 1. However, the results revealed a significant difference in the pore density of eggshells between the three different regions.

If there is significant genotypic variability among loon populations in the regions studied, then our results suggest that common loon eggshell thickness and water vapor conductance do not vary with genotype, while eggshell pore density does vary with genotype. It is also possible that because no significant differences were observed between either eggshell thickness or water vapor conductance, acceptable physiological limits exist for these eggshell characteristics such that an embryo cannot develop within an egg if thickness or water vapor conductance do not fall within those physiological limits. It is not clear, however, why physiological limits would exist for eggshell thickness and water vapor conductance, but not for eggshell pore density. This would be especially surprising since pore density was expected to be closely related to water vapor conductance.

Due to constraints such as small sample size, limited sample distribution, and unreliable methods, it is recommended that the findings of this study be validated by more comprehensive sample collection, modified methods, and further experimentation. With these improvements to the study, perhaps more concrete conclusions could be drawn from the data obtained.

7.0 References

A. R. Lindsay (personal communication, April 2, 2015)

Audubon Society (2014). Common Loon. Retrieved October 9, 2014 from http://climate.audubon.org/birds/comloo/common-loon

Anderson, D., Lumsden, H., & Hickey, J. (1970). Geographical variation in the eggshells of common loons. *The Canadian Field Naturalist, 84*, 351-356.

Ar, A. and Rahn, H. (1980). Water in the avian egg: overall budget of incubation. *Am. Zool.* 20: 373-384.

Ar, A. and Rahn, H. (1985). Pores in avian eggshells: Gas conductance, gas exchange and embryonic growth rate. *Respiration Physiology*, 61, 1-20.

Bachelet, D., Neilson, R., Lenihan, J.M., Drapek, R.J. (2001). Climate Change Effects on Vegetation Distribution and Carbon Budget in the United States. *Ecosystems*, 4 (3), 164-185.

Bard, E., Raisbeck, G., Yiou, F, Jouzel, J. (2000). Solar irradiance during the last 1200 years based on cosmogenic nuclides. *Tellus B,* 52 (3), 985.

Biodiversity Research Institute. (2013). Restore the Call: Wyoming status report for the common loon. Retrieved October 13, 2014 from http://www.briloon.org/uploads/BRI_Documents/Loon_Center/RCF/WY_Loon_Status_Report_092613.pdf

BirdLife International 2012. *Gavia immer*. The IUCN red list of threatened species. Version 2014.3. <www.iucnredlist.org>. Downloaded on 31 January 2015.

BirdLife International (2015). Species factsheet: *Gavia immer*. Downloaded from http://www.birdlife.org on 31/01/2015.

Board, R.G. (1982) Properties of avian eggshells and their adaptive value. *Biological reviews of the Cambridge Philosophical Society*, 57, 1-28.

Boersma, P.D. & Rebstock, G.A. (2009). Magellanic penguin eggshell pores: Does number matter? *International Journal of Avian Science*, 151(3), 535-540.

Booth, G. (2013). The uncommon loon. *Wisconsin Natural Resources Magazine*. Retrieved January 31, 2015, from http://dnr.wi.gov/wnrmag/2013/02/loon.htm.

Brinkhuis, H., Schouten, S. (2006). Episodic fresh surface waters in the Eocene Arctic Ocean. *Nature*, 441 (7093), 606-609.

Brown, C.J., Fulton, E.A., Hobday, A.J., Matear, R.J., Possingham, H.P., Bulman, C., Christensen, V., Forrest, R.E., Gehrke, P.C., Gribble, N.A., Griffiths, S.P., Lozano-Montes, H., Martin, J.M., Metcalf, S., Okey, T.A., Watson, R., & Richardson, A.J. (2010). Effects of climate-driven primary production change on marine food webs: Implications for fisheries and conservation. *Global Change Biology.* 16, 1194-1212.

Dawes, R. (2014). The not-so-common Loon: new study warns of decline. *Twin Cities Daily Planet.* Retrieved October 9, 2014 from http://www.tcdailyplanet.net/news/2014/09/30/ not-so-common-loon-new-study-warns-decline.

Diggles, M. (2005). The Cataclysmic 1991 Eruption of Mount Pinatubo, Philippines. *U.S. Geological Survey Fact Sheet 113-97*, United States Geological Survey.

Donaire, M., Lopez-Martinez, N. (2009). Porosity of Late Paleocene *Ornitholithus* eggshells (Tremp Fm, south-central Pyrenees, Spain): Palaeoclimatic implications. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 279(3–4)147–159.

Eaton, S. (2014). U.S. EPA allots nearly $12 Million to fight Lake Erie algal blooms. *The Plain Dealer.*

EPA(2007). Recent Climate Change: Atmosphere Changes. *Climate Change Science Program*.

Evers, David C., James D. Paruk, Judith W. Mcintyre and Jack F. Barr. 2010. Common Loon (*Gavia immer*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/313.

Evers, D. (2004). Status Assessment and Conservation Plan for the Common Loon (*Gavia Immer)* in North America. U.S. Fish and Wildlife Service. Retrieved April 22, 2015 from http://wdfw.wa.gov/conservation/loons/common_loon_status_assessment.pdf.

Francis, C., Marra, P., Mulvihill, R., Moore, F. (2004). The Influence of Climate on the Timing and Rate of Spring Bird Migration. *Oecologia*. 142, 307-315.

Great Northern Loon Eggs Hatching. (2012, June 15). Retrieved April 28, 2015, from https://naturallycuriouswithmaryholland.wordpress.com/tag/common-loon/.

Goodell, J. (2013). Goodby, Miami. *Rolling Stone.*

Hughes, M., Swetman, T., Diaz, H. (2010). *Dendroclimatology: Progress and Prospect*. New York: Springer.

Jaeckle, W.B., Kiefer, M., Childs, B., Harper, R.G., Rivers, J.W., and Peer, B.D. (2012). Comparison of eggshell porosity and estimated gas flux between the brown-headed cowbird and two common hosts. *Journal of Avian Biology*, 43(6), 486-490.

Journey North. (2014). Common Loon. Retrieved October 14, 2014 from http://www.learner.org/jnorth/search/LoonNotes2.html.

Kenow, K., Adams, D., Schoch, N., Evers, D., Hanson, W., Yates, D., ... Ozard, J. (2009, January 15). Migration Patterns and Wintering Range of Common Loons Breeding in the Northeastern United States. Retrieved October 13, 2014, from http://www.bioone.org/doi/full/10.1675/063.032.0204.

Koenig, W. (1982). Ecological and social factors affecting hatchability of eggs. *The Auk, 99*(3), 526-536. Retrieved September 18, 2014, from http://www.jstor.org/stable/4085932.

Maintaining egg shell quality. (2008, March 14). Retrieved January 31, 2015, from http://www.thepoultrysite.com/articles/979/maintaining-egg-shell-quality.

Miles, M.G., Grainger, R.G., Highwood, E.J. (2004). The significance of volcanic eruption strength and frequency for climate change. *Quarterly Journal of the Royal Meteorological Society,* 130 (602), 2361-2376.

Morales, J., Ruuskanen, S., Laaksonen T., Eeva T., Mateo R., Belskii E., Ivankina E., Jarvinen A., Kerimov A., Korpimaki E., Krams I., Mand R., Morosinotto C., Orell M., Qvarnstrom A., Siitari H., Slater F., Tilgar V., Visser M.E., Winkel W., Zang H., Moreno J. (2013). Variation in eggshell traits between geographically distant populations of pied flycatchers, *Ficedula hypoleuca*. *Journal of Avian Biology 44:*111-120.

New, M., Todd, M., Hulme, M., Jones, P. (2001). Review: Precipitation measurements and trends in the twentieth century. *International Journal of Climatology*, 21 (15), 1889-1922.

Nicholls, R.J., Cazenave, A. (2010). Sea-Level Rise and Its Impact on Coastal Zones. *Science Magazine*, 328 (5985), 1517-1520.

NSIDC. (2014). National Snow and Ice Data Center: State of the Cryosphere.

Nys, Y., Gautron, J., Garcia-Ruiz, J., & Hincke, M. (2004). Avian eggshell mineralization: Biochemical and functional characterization of matrix proteins. *General Palaeontology (Palaeobiochemistry), 3*, 549-562.

Paganelli, A., Reeves, R., Green, D., & Rahn, H. (1974). The Avian Egg: Water Vapor Conductance, Shell Thickness, and Functional Pore Area. *The Condor, 76*(2), 153-158.

Pollentier, C.D., Kenow, K.P., Meyer, M.W. (2007). Common Loon (*Gavia Immer*) Eggshell thickness and volume vary with acidity of Nest Lake in Northern Wisconsin. *The Waterbird Society* 30(3): 367-374.

Portugal, S., Maurer, G., & Cassey, P. (2010). Eggshell Permeability: A Standard Technique for Determining Interspecific Rates of Water Vapor Conductance. *Physiological and Biochemical Zoology, 83*(6), 1023-1031.

Rahn, H. and C.V. Paganelli (1982). Role of diffusion in gas exchange of the avian egg. *Fed. Proc.* 41:2134-2136.

Rahn, H., Greene, D.G., Toien, O., Krog, J., & Mehlum, F. (1984). Estimated Laying Dates and Eggshell Conductance of the Fulmar and Brunnich's Murre in Spitsbergen. *Ornis Scandinavica*, 15(2), 110-112.

Riley, A., Sturrock, C., Mooney, S., & Luck, M. (2014). Quantification of eggshell microstructure using X-ray micro computed tomography. *British Poultry Science, 55*(3), 311-320. Retrieved January 31, 2015, from http://dx.doi.org/10.1080/00071668.2014.924093.

Svingen, P., & Hertzel, A. (Eds.). (2000, January 1). The Common Loon Population Status and Fall Migration in Minnesota. Retrieved October 13, 2014, from http://files.dnr.state.mn.us/eco/nongame/projects/consgrant_reports/2000/2000_mou_loons.pdf.

Strode, P.K. (2009). Implications of climate change for North American wood warblers (Parulidae). *Global Change Biology*, 9, 1137-1144.

Sidor, I., Pokras, M., Major, A., Poppenga, R., Taylor, K. & Miconi, R. (2003). Mortality of Common Loons in New England, 1987 to 2000. *Journal of Wildlife Diseases* **39(2):** 306-315.

Stein, L. (2009). *Evolution of Eggshell Architecture Accompanying Rapid Range Expansion in a Passerine Bird* (Thesis). The University of Arizona, Arizona.

Third National Climate Assessment. (2014). *National Climate Assessment.*

Jones, P.D., Trenberth, K.E., Ambenje, P., Bojariu, R., Easterling, D., Klein Tank, A., Parker, D., Rahimzadeh, F., Renwick, J.A., Rusticucci, M., Soden, B., Zhai, P. (2007). *Climate Change 2007: The Physical Science Basis*, Observations: Surface and atmospheric climate change. (ed) Solomon S, et al. Cambridge Univ Press, Cambridge, UK, pp 747–845.

Tullett, S., & Board, R. (1977). Determinants of avian eggshell porosity. *Journal of Zoology, 183*(2), 203-211.

Vogel, H., & Taylor, K. (n.d). Species profile common loon. *New Hampshire Wildlife Action Plan*, pp 389-398.

Yahya, Harun. (2001). The Design in Nature. *Ta-Ha Publishers Ltd.* 72-73.

8.0 Appendices

8.1 Water Vapor Conductance Final Medians

Northeast Water Vapor Conductance Measurements

New Hampshire		Maine		Vermont		Massachusetts		New York	
Sample ID	**% Water Loss**	**Sample ID**	**% Water Loss**	**Sample ID**	**% Water Loss**	**Sample ID**	**% Water Loss**	**Sample ID**	**% Water Loss**
NHT0027	3.163371488	IND98E2	3.47970174	ISP98E1	12.64073695	QUABHWMA13086	2.522140932	ARBUNWNY13035	2.269993987
NHT0146	4.099895942	CPN98E1	2.967771853	HOL98E1	2.856122813	STODSPMA13072	1.779965916	BUCKBPNY13044	0.801220908
NHT0477	4.353710805	CPL98E3	2.330905307	MCC98E2	1.154939588	WACHCEMA13080	1.486748546	BUCKBPNY13052	1.941747573
NHT0047E4	8.730873087	JIM98E2	2.65654649	MOR98E1	1.193914885			CATLCLNY13040	2.776513427
NHT0171	3.891213389	AZI98E14	2.439431913	GROTGLVT13010	2.297063903			DEERDPNY13056	4.648925938
NHT0099	5.981475282	AZI98E3	3.073496659	BERLPDVT13019	2.119071645			KUSHKLNY13042	2.56269449
NHT0047E1	5.736813486	FLA98E3	1.243339254	COLEPDVT13017	3.222239773			LIMEISNY13050	2.190100745
NHT0097E2	2.017189967	IND98E1	1.997314535	MILEPDVT13013	4.0625			LIMEISNY13054	2.69541779
NHT0074	2.390722569	FLA98E6	11.66356877	NINVAVT13007	2.279888786			MOOSWTNY13048	1.27335869
NHT0213	2.622418194	FLA98E4	4.45226755	SPIRSLVT13006	1.204410517			SILVSLNY13046	2.924311927
NHT0297	2.685088634	LSB98E1	0.958826847	WOODWRVT13009	1.1465867			WOLFPINY13034	5.496879501
NHT0047E2	2.567033881	LJP98E1	1.66344294	MCC98E1	0.899280576				
NHT0090	2.145776567	JIM98E1	2.317260181	SPP98E1	1.600673968				
NHT0174	2.765838161	UMB98E4	0.762599469	GAV98E1	1.639910236				
NHT0020	2.357156327	FLA98E2	1.073529412	HOL98E2	2.703159317				
NHT0097E1	4.389435907	AZI98E10	1.725004187	GRR98E1	2.763229185				
NHT0014	1.29829501	AZI98E7	2.098039216						
NHT0071	1.765002521	FLA98E5	2.482394366						
NHT0297	2.866894198	IND98E4	2.616747182						
NHT0047E3	2.53709909	IND98E3	1.617250674						
NHT0003	1.640566741	AZI98E5	2.422990233						

NHT0063	1.974169742	UMB98E2	2.503744918						
NHT0674	0.470793374	AZI98E6	1.1035313						
NHT0055	2.229712212	FLA98E7	1.312828207						
SQU98E10	8.337361044	AZI98E4	5.591200733						
NHT0001	4.765085727	AZI98E9	1.991393131						
NHT0171	1.248093191								
UMB98E5	4.02669121								
UMB98E7	1.889859785								
SES98E1	0.95548317								
UMB98E10	1.486288466								
UMB98E6	1.996257018								
KAN98E1	2.035306334								
WHO98E1	7.901794925								
SQU98E9	1.981230448								
UMB98E1	4.884989329								
HIG98E2	1.741338654								
WAL98E1	0.709219858								
WIN98E4	0.864126409								
CHR98E1	1.369112815								
HIG98E1	1.226180613								
FOR98E1	1.703778677								
EAS98E1	1.24246988								
NOR98E1	1.342852286								
WIN98E5	2.304237454								
PEM98E1	1.963534362								
UMB98E1	1.704320254								
SUC98E1	1.73347779								
STR98E2	1.590852598								
WIN98E7	1.588180979								
BDU98E1	1.348865727								
SQU98E11	3.00524405								
WIN98E8	2.801894238								
MMP98E1	0.669925765								
WIN98E3	2.683142101								
UMB98E3	2.105924878								

SUN98E1	3.19784242								
PEM98E2	3.817302884								
STR98E1	2.552204176								

Region Summary:

NH Average:	**2.702627391**	**ME Average:**	**2.636351041**	**VT Average:**	**2.736483053**	**MA Average:**	**1.929618464**	**NY Average:**	**2.689196816**
NH STD:	**1.785143833**	**ME STD:**	**2.131590793**	**VT STD:**	**2.787006635**	**MA STD:**	**0.53367243**	**NY STD:**	**1.354810837**

Northeast Average:	**2.670903412**
Northeast STD:	**1.954908494**

Midwest Water/Vapor Conductance Measurements

Sample ID	% Water Loss
VER06	1.475935829
WAB06	5.245418159
GOT11	1.877022654
KAW06	2.648305085
GAY11	7.801552736
STA06	2.959674436
JIM11	1.646682654
POT11	1.30589632
OTT06	5.943102104
MAR10	1.938449241
TRO06	2.93508937
KEN11	1.217498968
FRA06	1.401515152
TUR06	1.818181818
CRA06	2.334197851
MAB06	1.179781362
Region Summary:	
Midwest Average:	**2.733018984**
Midwest STD:	**1.932969557**

Northwest Water/Vapor Conductance Measurements

Montana		Washington	
Sample ID	**% Water Loss**	**Sample ID**	**% Water Loss**
ALVOALMT13022	1.084524014	LYNCLLWA13001	1.97636117
BLANBLMT13030	1.138384916		
CLEACLMT13032	2.385722091		
HOWEHLMT11018	1.876453006		
ROGERLMT12024	1.444196666		
STILLLSMT13026	7.515758849		
THOMLTMT13028	3.044174868		
MT Average:	**2.641316344**		
MT STD:	**2.261575035**		
Region Summary:			
Northwest summary:	**2.558196948**		
Northwest STD:	**2.106968864**		

8.2 Pore Density Final Averages

Northeast Pore Density Data

New Hampshire		Maine		Vermont		Massachusetts		New York	
Sample ID	**Final Average**	**Sample ID**	**Final Average**	**Sample ID**	**Final Average**	**Sample ID**	**Final Average**	**Sample ID**	**Final Average**
NHT0097E2	129.25	IND98E2	215	COLEPDVT13017	268.5	QUABHWMA13086	244	ARBUNWNY13038	394.3333333
NHT0074	170.75	UMB98E10	136.75	NINVAVT13007	191.3333333	STODSPMA13072	266	BUCKBPNY13044	395.5
NHT0020	202	CPL98E3	218.25	SPIRSLVT13006	511.3333333	WACHCEMA13080	230.5	CATLCLNY13040	108.75
NHT0071	243.75	UMB98E1	286.5	WOODWRVT13009	132			KUSHKLNY13042	456.6666667
NHT0047E3	168.5	AZI98E15	227.75	HOL98E2	106.25			LIMEISNY13054	546
NHT0063	100.75	IND98E4	333.5	MCC98E1	117.6666667			MOOSWTNY13048	283
NHT0171	97.25	IND98E3	96.5	SPP98E1	287.25			SILVSLNY13046	338
SQU98E9	307	AZI98E5	391	MILEPDVT13013	0.5525			WOLFPINY13034	185.25

Region Summary:

NH Average:	**177.4063**	**ME Average:**	**238.1563**	**VT Average:**	**230.6191**	**MA Average:**	**246.8333**	**NY Average:**	**338.4375**
NH STD:	**72.25426**	**ME STD:**	**97.23259**	**VT STD:**	**143.1418**	**MA STD:**	**17.91879**	**NY STD:**	**142.7971**
Northeast Average:	**246.6716**								
Northeast STD:	**120.2490**								

Midwest Pore Density Data

Sample ID	Final Average
VER06	89.16666667
GOT11	146.6666667
KAW06	170.5
STA06	74.66666667
JIM11	223.1666667
POT11	287.8333333
TRO06	148.6666667
KEN11	238.3333333
FRA06	104.5
TUR06	183.8333333
CRA06	121
Region Summary:	
Midwest Average:	**162.5758**
Midwest STD:	**66.54278**

Northwest Pore Density Data

Montana		Washington	
Sample ID	**Final Average**	**Sample ID**	**Final Average**
BLANBLMT13030	510.5	LYNCLLWA13001	248.3333333
CLEACLMT13032	357		
HOWEHLMT11018	193.8333333		
ROGERLMT12024	427.3333333		
STILLLSMT13026	143.1666667		
THOMLTMT13028	200.75		
MT Average:	**305.4306**		
MT STD:	**147.8612**		
Region Summary:			
Northwest summary:	**297.2738095**		
Northwest STD:	**136.6925171**		

8.3 Thickness Final Averages

Northeast Thickness Measurements

New Hampshire		Maine		Vermont		Massachusetts		New York	
Sample ID	**Final Average**	**Sample ID**	**Final Average**	**Sample ID**	**Final Average**	**Sample ID**	**Final Average**	**Sample ID**	**Final Average**
NHT0027	0.493888889	IND98E2	0.526388889	ISP98E1	0.4925	QUABHWMA13086	0.503888889	ARBUNWNY13038	0.521944444
NHT0146	0.554722222	CPN98E1	0.409166667	HOL98E1	0.534444444	STODSPMA13072	0.500555556	BUCKBPNY13044	0.464166667
NHT0477	0.47	FLA98E6	0.528958333	MCC98E2	0.560833333	WACHCEMA13080	0.515555556	BUCKBPNY13052	0.500555556
NHT0047E4	0.491388889	CPL98E3	0.516111111	MOR98E1	0.454166667			CATLCLNY13040	0.445
NHT0171	0.470555556	JIM98E2	0.491111111	GROTGLVT13010	0.53			DEERDPNY13056	0.420833333
NHT0099	0.535833333	AZI98E14	0.581944444	BERLPDVT13019	0.430555556			KUSHKLNY13042	0.469444444
NHT0047E1	0.503888889	UMB98E1	0.438611111	COLEPDVT13017	0.450833333			LIMEISNY13050	0.4675
NHT0097E2	0.477222222	AZI98E3	0.4325	MILEPDVT13013	0.5525			LIMEISNY13054	0.435833333
NHT0074	0.494444444	FLA98E3	0.481666667	NINVAVT13007	0.481111111			MOOSWTNY13048	0.539166667
NHT0213	0.478055556	IND98E2	0.526388889	SPIRSLVT13006	0.546944444			SILVSLNY13046	0.553888889
NHT0297	0.497777778	CPN98E1	0.409166667	WOODWRVT13009	0.574444444			WOLFPINY13034	0.351944444
NHT0047E2	0.515416667	FLA98E6	0.528958333	MCC98E1	0.512604167				
NHT0090	0.557222222	CPL98E3	0.516111111	SPP98E1	0.485277778				
NHT0174	0.495	JIM98E2	0.491111111	GAV98E1	0.524166667				
NHT0020	0.555277778	AZI98E14	0.581944444	HOL98E2	0.47				
NHT0097E1	0.559888889	AZI98E3	0.4325	GRR98E1	0.410277778				
NHT0014	0.531944444	FLA98E3	0.481666667						
NHT0071	0.535333333	FLA98E4	0.51						
NHT0297	0.487222222	LSB98E1	0.503888889						
NHT0047E3	0.551875	LJP98E1	0.505277778						
NHT0003	0.516666667	JIM98E1	0.498055556						
NHT0063	0.511111111	UMB98E4	0.469166667						
NHT0674	0.509583333	FLA98E2	0.556666667						
NHT0055	0.448958333	AZI98E10	0.524722222						

SQU98E10	0.57	AZI98E7	0.523125						
NHT0001	0.544833333	IND98E1	0.536666667						
NHT0171	0.525	FLA98E5	0.55125						
UMB98E5	0.492083333	IND98E4	0.392777778						
UMB98E7	0.483888889	IND98E3	0.520555556						
SES98E1	0.4975	AZI98E5	0.4875						
UMB98E7	0.603888889	UMB98E2	0.429444444						
UMB98E6	0.436388889	AZI98E6	0.506944444						
KAN98E1	0.477777778	FLA98E7	0.472222222						
WHO98E1	0.468055556	AZI98E15	0.566666667						
SQU98E9	0.525833333	AZI98E4	0.403888889						
UMB98E7	0.483888889	AZI98E9	0.502666667						
SES98E1	0.4975								
UMB98E7	0.603888889								
UMB98E6	0.436388889								
KAN98E1	0.477777778								
UMB98E1	0.438611111								
WHO98E1	0.468055556								
SQU98E9	0.525833333								
HIG98E2	0.488611111								
WAL98E1	0.51								
WIN98E4	0.53								
CHR98E1	0.495833333								
HIG98E1	0.503333333								
FOR98E1	0.529444444								
EAS98E1	0.510833333								
NOR98E1	0.535								
WIN98E5	0.483888889								
PEM98E1	0.4875								
UMB98E1	0.435								
SUC98E1	0.57								
STR98E2	0.425277778								
WIN98E7	0.548888889								
BDU98E1	0.523888889								
SQU98E11	0.533333333								

WIN98E8	0.460138889								
MMP98E1	0.574166667								
WIN98E3	0.523888889								
UMB98E3	0.578055556								
SUN98E1	0.526111111								
PEM98E2	0.458888889								
STR98E1	0.445833333								

Region Summary:

NH Average:	**0.507248316**	**ME Average:**	**0.495438657**	**VT Average:**	**0.500666233**	**MA Average:**	**0.506666667**	**NY Average:**	**0.470025253**
NH STD:	**0.041082281**	**ME STD:**	**0.04999151**	**VT STD:**	**0.048952602**	**MA STD:**	**0.007876359**	**NY STD:**	**0.058000566**

Northeast Average:	0.500114531
Northeast STD:	0.046324687

Midwest Thickness Measurements

Sample ID	Final Average
VER06	0.419166667
WAB06	0.585833333
GOT11	0.445277778
KAW06	0.505833333
GAY11	0.541111111
STA06	0.589722222
JIM11	0.465277778
POT11	0.504722222
OTT06	0.470277778
MAR10	0.456388889
TRO06	0.411111111
KEN11	0.443333333
FRA06	0.508333333
TUR06	0.478888889
CRA06	0.442777778
MAB06	0.460555556
Region Summary:	
Midwest Average:	**0.483038194**
Midwest STD:	**0.053252781**

Northwest Thickness Measurements

Montana		Washington	
Sample ID	**Final Average**	**Sample ID**	**Final Average**
ALVOALMT13022	0.5675	LYNCLLWA13001	0.526666667
BLANBLMT13030	0.406111111		
CLEACLMT13032	0.562222222		
HOWEHLMT11018	0.536666667		
ROGERLMT12024	0.446944444		
STILLLSMT13026	0.561388889		
THOMLTMT13028	0.422833333		
MT Average:	**0.50052381**		
MT STD:	**0.072022779**		
Region Summary:			
Northwest summary:	0.503791667		
Northwest STD:	0.067317693		