An Investigation of Herring Gull Population Decline in Pukaskwa National Park, Lake Superior

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Abstract

In Pukaskwa National Park on Lake Superior, Herring Gull (*Larus argentatus*) population size is used as an indicator of ecological integrity. Since the 1970s, their populations have declined by 70%. One factor that may be regulating these declines is food availability. Lake-wide declines in surface-schooling prey fish may be limiting natural food sources for Pukaskwa gulls. Birds in the southern section of the park have little to no access to human sources of food. In the northern section of the park, impacts of natural food declines may be buffered as birds can obtain anthropogenic food from nearby dumps. To assess regional differences in gull diets, Herring Gull eggs were collected from northern and southern parts of Pukaskwa National Park. Markers of diet composition, i.e. stable isotopes of nitrogen and carbon; fatty acids, were measured in the eggs. These analyses support the hypothesis that gulls from the southern end of the park rely to a greater extent on natural foods. The lack of alternative foods in the south may be changing reproductive endpoints and be contributing to more extreme population declines in that region. Understanding the degree to which anthropogenic food subsidies support Herring Gull populations is critical when utilizing gulls as an indicator of ecological integrity in Pukaskwa National Park.

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Introduction

Ecosystem perturbations, e.g. exotic species introductions, overharvest and habitat destruction may cause alterations in food web structure. Altered food webs can, in turn, force organisms to adjust their diets to reflect food availability, e.g. changes in relative abundance of aquatic versus terrestrial food. Predators that usually feed primarily on fish may be forced to forage on less nutritious food sources such as terrestrial organisms or anthropogenic food, e.g. garbage. This shift in foraging habits can affect an organism's behaviour and fitness.

Top-level predators are often used to provide information regarding the state of an ecosystem. Ecosystem change may be preceded by a significant decrease in the abundance of top trophic level predators (Chapdelaine and Rail, 1997; Weimerskirch *et al,* 2003; Miller and Sydeman, 2004). For example, in the 1980s and 1990s Atlantic Cod (*Gadus morhua*) abundance in the St. Lawrence River plummeted. This resulted in a collapse of the cod fishery with significant environmental and social consequences. Research performed after the collapse showed that populations of a top trophic level avian predator, the Herring Gull (*Larus argentatus*), dropped by over 80% in conjunction with the collapse of the cod stock (Chapdelaine and Rail, 1997). This example highlights the utility of using top level predators and colonial waterbirds as indicators of ecosystem change. Understanding the utility of data obtained by monitoring these species may help manage ecosystems more effectively.

In Pukaskwa National Park (PNP), Herring Gulls are used by the Parks Canada Agency as an indicator of ecological integrity. Ecological integrity is defined by Parks Canada as: "a condition that is determined to be characteristic of its natural region and likely to persist, including abiotic components and the composition and abundance of native species and biological communities, rates of change and supporting processes." (Parks Canada, 2013). In

PNP there are 15 measures of ecological integrity. Of those 15, the only indicators listed in poor condition are those associated with colonial waterbirds, i.e. Herring Gull and Great Blue Heron (*Ardea herodias*) populations. The other 13 indicators of ecological integrity are all listed as fair or good. Herring Gull populations in PNP have decreased by approximately 70% since the 1970s (Figure 1.0). This precipitous decline in Herring Gull populations suggests that the ecological integrity of PNP has undergone a significant decline through time. Understanding the reasons for Herring Gull population decline is important if we are going to gain insights into factors influencing park ecological integrity. Clues to addressing this issue may be found in the nature of population declines in southern and northern PNP (Figure 1.1). The population decrease has not been uniform across the park (Figure 1.2). Populations in the southern part of the park have decreased to a greater extent than northern populations (Figure 1.2). These areas are approximately 100 km apart minimizing overlap in gull populations during the breeding season. The typical maximum foraging range for Herring Gulls is approximately 30-40 km but can vary depending on the availability of food (Nisbet *et al,* 2017). The geographic differences in population trends outlined above may help identify factors regulating population declines.

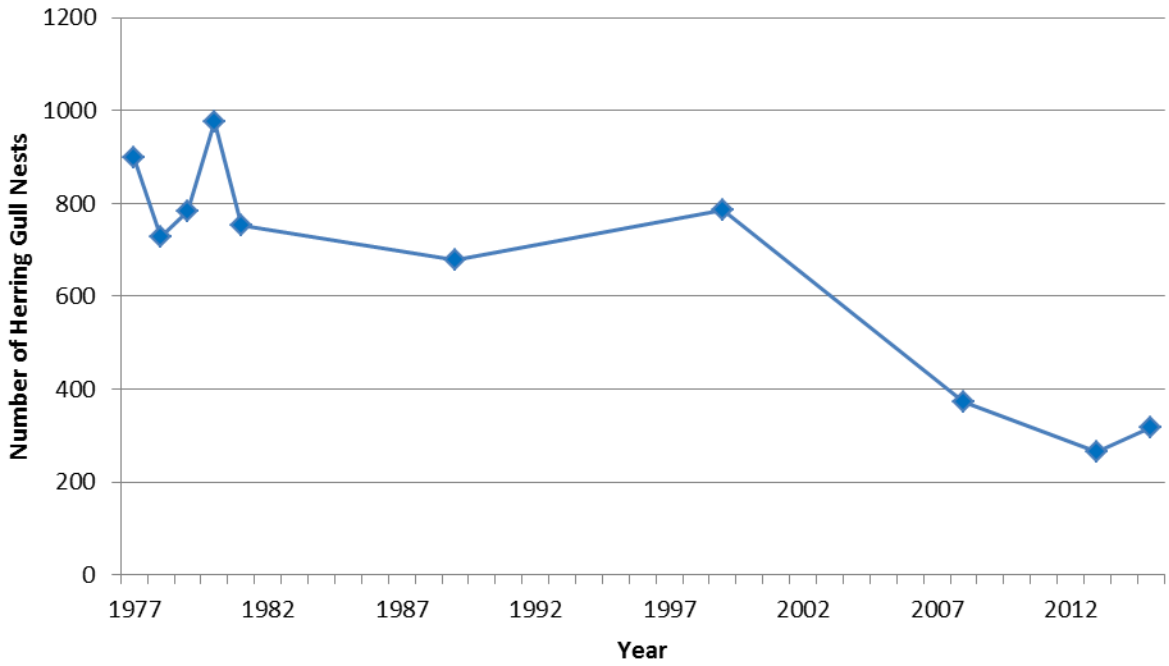

Figure 1.0: Temporal trends in Herring Gull nest numbers in Pukaskwa National Park. Source: Parks Canada Agency.

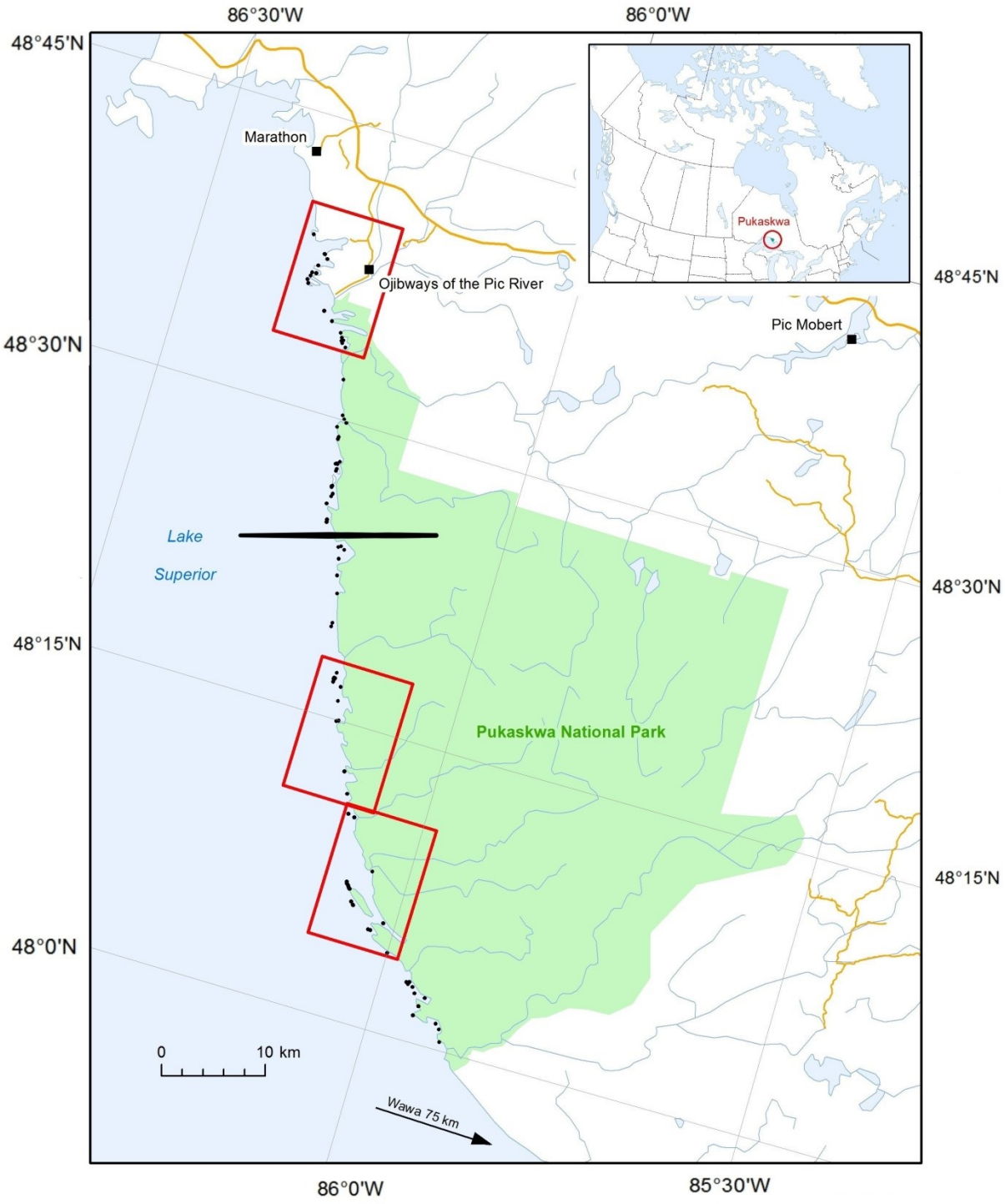

Figure 1.1: Map of Pukaskwa National Park (PNP). Black dots indicate locations of nesting sites where nest counts were conducted from 1977 to 2015. Black line indicates arbitrary division between north and south PNP. Red boxes show northern (top box) and southern (bottom two boxes) areas in PNP where samples were collected.

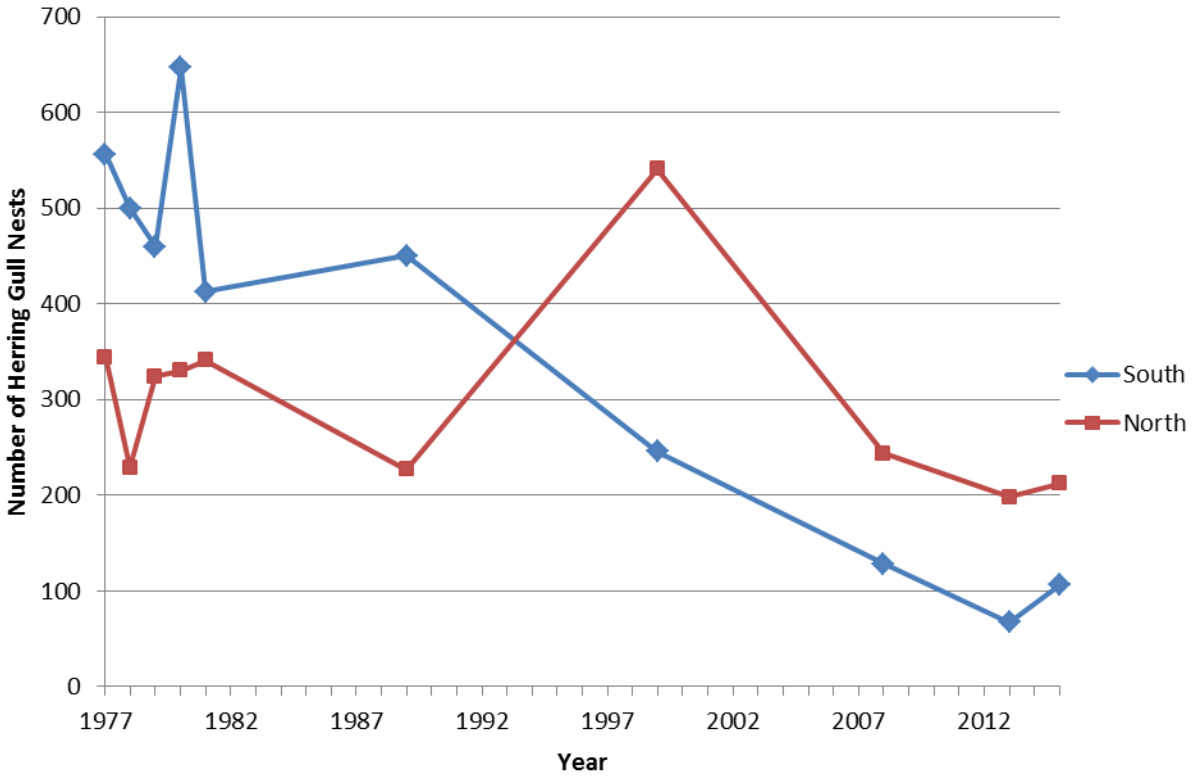

Figure 1.2: Temporal trends in Herring Gull nest numbers in southern and northern regions of Pukaskwa National Park. Source: Parks Canada Agency

One of the possible causes of the geographic differences in population trends is proximity to anthropogenic food sources. Southern birds are removed from anthropogenic influences as they inhabit the longest stretch of undeveloped coastline on the Great Lakes. However, Herring Gulls in northern PNP breed in an area influenced by humans, e.g. towns and garbage dumps. Herring Gulls are known to eat garbage and in some cases when dumps have closed, populations have declined (Kilpi and Öst, 1998). Previous studies have shown that as anthropogenic food sources, e.g. garbage, decrease, Herring Gull clutch size, egg size, hatchling body mass, and number of young fledged may also decrease (Spaans *et al,* 1987; Kilpi and Öst, 1998). Herring Gulls that lack a reliable food supply, whether natural or not, can suffer increased predation on their eggs as they spend more time foraging forcing them to be less attentive to protecting their

nests. Lower chick survival may also result from lower food availability (Kilpi and Öst; 1998; Hunt, 1972). Garbage dumps in northern PNP could be sustaining Herring Gull populations in that region and could be one of the reasons that there are geographic differences in population trends between northern and southern PNP.

The goal of my thesis is to investigate factors that may be contributing to Herring Gull population declines in Pukaskwa National Park. As alluded to above, one possible factor that may be important in regulating gull population size is food availability. I will evaluate possible food stress by measuring a variety of intrinsic endpoints in gull eggs that reflect diet composition. Herring Gulls rely on local food sources for egg formation (Hobson *et al,* 1997), therefore, the chemical composition of their eggs will reflect the local environment that is used for foraging. Herring Gulls forage near their nesting colonies, preferring local foraging sites versus those further away (Sibly and McCleery, 1983).

In addition to their use as ecological indicators in PNP, Herring Gulls have been used in a similar role across the Laurentian Great Lakes for the past five decades. Environment and Climate Change Canada's Great Lakes Herring Gull Monitoring Program (GLHGMP) was started in the 1970s to monitor chemical contaminants in the environment (Figure 1.3). Egg samples are collected annually from all the lakes including Lake Superior. These samples are stored frozen in the National Wildlife Specimen Bank at the National Wildlife Research Centre, Ottawa. In recent decades, these egg samples have also been used to assess gull diets. As a result, I can interpret the PNP results in a larger spatial and temporal context. This may be important in terms of assisting the Parks Canada Agency in formulating a strategy for addressing the decline in Herring Gull populations as it pertains to park ecological integrity.

Figure 1.3: Map showing sites monitored as part of the Great Lakes Herring Gull Monitoring Program. Colony names are as follows: 1, Granite Island; 2, Agawa Rocks; 3, Gull Island; 4, Big Sister Island, Green Bay; 5, Double Island; 6, Chantry Island; 7, Channel/Shelter Island, Saginaw Bay; 8, Fighting Island, Detroit River; 9, Middle Island; 10, Port Colborne; 11, Niagara River; 12, Muggs Island/Leslie Street Spit in Toronto Harbour; 13, Snake Island (Hebert *et al,* 1999)

Diet Indicators

Numerous techniques can be used to study bird diets. Here, I focus on three methods: regurgitated pellets, egg stable nitrogen and carbon isotopes, and egg fatty acids.

Pellets

Pellets are regurgitated material that birds cannot digest. They can consist of many things including anthropogenic material, bones, bird feathers, fish scales and fish bones such as vertebrae. Pellets can be used to assess diet as they can be collected from the nest itself or the area immediately surrounding the nest. Since pellets are comprised of undigestible material which the Herring Gulls consumed they provide a snap-shot of the Herring Gull diet (Resano-

Mayer *et al,* 2013). This limits the temporal scope of the analysis as pellets do not provide long term dietary data unless collected frequently over a longer period. Such frequent collections can be a source of significant disturbance in nesting colonies. Although not all material will remain in the pellets as some of it will be digested, pellets can provide a general indication of what Herring Gulls are eating. For example, the presence of fish vertebrae indicates utilization of aquatic food sources while the presence of plastic or paper indicates use of anthropogenic food sources. Although pellet analysis is rather easy to conduct there are limitations to their use. Some pellet contents may be impossible to identify. Furthermore, some types of food will be completely digested, e.g. soft-bodied fish, and will not be found in pellets. This can lead to biases in estimation of food types in pellets (Real, 1996; Resano-Mayer *et al,* 2013).

Stable Isotopes

Biochemical markers are used to track bird diet (Hebert *et al,* 2006). For my research, I collected Herring Gull eggs from northern and southern sections of PNP in 2015 and 2016 (Figure 1.1) and examined stable isotopes of carbon and nitrogen. Stable isotopes are useful biochemical markers as they provide information on food origins and trophic position. A stable isotope is a stable, i.e. non-radioactive, form of an element. Stable isotopes of an element have the same number of protons but differ in their number of neutrons creating differences in the atomic mass of the isotopes. For example, there are two stable isotopes of carbon: ^{12}C and ^{13}C. ^{12}C has six protons and six neutrons resulting in an atomic mass of 12. ^{13}C has six protons and seven neutrons giving it an atomic mass of 13. Mass differences between isotopes, such as the lighter ^{12}C isotope and the heavier ^{13}C isotope result in those isotopes reacting at different rates in chemical and biological processes. These rate differences occur because the chemical bonds of

atoms associated with heavy stable isotopes are stronger than those of lighter isotopes. This causes chemical reactions involving the lighter isotopes to occur more rapidly as their bonds are easier to break during biological processes such as photosynthesis. As a result, the heavier isotopes react more slowly than the lighter isotopes leading to isotopic fractionation between reactant and product in chemical and biological reactions. Stable carbon ($^{13}C/^{12}C$) isotopes are used to provide information on the origins of food an organism is eating while nitrogen isotopes ($^{15}N/^{14}N$) provide information on an organism's position in the food chain. Stable isotope values in eggs provide one method for evaluating gull diets in PNP.

Stable carbon isotopes (expressed as $\delta^{13}C$ values) provide information on what food sources a consumer is feeding on. If they are feeding on prey associated with a food web based on aquatic primary producers, such as phytoplankton, they will have less of the ^{13}C isotope present in their tissues resulting in more negative $\delta^{13}C$ values. If a consumer is feeding on terrestrial prey, the ratio of $^{13}C/^{12}C$ will shift with the consumer having more ^{13}C in their tissue resulting in less negative $\delta^{13}C$ values (Rau 1980, France 1995). These differences may be caused by the different photosynthetic pathways used by aquatic and terrestrial plants to fix CO_2. For example, in human food webs, a plant of very high importance is corn, a C4 plant. C4 plants have higher relative amounts of ^{13}C than aquatic plants, which use C3 photosynthetic pathways (Van der Merwe, 1982). This can result in animals that feed on terrestrial plants having less negative $\delta^{13}C$ values than animals feeding on aquatic plants (Schoeninger and DeNiro, 1983). The $\delta^{13}C$ values of aquatic plants can vary as well. Primary producers that live in turbulent, open waters, such as phytoplankton, have reduced boundary layers around them resulting in constant replenishment of CO_2 in the water for carbon fixation. This results in greater discrimination against the heavier ^{13}C isotope as phytoplankton preferentially use ^{12}C instead of ^{13}C during

photosynthesis. Plants that live in less turbulent areas, e.g. benthic plants, may be surrounded by well-defined boundary layers resulting in less discrimination against the heavier ^{13}C isotope as carbon supplies for fixation may be more constrained (Post, 2002). This means that based on where a consumer is feeding, either terrestrial or aquatic food sources, $\delta^{13}C$ values can differ.

Stable nitrogen isotopes (expressed as $\delta^{15}N$ values) are an effective indicator of organism trophic position. As matter and energy are passed through food webs the relative amount of ^{15}N is increased at each trophic level resulting in increasing $\delta^{15}N$ values. This is in part due to differences in the rates at which isotopes undergo biochemical reactions, e.g. transamination/deamination processes, associated with the metabolism of amino acids (Gannes *et al,* 1997). Isotopic differences in reaction rates ultimately result in the preferential elimination of the lighter ^{14}N isotope in nitrogenous wastes (uric acid in birds) while the heavier ^{15}N isotope is conserved during trophic interactions (Mill *et al,* 2007, DeNiro and Epstein 1981; Minagawa and Wade, 1984; Schoeninger and DeNiro, 1983). At each trophic level, $\delta^{15}N$ values increase by approximately 3-4‰ (Hobson *et al,* 1994; Minagawa and Wade, 1984).

When used in conjunction with pellets, stable isotope analysis can provide a clearer picture of the diets of Herring Gulls. While pellets provide insight into the diet through the remains of the meal, stable isotopes provide insight into the assimilated portion of the diet. Pellets provide insight into the short term diet of the birds while stable isotopes provide longer term insights into bird diets. Egg isotope values reflect diet composition during the period of egg formation, similar to other tissues with relatively rapid rates of isotopic turnover (Hobson, 1995).

Fatty Acids

Another group of biochemical markers that can help understand bird diets is fatty acids. Fatty acids are the main components of all lipids and are required by consumers for normal growth and development. For my research, Herring Gull eggs from northern and southern sections of PNP in 2015 and 2016 were analyzed for a variety of fatty acids. Some of these fatty acids are termed essential fatty acids as they cannot be synthesized by consumers (Kainz *et al*, 2004). These fatty acids are only synthesized by primary producers and consumers must meet their requirements from their diets (Kainz *et al,* 2004). This results in significant conservation of prey fatty acid patterns in consumer tissues (Napolitano 1999, Iverson *et al*, 2004).

Fatty acids consist of a methyl end and a carboxyl end. They are defined based upon their carbon skeleton length and number of double bonds. Those with no double bonds are saturated fatty acids. Those with one double bond are monounsaturated fatty acids and those with two or more double bonds are polyunsaturated fatty acids. Since the fatty acid composition of an organism varies from species to species the fatty acid pattern present in a predator provides insight into what they were eating (Napolitano 1999, Iverson *et al*, 2004). For example, terrestrial food sources have lower Omega-3 to Omega-6 fatty acid ratios than aquatic food sources (Meyer *et al,* 2003). An Omega-3 fatty acid is a fatty acid that has its first double bond between the third and fourth carbon atom in the carbon chain relative to the methyl end of the molecule. An Omega-6 fatty acid has its first double bond between the sixth and seventh carbon atom in the chain (Figure 1.4 and 1.5). Fatty acids can also provide insights into whether an organism is feeding on natural or anthropogenic food sources. Trans-fats are produced by natural and anthropogenic processes but the distribution and abundance of trans-fats differs greatly between the two (Quemeneur and Marty, 1991; Ledoux *et al,* 2007). Certain fatty acids, such as

the trans-fat elaidic acid, are rarely present in natural ecosystems but are found in anthropogenic food sources. Therefore the proportions of different fatty acids in an organism can indicate whether they have been feeding on terrestrial, aquatic or anthropogenic food sources.

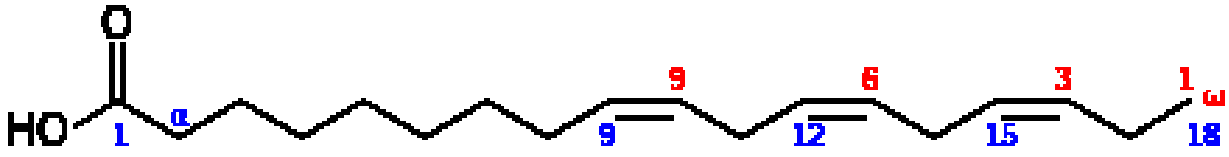

Figure 1.4: Structural drawing of α-linolenic acid (C18:3n3), an Omega-3 fatty acid

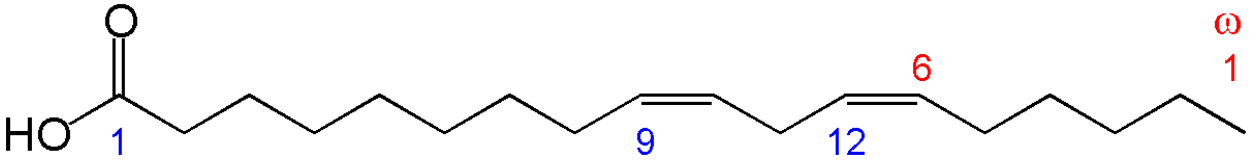

Figure 1.5: Structural drawing of linoleic acid (C18:2n6), an Omega-6 fatty acid

Fatty acid concentrations can also provide information on the quality of the Herring Gull diet. Some fatty acids, particularly the polyunsaturated Omega-3 fatty acids (n-3 PUFAs), such as docosapentaenoic acid (DPA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are required for normal growth and development. Omega-3 and Omega-6 fatty acids are important as they can be essential fatty acids that Herring Gulls can only obtain through their diet. In birds, lower availability of n-3 PUFAs can alter the development of neural tissue and can hamper immune system function (Twining *et al,* 2016). Therefore, if food consumed by gulls has low n-3 PUFA levels it could affect the quality of eggs and chicks. Past studies with swallows (*Tachycineta bicolor*) showed that fatty acid composition was more important to chick survival than food quantity (Twining *et al,* 2016b).This is important because although Herring Gulls from northern PNP may have access to food through human sources, this “junk food” may not meet their nutritional requirements.

Through the application of these three groups of dietary indicators I will assess possible dietary differences in gulls nesting in north and south PNP. I will then examine how diet composition may affect endpoints associated with gull reproductive success, physiology, and population trends.

Effects of Diet

Clutch Size

Clutch size is one way to measure the effects of dietary differences. Clutch size refers to the number of eggs in a nest. For Herring Gulls, modal clutch size is three eggs. However, clutch size can vary depending on food availability and nutrient stores. If food availability is low, clutch size will decrease as the maternal bird does not have the resources required to lay a complete 3-egg clutch (Ankney and MacInnes, 1978). Smaller clutch sizes may indicate lower food availability and can have negative impacts on population sustainability as smaller clutches can lead to reduced recruitment of chicks into gull populations.

Egg Volume and Mass

Egg volume reflects egg size which is determined to a significant extent by the resources available to the female for egg formation. As such, it can be used as an indicator of food availability. Birds with access to more food are able to produce larger and higher quality eggs (Moss and Watson, 1984; Hiom *et al*, 1991). Therefore, when food sources become scarce egg size decreases. Furthermore, increased egg size and quality have been linked with an increase in offspring survival rates (Hiom *et al*, 1991; Williams, 1994). As the availability of food to the

female increases, offspring survival may also increase, as increased egg size may benefit offspring through increased skeletal size and mass (Bolton, 1991).

Egg mass may also affect reproductive success. Lighter eggs may have increased rates of mortality (Williams, 1980). Furthermore, lighter eggs produce smaller chicks. Smaller chicks may have increased rates of mortality during the first few days post-hatch (Schifferli, 1973; Ankney, 1980; Williams, 1980). Furthermore, egg mass may provide information on the quality of the maternal bird as egg mass has been correlated with condition in laying females (Houston *et al*, 1983).

Egg Energy Density

Egg energy density is an important endpoint as eggs with lower energy densities are considered to be of lower quality and may reflect constraints on the availability of energy to gulls during the period of egg formation (Moss and Watson, 1984). Egg energy density reflects the amount of energy in an egg and can be expressed as kJ/egg or kJ/g. Egg energy density is a valuable tool for assessing the impacts of dietary differences as egg energy density depends on food availability during egg development. During periods of low food availability egg energy density may decrease as the availability of energy for the female to invest in her eggs decreases. This can have dramatic impacts on birds as higher quality eggs, e.g. increased energy density, may lead to larger and more developed offspring at hatch (Martin, 1987). Egg energy density will be calculated for Herring Gull eggs collected from PNP and Agawa Rocks (colony monitored in eastern Lake Superior as part of the Great Lakes Herring Gull monitoring program).

Nest Attentiveness

Nest attentiveness is a measure of the amount of time Herring Gulls spend on or near their nest. Both parents take turns incubating their eggs. Incubation ensures that the eggs are kept at the correct temperature for embryonic development while the presence of a parent also acts as a deterrent to predators. However, if food becomes scarce, both parents may be forced to spend more time foraging which can result in neither gull incubating or guarding the eggs. I examined nest attentiveness by setting up remote game cameras which took nest photos each minute, 24 hours per day. These photos were examined to determine how often gulls were absent from their nests.

Population Distribution

The population distribution of breeding Herring Gulls may provide insights into the availability of food. Herring Gulls feed within a 30-40 km range of their breeding colony. If food sources become low in this area they may be forced to relocate to a new site where food is more readily available or reproductive success may be so low as to reduce recruitment ultimately resulting in population declines over the long-term.

Stress (Corticosterone)

Corticosterone is a stress hormone found in birds. With increasing exposure to environmental stress, levels of corticosterone in blood (and other tissues) may increase. As such, measuring corticosterone levels in avian samples may provide insights into the degree of stress being experienced by a bird (Marra and Holberton, 1998). Here, I investigate the degree to which egg contents and eggshell can be used to measure corticosterone in birds. I also investigate the

degree to which corticosterone levels are related to gull diets as inferred from my dietary markers. The use of eggs for evaluating corticosterone levels in birds, as opposed to blood, removes the possible effects of investigator disturbance. Corticosterone levels in eggs during early stages of development should only reflect maternally contributed corticosterone as embryonic contributions do not become important until later in development (Kalliecharan and Hall, 1974). Hence, egg corticosterone should reflect the level of stress experienced by the laying female during egg formation (Paitz and Casto, 2012).

Current Study

For my project I collected Herring Gull eggs from northern and southern sections of Pukaskwa National Park (Figure 1.1) in 2015 and 2016. By examining pellets adjacent to sampled nests and by measuring intrinsic endpoints in eggs, i.e. stable carbon and nitrogen isotopes and fatty acids, I gain insights into Herring Gull diets. I then examine the possible effects of diet on factors associated with Herring Gull population sustainability. I use this information to evaluate factors affecting Herring Gull populations in Pukaskwa National Park.

In Chapter 2, I examine indicators of diet, i.e. pellets, egg stable isotopes, and egg fatty acids in samples collected in 2015 and 2016 from northern and southern PNP. I predict that there will be differences in diets between the two regions of the park with a greater reliance on natural foods in the southern part of the park and more evidence of anthropogenic foods in gull diets from northern PNP. To generate the results summarized in Chapter 2, I collected pellet samples in 2016 and dissected them for food remains, I collected eggs in 2015 and 2016, processed them in preparation for laboratory analysis, encapsulated egg subsamples prior to stable isotope analysis, and conducted the egg fatty acid analyses. My predictions here include:

1) south PNP pellets will contain less anthropogenic food than north PNP

2) south PNP egg $\delta^{15}N$ values will be greater than north PNP

3) south PNP egg $\delta^{13}C$ values will be more negative than north PNP

4) south PNP egg fatty acid profiles will have higher Omega 3/Omega 6 fatty acid ratios than north PNP.

In Chapter 3, I examine endpoints that could contribute to declines in gull populations, i.e. egg and clutch size, egg energy density, nest attentiveness, stress, and their possible associations with gull diets. My working hypothesis here is that gulls nesting in south PNP are food stressed compared to gulls breeding in north PNP. For Chapter 3, I measured eggs so that I could calculate egg size and energy density, I assessed nest attentiveness by setting up remote-controlled cameras in the field and summarized photographic results. I also performed laboratory analyses to measure corticosterone in both egg contents and eggshells. I compared results between northern and southern PNP. My predictions here include:

5) south PNP clutch sizes and egg volumes will be lower than north PNP

6) south PNP egg energy density will be lower than north PNP

7) south PNP nest attentiveness will be lower than north PNP as south PNP birds may spend more time foraging for food.

8) south PNP eggs will have higher levels of corticosterone than north PNP.

I interpreted my PNP results in a larger temporal context by comparing them to a larger database generated through Environment and Climate Change Canada's GLHGMP (Figure 1.3). As part of that program, samples have been collected from across all the Great Lakes. I pay particular attention to results obtained from the analysis of samples from another site in eastern Lake Superior, i.e. Agawa Rocks (approximately 150 km to the southeast). Egg collections have

been made there for decades allowing me to incorporate a temporal component in my study. I also compare changes in egg endpoints at Agawa Rocks with an index of prey fish availability collected through time by the U.S. Geological Survey (USGS). This allows me to investigate the possible role of changes in food availability on egg endpoints.

Integrating these different data sources enables me to interpret the PNP situation in a larger spatial and temporal context so that I may better understand if diet is contributing to the decline in Herring Gull populations in the park. This research is important as it investigates possible factors contributing to the significant population decline observed in Pukaskwa National Park Herring Gull populations. If I can determine what factors are underlying declines in Herring Gull populations I may be able to provide insights into how the PNP ecosystem is changing and what the broader implications may be for other biota in the same ecosystem. For example, Double-Crested Cormorants (*Phalacrocorax auritus*) feed exclusively on fish. By understanding what impact prey fish abundance is having on Herring Gulls we will be able to better understand the potential impacts on cormorant populations. Similar connections could exist for other species, e.g. Bald Eagles (*Haliaeetus leucocephalus*), so understanding the factors contributing to Herring Gull population decreases will improve our ability to predict possible impacts on other biota. In Chapter 4, I discuss how my results are relevant to the management of PNP and I suggest possible avenues for future research.

Chapter 2 – Pellets, Stable Isotopes, and Fatty Acids as Diet Indicators in Herring Gulls in Pukaskwa National Park

Introduction

In Pukaskwa National Park (PNP), Herring Gulls are used to monitor park ecological integrity. Monitoring dietary changes in top-level predators, such as Herring Gulls, provides information on the state of an ecosystem. By understanding how the diet of top-level predators is changing it is possible to assess how the ecosystem is changing and the stresses it is under (Weimerskirch *et al,* 2003; Miller and Sydeman, 2004). Here, I examine gull diets through the use of regurgitated pellets and biochemical markers in eggs.

A traditional approach to assessing gull diets is through the use of regurgitated pellets. Pellets consist of undigestible food remains regurgitated by gulls and include items, such as bones, feathers, or garbage. These pellets are often regurgitated adjacent to nests. Pellets provide insights into Herring Gull diets as they represent the remains of food that could not be digested. For example, pellets which contain garbage are indicative of Herring Gulls feeding on anthropogenic food sources while pellets containing fish vertebrae or feathers indicate the consumption of more "natural" food types. Pellet analysis provides a "snap-shot" of the Herring Gull diet at the time of regurgitation. However, pellet analysis can over-represent more undigestible food types, e.g. bones, feathers, while under-representing others, e.g. soft-bodied fish (Real, 1996; Resano-Mayer *et al,* 2013).

Biochemical markers, i.e. stable isotopes and fatty acids, are also useful in terms of understanding bird diets and trophic position (Hebert *et al*, 2006). These biochemical markers have been used in wildlife monitoring studies to understand how bird diets may be changing through time or differ spatially (Hebert *et al,* 2008). By studying biochemical markers in top trophic level predators it is possible to make inferences regarding larger ecosystem level changes (Chapdelaine and Rail, 1997; Furness and Kees, 1997). Eggs are a useful matrix for measuring

these markers because the biochemical composition of gull eggs reflects the local environment. Gulls arrive at their breeding grounds approximately one month prior to egg laying and typically feed within a 30-40 km radius meaning egg biochemical profiles are representative of their local breeding ground diet.

Biochemical markers that can be used to understand bird diets include stable isotopes of carbon (^{12}C, ^{13}C) and nitrogen (^{14}N, ^{15}N). A stable isotope is a stable, non-radioactive, form of an element. Stable isotopes of an element have the same number of protons but differ in their number of neutrons creating differences in the atomic mass of the isotopes. These isotopes react at different rates in kinetic reactions leading to isotopic fractionation during processes such as nitrogenous waste formation and photosynthesis (Mill *et al,* 2007, DeNiro and Epstein 1981; Minagawa and Wade, 1984; Schoeninger and DeNiro, 1983). Stable isotope data are usually expressed in delta (δ) notation ($\delta^{13}C$ or $\delta^{15}N$) as parts per thousand (or per mil) deviation from a standard. Previous studies have used carbon and nitrogen isotopes to provide information on the diet and trophic position of birds (Hebert *et al*, 2008).

Carbon isotopes can provide insights into food sources used by organisms. For example, organisms associated with phytoplankton-based aquatic food webs are depleted in ^{13}C compared to organisms relying on terrestrial food (Schoeninger and DeNiro, 1983, Rau, 1980, France, 1995). Nitrogen isotopes provide insight into the trophic position of an organism because the ratio of ^{15}N to^{14}N increases with increasing trophic position. This occurs during transamination and deamination of amino acids in food when the nitrogen pool in consumers becomes selectively enriched in ^{15}N. This occurs because ^{14}N is preferentially eliminated during the formation of nitrogenous waste, i.e. uric acid in birds (Gannes *et al,* 1997; Mill *et al,* 2007; DeNiro and Epstein 1981; Minagawa and Wade, 1984; Schoeninger and DeNiro, 1983). This

results in a predictable increase in $\delta^{15}N$ values of 3-4‰ per trophic level (Hobson *et al,* 1994; Minagawa and Wade, 1984). $\delta^{15}N$ values are larger in fish as there are more trophic levels in aquatic food webs, thus, there is more scope for increases in $\delta^{15}N$ values with the additional trophic levels. Terrestrial food webs often have fewer steps with agricultural animals being primary consumers. Stable isotope analysis is a potentially useful method for understanding the diet of Herring Gulls. However, $\delta^{13}C$ or $\delta^{15}N$ values for one species, particularly omnivores, may be hard to interpret in isolation. To aid in this it is useful to have a reference sample or species for which diet has been well characterized (Post, 2002). Double-Crested Cormorants (*Phalacrocorax auritus*), as piscivores, provide baseline isotope values reflective of a totally aquatic, primarily fish diet, for comparison with Herring Gull data. Even so, interpretation of stable isotope data is not always straight-forward and other dietary markers, e.g. fatty acids, can help to further elucidate gull diets.

Fatty acids are the main components of lipids. They consist of a hydrocarbon skeleton with a carboxyl and a methyl end. They are an important source of fuel for animals as some fatty acids are metabolized to provide energy. They are also necessary for normal growth and development in birds (Twining *et al,* 2016b). Certain fatty acids, e.g. omega 3 polyunsaturated fatty acids (n3-PUFAs) are termed essential fatty acids because they cannot be synthesized by consumers (Arts *et al,* 2001; Kainz *et al,* 2004). Consumers must obtain their requirements for these fatty acids from their diets. Because of this, these fatty acids are conserved in the tissues of consumers after food web transfer. This means that insights into predatory bird diets can be gained by looking at the fatty acid composition of their tissues, e.g. eggs (Napolitano, 1999; Iverson *et al,* 2004). For example, omega 3 fatty acids are much more abundant in aquatic systems than terrestrial biomes while omega 6 fatty acids are more common in terrestrial systems

(Nettleton, 1991; Simopoulos, 2002; Leitzmann *et al,* 2004). Hence, foods originating from aquatic sources would be expected to have greater omega 3/omega 6 fatty acid ratios. Fatty acids may also be useful in examining the presence of anthropogenic food sources in an organism's diet. For example, trans-fats are fatty acids with a trans bond instead of a cis bond in the hydrocarbon chain of the fatty acid and are found primarily in foods of anthropogenic origin (Quemeneur and Marty, 1991; Ledoux *et al,* 2007). Therefore, the presence of anthropogenic trans-fats such as elaidic acid in eggs may indicate that anthropogenic food is being consumed by an organism. Fatty acids are useful for providing more detailed information on diet composition in birds. Fatty acids profiles may show shifts in fatty acid ratios, presence or absence of certain fatty acids and changes in the concentration of fatty acids. Thus, fatty acid profiles may be extremely versatile in providing information regarding diet composition and differences in diet between geographic regions. Using pellets, stable isotopes, and fatty acids in concert will allow me to gain improved insights regarding bird diets. Here, I use these methods to investigate diet composition in Herring Gulls on Lake Superior, specifically in PNP.

In PNP on Lake Superior, Herring Gull populations have been censused semi-regularly by the Parks Canada Agency (Figure 1.1). These surveys identified a significant decline in Herring Gull populations of approximately 70% since surveys began in the 1970s (Figure 1.0). However, this decline varies across different regions of the park (Figure 1.2). The southern portion of the park has seen Herring Gull populations decrease by approximately 80% while populations in the northern section of the park have only decreased by approximately 40%. Studying dietary differences of gulls between these two regions may provide insights into the factors contributing to Herring Gull population trends.

Since Herring Gulls are top trophic level predators, their diets and trophic position may be useful in identifying ecosystem change (Chapdelaine and Rail, 1997; Furness and Kees, 1997; Hebert *et al,* 2008). Changes in diet may reflect larger scale factors regulating relative availability of different food types in northern and southern regions of PNP.

Methods

Egg Collection

In 2015, I collected 15 eggs from the northern and 13 eggs from the southern regions of PNP, respectively. I conducted similar collections in 2016 with 15 eggs collected from both regions. For these collections, one egg was chosen randomly from multiple egg nests whenever possible to minimize impacts on reproductive success. Nests which had three eggs were preferred for egg collection over two egg nests while two egg nests were preferred over one egg nests. Once collected, eggs were measured, labelled, and placed in a padded case for transport to the Tissue Preparation Laboratory at the National Wildlife Research Centre.

In 2016, pellets were collected from as many sampled nests as possible (north PNP n = 8; south PNP n = 11). Pellets were only collected if they were within the nest itself or less than one meter from the nest in order to associate pellets with a particular nest and egg. Pellet analysis was completed at the NWRC using a dissecting microscope. Pellets were broken apart and the contents were sorted into three categories: aquatic, terrestrial and anthropogenic. Pellets were classified based on which food types were present, individual pellets could be classified into more than one category. Percent occurrence of each food type in the diets of northern and southern PNP Herring Gulls was estimated from the pellet analysis.

In addition to Herring Gull eggs, 13 eggs of Double-crested Cormorants were also collected on Lake Superior. Because no cormorants nested in 2015 and 2016 in PNP, collections were made in Batchawana Bay which is located approximately 185km south-east of PNP. Cormorants are obligate aquatic feeders that primarily consume fish. This makes them an ideal species for comparison with Herring Gulls for the evaluation of biochemical markers of diet. Gull eggs that are more similar to cormorant eggs in terms of their biochemical signatures should reflect diets with greater reliance on aquatic resources.

Egg Processing and Analysis

All laboratory equipment was chemically cleaned and all work was conducted in a biological safety cabinet. Tools were washed with soap and water then rinsed with distilled water. After drying, they were sprayed three times with ethanol. They were then rinsed three times with hexane. Eggs were opened along their equator using a scalpel and the egg contents, i.e. yolk and albumen, were placed in a pre-weighed container. The container was then weighed and egg contents mass was determined using an electronic balance. Once the egg contents were removed, the shell was retained and allowed to dry. Egg contents were transferred into a pre-weighed Teflon cup and weighed to 1 decimal place. Egg contents were homogenized using a Mettler PB3001-Sl and Polytron 20 mm shaft. Homogenization was conducted for approximately 10-20 seconds depending on the egg. In certain situations extra time was required to completely homogenize the egg contents. The egg contents were aliquoted into various storage containers (glass, polyethylene vial, cryovials) depending on how they were to be stored prior to specific laboratory analyses. Samples for stable isotope and fatty acid analyses were stored at -80°C.

Samples were placed into individual vials which had small holes poked in their lids. The samples were then placed in a -80°C freezer. They were left at -80°C for between 30 minutes to a

few hours depending upon the size of the sample. The samples were then placed in a Labconco FreezeZone 6 Litre Freeze Dry System (Model 7753) and left for at least 48 hours. When freeze-drying was completed, samples were stored at -80°C until fatty acid analysis was performed.

The first phase of the fatty acid methyl ester (FAME) protocol involved the extraction of lipids from each sample. Approximately 0.2g (dry weight) of each sample was placed in a test tube. Two ml $CHCl_3$:MeOH and 100 µL of 1000 µg/mL 5a-Cholestane were added. The 5a-Cholestane was used as an internal standard. Each sample was extracted three times. Each extraction involved the homogenization of the sample before centrifuging at 3500 rpm for 10 minutes. Once centrifugation was completed, the supernatant was removed from the test tube and transferred to a screw cap tube. The pellet was not disturbed to avoid transfer of particulates into the screw cap tube. The extraction process was repeated two more times and the liquid in the screw cap tube was then evaporated under a N_2 stream. The pellet remaining in the test tube was left to dry over-night before being transferred into individual vials. These lipid-free pellets were used for stable isotope analysis (see below). The screw cap tubes containing the supernatant were placed in a water bath at 40°C with the N_2 stream blowing on the surface of each sample. Once the sample was completely evaporated the residue was reconstituted in 2ml of $CHCl_3$:MeOH to allow lipid content analysis. Using pre-weighed vials, 200 µL of each sample was transferred into individual vials. The vials were left to dry for at least 3 days. The lipid content analysis was completed in duplicate for each sample. The liquid remaining once the 200 µL were removed was then evaporated to dryness again.

The second part of the preparation for FAME analysis was the methylation of fatty acids. This process was completed under acidic conditions. Each sample had 1.5 ml of toluene added to it. The samples were vortexed for 10 seconds before having 2 ml of acid (H_2SO_4-MeOH) added.

The samples were placed in a water bath at 50°C overnight or for a minimum of 16 hours. Two ml of a base ($KHCO_3$) were added to neutralize the acid. Samples were vortexed for 10 seconds before capping the tube. This was done in order to allow the CO_2 produced by the acid neutralization to vent so the tubes did not explode. Each tube was then placed in a 3500 rpm centrifuge for 5 minutes. Once centrifuged, the tube had two layers: toluene on the top, water at the bottom. The top layer of toluene was transferred to a new test tube using a pipette. Next, 2 mL of hexane were added to the original screw cap tubes. The screw cap tubes were centrifuged again for 5 minutes. The top layer, this time, hexane, was transferred into the new test tubes. Another 2 mL of hexane were added to the screw cap tubes and they were centrifuged again. The hexane was then transferred to the new test tubes. The samples were evaporated in a hot water bath at 40°C under a N_2 stream. The final step of the preparation was to reconstitute the residue in 2mL of hexane. 200 µL of each sample was placed in a gas chromatography (GC) mini-vial for GC analysis. The remaining liquid was transferred to a second vial and stored in a freezer at -80°C. The GC analysis was completed using an Agilent 6890N High Resolution Gas Chromatograph equipped with a Flame Ionization Detector. Compared to a standard fatty acid mixture, each substance was identified as it passed through the GC column based upon its time of elution.

During fatty acid analysis a blank, two quality control (QC) samples and a duplicate sample were run. The QC sample consisted of Herring Gull egg sample that had been well-characterized regarding its fatty acid composition. Results from these QC samples ensured that results from "unknown" samples were accurate. Duplicate samples were chosen arbitrarily with the duplicate being run last to ensure that the results remained consistent throughout the procedure.

Stable isotope analysis used the lipid-free pellet created during FAME preparation. A lipid-free pellet was used as the presence of lipids can alter the stable isotope values of a sample (Logan *et al,* 2008). One mg (±0.2mg) of freeze-dried egg was encapsulated in tin using a Mettler Toledo MX5 microbalance. The balance was zeroed before the sample was added. Once the correct weight was obtained the capsule was pinched closed using tweezers. Once the capsule was closed it was folded so that it resembled a ball. Once the desired shape was obtained the capsule was re-weighed to ensure that no sample was lost. Capsules were placed in a 96-well plate and sent to the G.G. Hatch Stable Isotope Laboratory at the University of Ottawa for analysis. Stable isotope analysis was conducted using an Elementar Isotope Cube Elemental Analyser followed by "trap and purge" separation and on-line analysis by continuous-flow with a DeltaPlus Advantage isotope ratio mass spectrometer (Thermo Scientific) coupled with a ConFlo III. Stable isotope values were reported in delta notation, as parts per thousand (‰, per mil) deviation from standard material (N - atmospheric air, C - PeeDee Belemnite limestone). δ values were calculated by comparing the isotope ratio found in the sample to the isotope ratio found in a standard using the following equation: δ (‰) = ($R_{sample}/R_{standard}$ - 1)*1000 (Dawson and Brooks, 2001). Where R is the ratio of the heavy to light isotope, e.g. $^{15}N/^{14}N$, and R_{sample} and $R_{standard}$ are the heavy to light isotope ratios of the sample and standard respectively. Quality control was maintained through the analysis of QC samples as well as by analyzing every tenth sample in duplicate. Analytical precision was ±0.2 ‰.

All statistical tests were performed using Statistica 13 Academic package with $\alpha = 0.05$. Graphs were produced using Microsoft Excel and Statistica 13. Inter-year and inter-region differences were assessed using either parametric or non-parametric statistics depending upon whether data met parametric assumptions. The degree to which data could be combined across

years was evaluated by determining whether there were significant differences in dietary variables between years within regions. Principal components analysis (PCA) was conducted on both fatty acid percent composition data and concentration data. These analyses summarized variation in the fatty acid data and were useful in assessing the degree to which samples were similar to one another. Here, I use PCA to investigate the degree to which eggs from north and south Pukaskwa populations could be distinguished based upon their fatty acid profiles. Principal components included were those that had eigenvalues greater than 1.0. Selection of principal components for further interpretation is somewhat arbitrary, but this method provides an objective way of making this selection. Variable loadings on the principal components provided the means to identify which fatty acids were most important in differentiating samples from the two regions. Fatty acids included in the percent composition PCA were those that represented at least 0.1% of total fatty acid content. The same fatty acids were used in the concentration data PCA. Principal components with eigenvalues greater than 1 were included in the interpretation of the PCA results.

Results

Pellet Analysis

Analysis of pellets collected in 2016 indicated dietary differences in northern versus southern PNP Herring Gulls. As inferred from pellets, there was a significant difference in the degree to which anthropogenic food occurred in the diets of northern gulls (approximately 70% of pellets contained food from human sources) versus southern gulls (20%) ($z = 3.0$, $p = 0.003$).Terrestrial foods, i.e. birds, contributed more to diets of southern nesting gulls (50% of pellets contained terrestrial food) than northern birds (20%) ($z = -2.0$, $p = 0.049$) (Figure 2.0).

There was no significant difference in proportions of pellets containing aquatic food between northern and southern PNP (z = -1.4, p = 0.17).

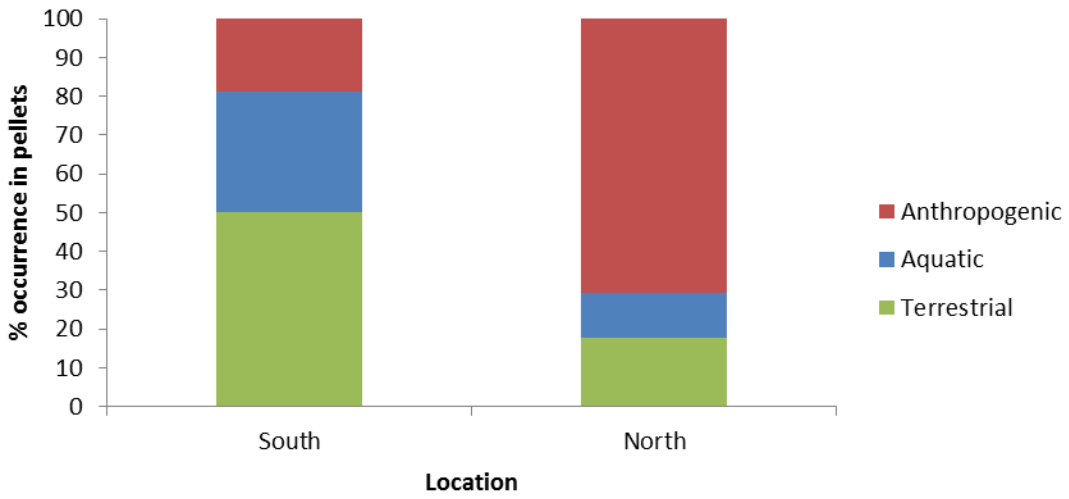

Figure 2.0: Pellet analysis results for southern and northern PNP showing the percentage of pellets containing anthropogenic, aquatic or terrestrial foods.

Stable Isotopes

Data from 2015 and 2016 were combined based upon the lack of inter-year statistical differences in dietary endpoints within northern and southern regions of the park (see Appendix, Tables A3, A6). Comparison of $\delta^{13}C$ values in Herring Gull eggs between north and south PNP showed no statistical difference ($t(56) = 0.1$, $p = 0.94$) (Figure 2.1). However, egg $\delta^{15}N$ values were higher in southern samples than in northern samples ($t(56) = 5.3$, $p < 0.001$) (Figure 2.2). $\delta^{13}C$ values in cormorant eggs (mean ± std error $\delta^{13}C$ = -23.5 ± 0.3) were significantly more negative than in Herring Gull eggs from north and south PNP (ANOVA $F_{2,68} = 4.0$, $p = 0.02$ followed by Tukey's HSD test). $\delta^{15}N$ values in cormorant eggs (mean ± std error $\delta^{15}N$ = 12.2 ± 0.1) were significantly greater than in gull eggs from north and south PNP (ANOVA $F_{2,68}$ = 184.3, $p < 0.001$ followed by Tukey's HSD test).

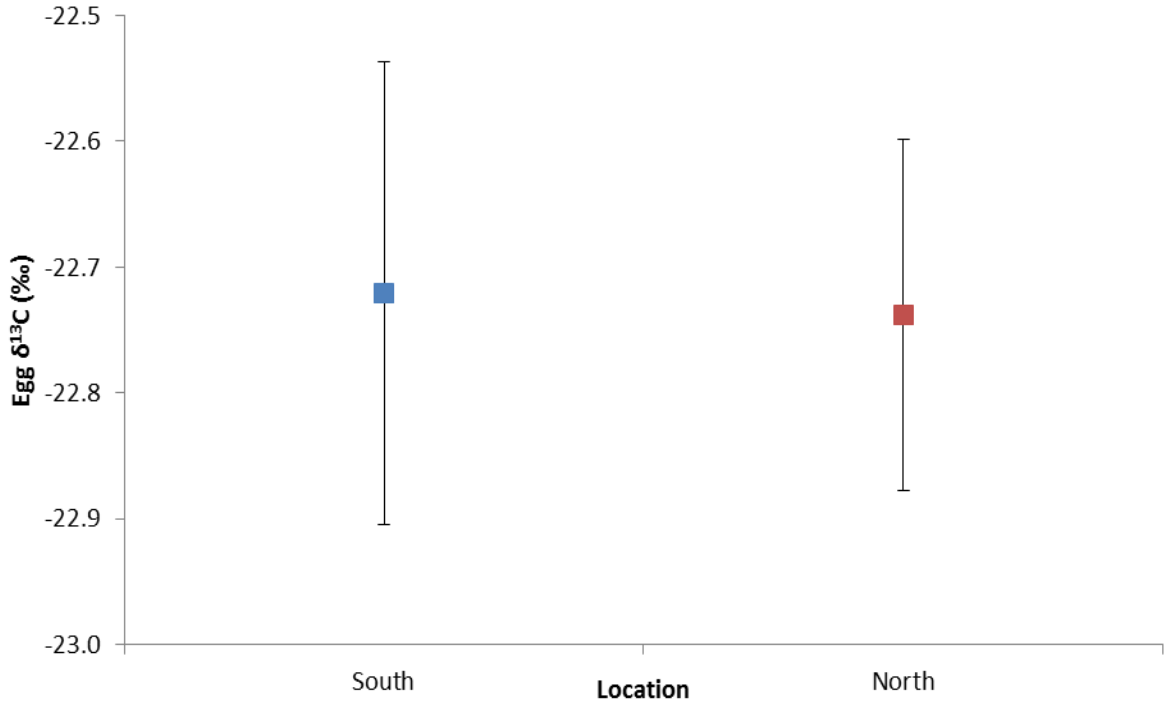

Figure 2.1: Mean (± standard error) $\delta^{13}C$ values (‰) in Herring Gull eggs from south and north PNP, 2015 and 2016.

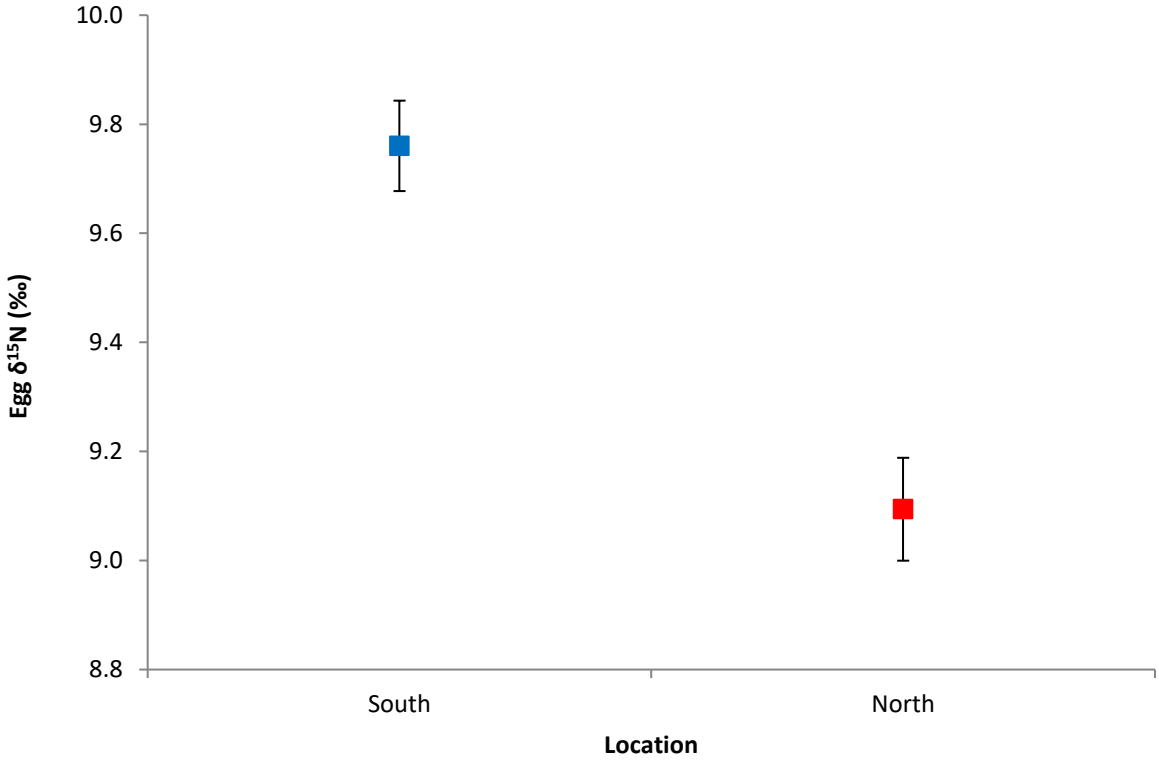

Figure 2.2: Mean (± standard error) δ15N values (‰) in Herring Gull eggs from south and north PNP, 2015 and 2016.

Fatty Acid Results

Data from 2015 and 2016 were combined based upon the lack of inter-year statistical differences in fatty acid results within northern and southern regions of the park (see Appendix, Tables A1-2, A4-5). Principal components analysis (PCA) of both concentration and percent composition data indicated that there were 5 principal components with an eigenvalue greater than 1 (Table 2.0, Table 2.1).

Table 2.0: Principal component (PC) eigenvalues and percent variance explained from the principal components analysis of egg fatty acid concentration data from north and south PNP, 2015 and 2016.

PC	Eigenvalue	% Total Variance	Cumulative Variance
1	7.6	38.0	38.1
2	2.7	13.6	51.6
3	1.9	9.4	61.0
4	1.5	7.4	68.5
5	1.1	5.7	74.2

Table 2.1: Principal component (PC) eigenvalues and percent variance explained from the principal components analysis of egg fatty acid percent composition data from north and south PNP, 2015 and 2016.

PC	Eigenvalue	% Total Variance	Cumulative Variance
1	7.8	39.1	39.1
2	2.4	12	51
3	2	10.1	61.1
4	1.5	7.5	68.6
5	1.3	6.4	75

In both PCAs, only principal component 1 was useful in separating egg samples from north and south PNP ($t(56) = -5.3$, $p < 0.001$). Fatty acids which had principal component loadings greater than 0.6 or lower than -0.6 were deemed to be important in defining differences in fatty acids between north and south PNP and these fatty acids were examined in further detail (Table 2.2, 2.3; Figures 2.3, 2.4).

Table 2.2: Variable loadings on individual principal components (PC) from the principal components analysis of egg fatty acid concentration data from north and south PNP, 2015 and 2016.

Variable	PC1	PC2	PC3	PC4	PC5
Myristic acid	-0.63	0.34	0.50	0.07	-0.07
Pentadecanoic acid	-0.47	0.71	-0.05	0.40	0.08
Palmitic acid	0.10	0.63	-0.35	-0.52	0.05
Palmitoleic acid	-0.79	0.38	-0.20	-0.06	-0.01
Heptadecanoic acid	0.07	0.73	0.41	0.33	0.00
Stearic acid	0.57	0.49	-0.24	-0.06	-0.23
Elaidic acid	0.17	0.27	0.35	-0.36	0.62
Oleic acid	0.40	0.49	-0.11	-0.57	-0.26
Linoleic acid ‡	0.60	0.29	0.08	0.31	0.37
Arachidic acid	0.03	0.04	-0.67	-0.04	0.51
γ-linolenic acid (GLA) ‡	0.81	0.24	-0.01	0.18	0.03
Eicosenoic acid	-0.76	0.18	-0.04	-0.04	-0.16
α-linolenic acid (ALA) †	-0.85	0.18	-0.02	0.18	0.08
Cis-11,14-eicosadienoic acid ‡	-0.78	0.03	0.07	-0.02	0.11
Cis-8,11,14-eicosatrienoic acid (DGLA)‡	0.08	-0.17	0.63	-0.47	0.06
Cis-11,14,17 eicosatrienoic acid (ETE)†	-0.66	0.10	-0.39	-0.03	0.00
Arachidonic acid (ARA) ‡	0.67	0.39	0.07	0.04	-0.38
Eicosapentaenoic acid (EPA) †	-0.92	-0.06	0.02	0.01	-0.13
Docosapentaenoic acid (DPA) †	-0.85	-0.08	-0.11	-0.17	-0.09
Docosahexaenoic acid (DHA) †	-0.66	0.23	0.29	-0.25	0.10

† Omega 3 fatty acid

‡Omega 6 fatty acid

Table 2.3: Variable loadings on individual principal components (PC) from the principal components analysis of egg fatty acid percent composition data from north and south PNP, 2015 and 2016.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5
Myristic acid	-0.69	-0.38	0.35	0.06	0.16
Pentadecanoic acid	-0.57	-0.63	-0.14	-0.18	-0.28
Palmitic acid	-0.29	0.07	-0.59	0.58	0.18
Palmitoleic acid	-0.86	-0.04	-0.16	-0.16	-0.14
Heptadecanoic acid	-0.05	-0.76	0.34	-0.17	-0.24
Stearic acid	0.45	-0.36	-0.34	-0.05	0.52
Elaidic acid	0.15	-0.11	0.31	0.68	-0.35
Oleic acid	0.40	0.67	0.33	-0.39	-0.16
Linoleic acid‡	0.53	-0.51	-0.09	0.16	-0.22
Arachidic acid	0.01	0.17	-0.59	-0.03	-0.48
γ-linolenic acid (GLA) ‡	0.77	-0.39	-0.15	0.12	0.14
Eicosenoic acid	-0.79	0.03	0.11	-0.25	0.05
α -linolenic acid (ALA) †	-0.86	-0.22	-0.05	-0.06	-0.06
Cis-11,14-eicosadienoic acid ‡	-0.80	-0.09	0.01	0.15	0.21
Cis-8,11,14-eicosatrienoic acid (DGLA)‡	0.02	0.14	0.64	0.39	0.26
Cis-11,14,17 eicosatrienoic acid (ETE)†	-0.67	0.07	-0.36	-0.08	0.10
Arachidonic acid (ARA) ‡	0.59	-0.34	0.15	-0.36	0.35
Eicosapentaenoic acid (EPA) †	-0.91	0.04	0.05	-0.04	0.19
Docosapentaenoic acid (DPA) †	-0.85	0.18	-0.05	0.07	0.22
Docosahexaenoic acid (DHA) †	-0.74	0.03	0.35	0.13	-0.17

† Omega 3 fatty acid

‡Omega 6 fatty acid

Sample scores for both PCAs indicated that, in general, Factor 1 separated northern and southern egg samples (Figures 2.3, 2.4) while Factors 2 through 5 were not useful in that regard.

The omega 3 fatty acids loaded negatively on Factor 1 while most of the omega 6 fatty acids loaded positively (Tables 2.2, 2.3; Figures 2.3, 2.4).

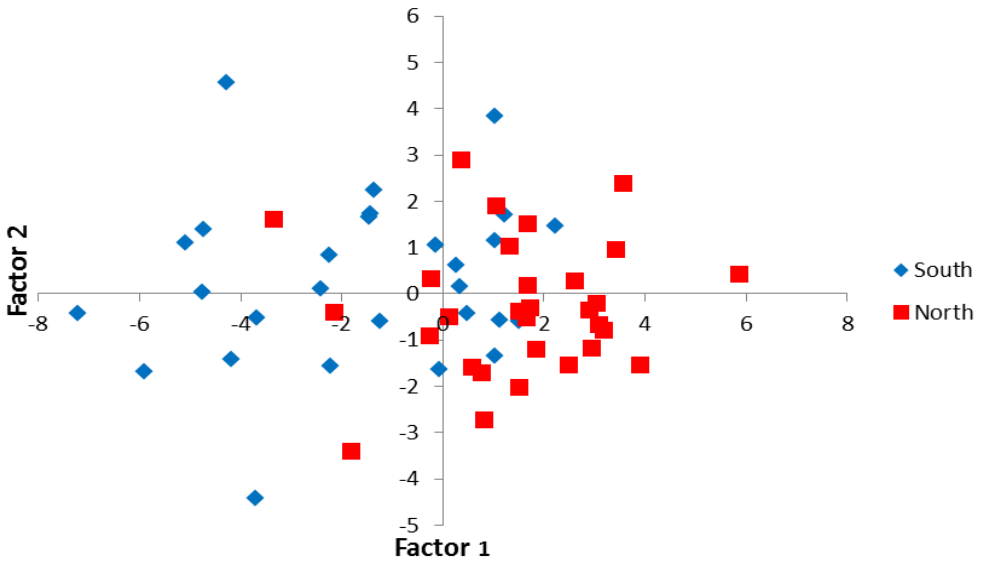

Figure 2.3: Sample scores from the principal components analysis of egg fatty acid concentration data. Samples from north and south PNP are indicated. Data from 2015 and 2016 are included.

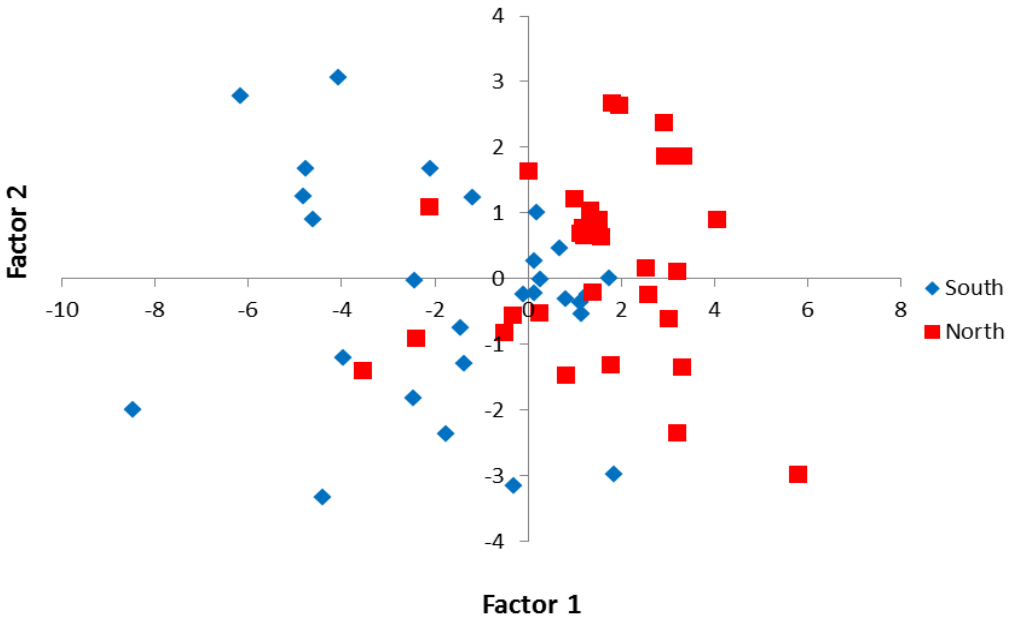

Figure 2.4: Sample scores from the principal components analysis of egg fatty acid percent composition data. Samples from north and south PNP are indicated. Data from 2015 and 2016 are included.

Statistical comparison of egg fatty acid concentration data and fatty acid percent composition data between north and south PNP revealed consistent differences (Tables 2.4 and 2.5). Most of the fatty acids that were important in separating eggs from north and south PNP were greater in eggs from south PNP. Only GLA was greater in north PNP (Tables 2.4 and 2.5). There were no differences in mean egg concentrations of the trans-fat, elaidic acid, between north and south PNP ($t(56) = -0.5$, $p = 0.64$) nor was there a regional difference in percent contribution of elaidic acid to overall fatty acid levels ($t(56) = -0.7$, $p = 0.49$). Eggs from south PNP had higher concentrations of the important polyunsaturated omega 3 fatty acids, i.e. EPA, DPA, and DHA (Table 2.4). These omega 3 fatty acids also contributed greater overall percentages of total fatty acid levels in eggs from south PNP compared to north PNP (Table 2.5). The ratio of Omega 3/Omega 6 fatty acids in southern Herring Gull eggs was greater than in northern eggs ($t(56) = 4.2$, $p < 0.001$) (Figure 2.5). When compared with Double-crested Cormorant eggs (mean ± std error O3/O6 = 0.79 ± 0.02), egg omega 3/omega 6 fatty acid ratios were lower in both northern (mean=0.34) and southern Herring Gull eggs (mean=0.50) (Welch's ANOVA, $F_{2,68} = 46.9$, $p < 0.001$, followed by Dunnett's post-hoc test, $p < 0.05$).

Table 2.4: Comparison of fatty acid concentrations (mg/g) in Herring Gull eggs from north and south PNP. Fatty acids included here were those identified through principal components analysis.

Variable	Mean South	Mean North	p	Std.Dev. South	Std.Dev. North
Myristic acid	1.38	1.11	0.005	0.39	0.32
Palmitoleic acid	7.62	5.92	0.001	1.92	1.8
γ-Linolenic acid (GLA) ‡	0.38	0.61	<0.001	0.18	0.26
Eicosenoic acid*	1.03	0.88	0.008	0.2	0.16
α-linolenic acid (ALA) †*	2.12	1.45	<0.001	0.8	0.59
Cis-11,14 eicosadienoic acid	0.59	0.33	<0.001	0.25	0.12
Cis-11,14,17 eicosatrienoic acid (ETE) †	0.36	0.14	<0.001	0.17	0.1
Arachidonic acid (ARA) ‡	19.02	20.35	0.02	1.78	2.32
Eicosapentaenoic acid (EPA) †*	1.79	0.74	<0.001	0.97	0.45
Docosapentaenoic acid (DPA) †	2.19	1.61	<0.001	0.78	0.38
Docosahexaenoic acid (DHA) †	13.48	10.96	<0.001	2.41	2.65
Pentadecanoic acid	0.44	0.36	0.001	0.1	0.08
Omega 3	19.94	14.88	<0.001	4.23	3.43
Omega 6	42.1	45.05	0.1	7.89	5.19

† Omega 3 fatty acid

‡Omega 6 fatty acid

t-test used for all comparisons except where indicated by * - Mann-Whitney U Test

Table 2.5: Comparison of fatty acid percent composition in Herring Gull eggs from north and south PNP. Fatty acids included here were those identified through principal components analysis.

Variable	Mean South	Mean North	p	Std.Dev. South	Std.Dev. North
Myristic acid	0.41	0.33	0.007	0.12	0.1
Palmitoleic acid	2.23	1.72	<0.001	0.56	0.53
γ-Linolenic acid (GLA) ‡	0.11	0.18	<0.001	0.05	0.08
Eicosenoic acid*	0.3	0.26	0.01	0.06	0.05
α-linolenic acid (ALA) †*	0.61	0.43	<0.001	0.23	0.19
Cis-11,14 eicosadienoic acid	0.17	0.1	<0.001	0.08	0.04
Cis-11,14,17 eicosatrienoic acid (ETE) †	0.11	0	<0.001	0.05	0.03
Eicosapentaenoic acid (EPA) †*	0.51	0.22	<0.001	0.3	0.14
Docosapentaenoic acid (DPA) †	0.63	0.47	0.002	0.24	0.12
Docosahexaenoic acid (DHA) †	3.91	3.18	<0.001	0.75	0.77
Pentadecanoic acid	0.13	0.1	0.002	0.03	0.02
Oleic acid	43.1	44.46	0.008	1.73	2.01
Omega 3	5.77	4.34	<0.001	1.34	1.06
Omega 6	12.46	13.11	0.2	2.03	1.53

† Omega 3 fatty acid

‡Omega 6 fatty acid

t-test used for all comparisons except where indicated by * - Mann-Whitney U Test

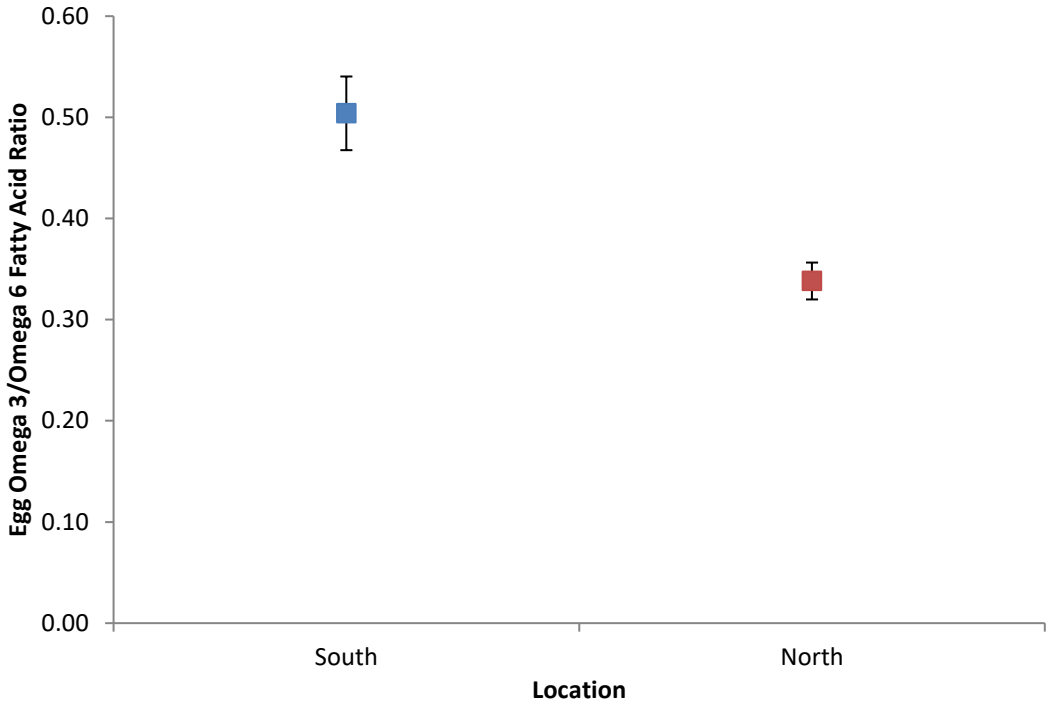

Figure 2.5: Mean (± standard error) omega 3/omega 6 fatty acid ratio in Herring Gull eggs from south and north PNP, 2015 and 2016.

Relationships between egg $\delta^{15}N$ values and key egg fatty acids, identified through principal components analysis, were also examined. Omega 3 fatty acids showed positive relationships with $\delta^{15}N$ (Figure 2.6 to 2.8). However, omega 6 fatty acids exhibited negative relationships with $\delta^{15}N$ (Figure 2.9 and 2.10). The ratio of omega 3/omega 6 fatty acids increased as $\delta^{15}N$ increased (Figure 2.11).

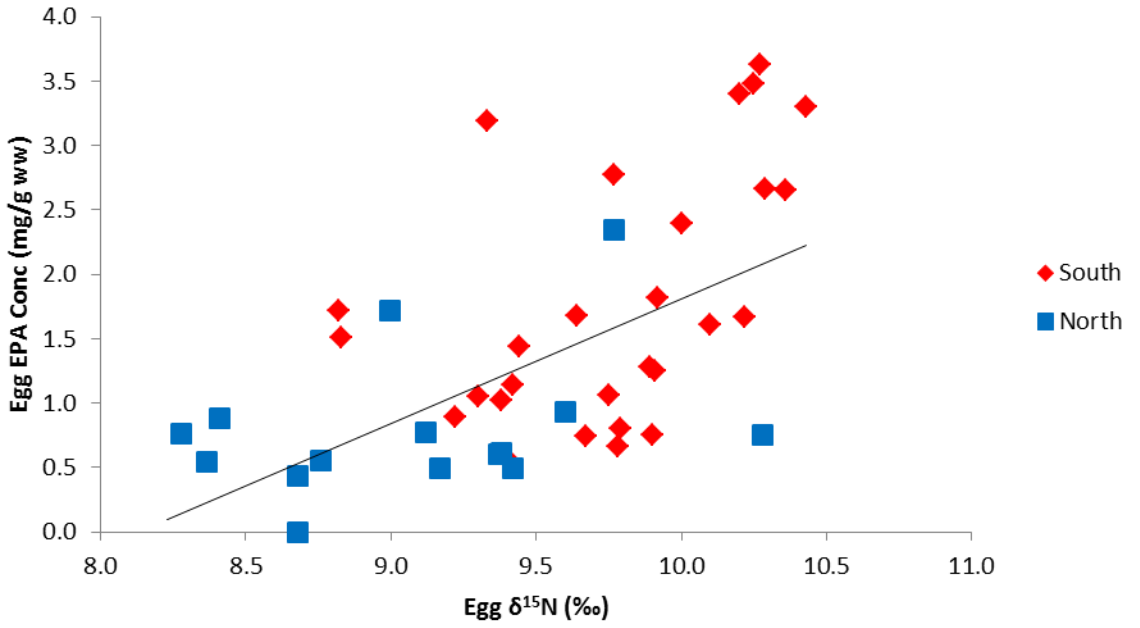

Figure 2.6: The relationship between the concentration of eicosapentaenoic acid (EPA) and $\delta^{15}N$ values in southern and northern Pukaskwa eggs, 2015 and 2016 ($r = 0.62$, $p < 0.001$).

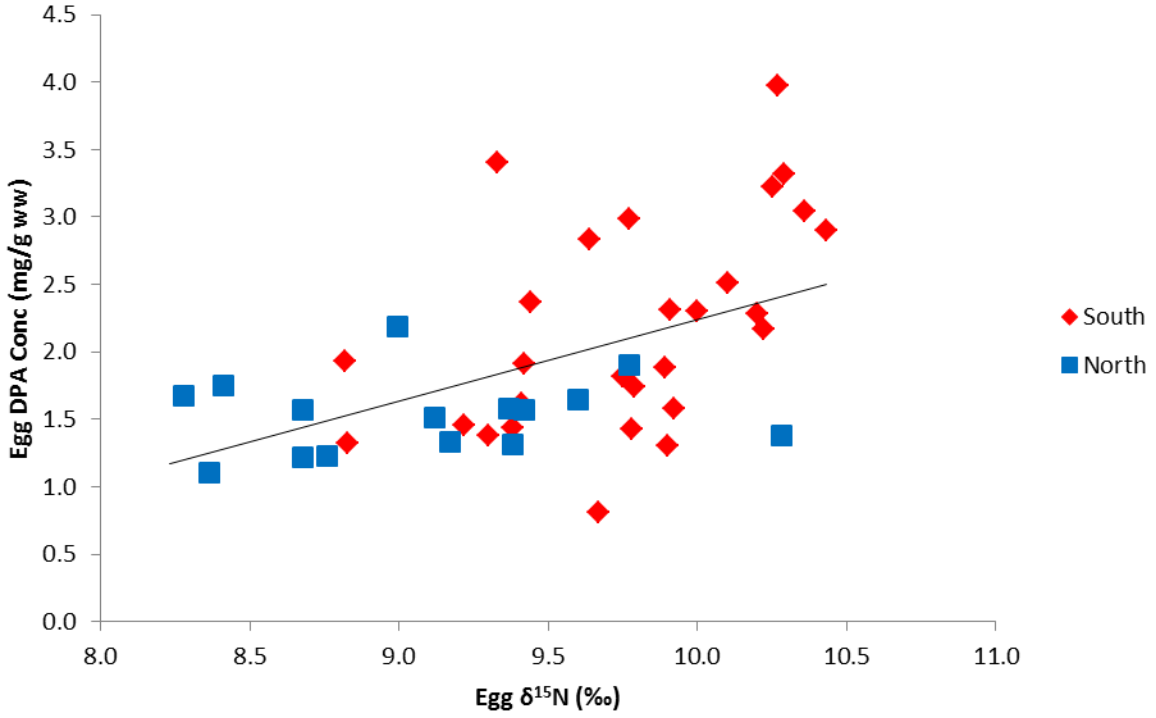

Figure 2.7: The relationship between the concentration of docosapentaenoic acid (DPA) and $\delta^{15}N$ values in southern and northern Pukaskwa eggs, 2015 and 2016 ($r = 0.52$, $p < 0.001$).

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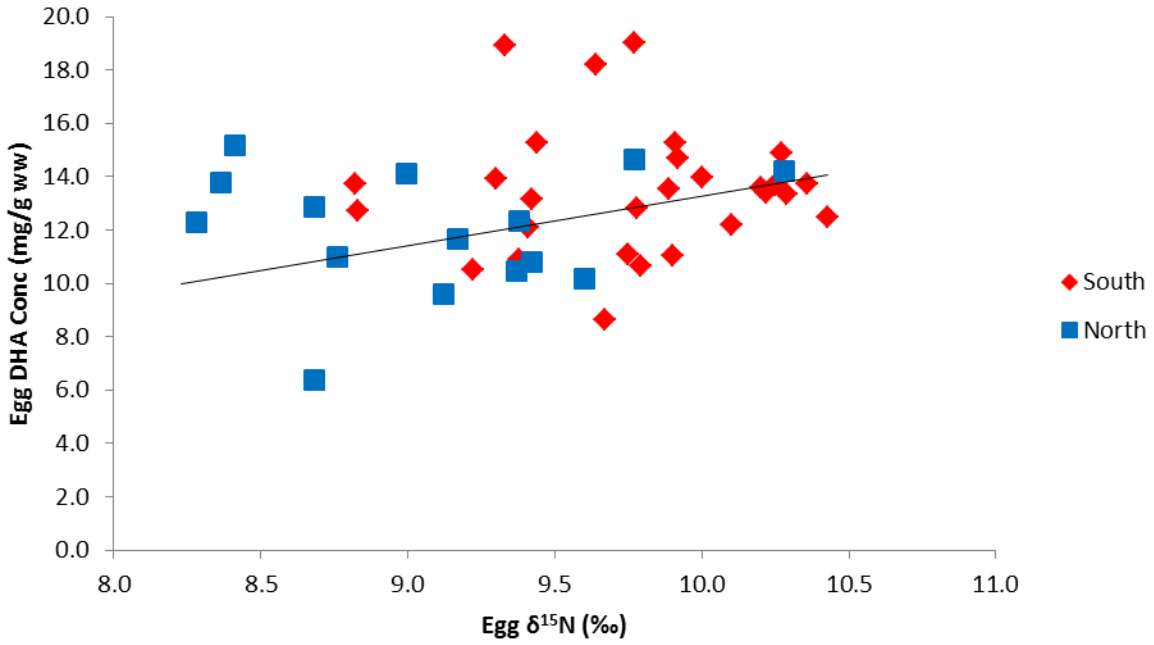

Figure 2.8: The relationship between the concentration of docosahexaenoic acid (DHA) and $\delta^{15}N$ values in southern and northern Pukaskwa eggs, 2015 and 2016 (r = 0.38, p = 0.003).

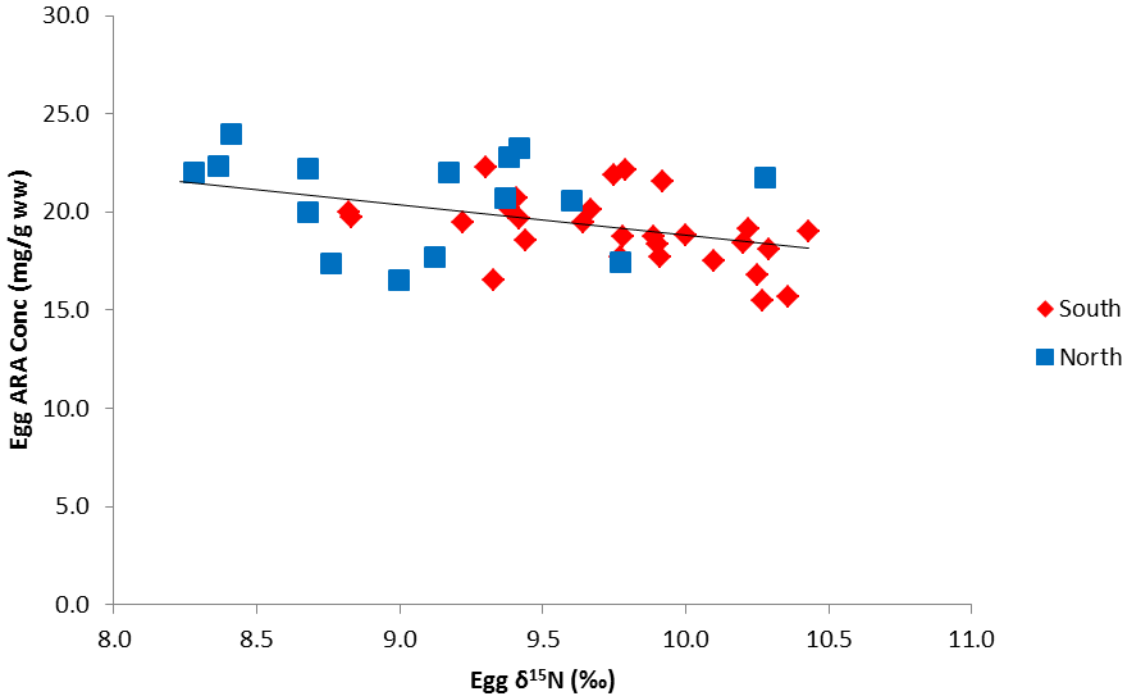

Figure 2.9: The relationship between the concentration of arachidonic acid (ARA) and $\delta^{15}N$ values in southern and northern Pukaskwa eggs, 2015 and 2016 (r = -0.42, p = 0.001).

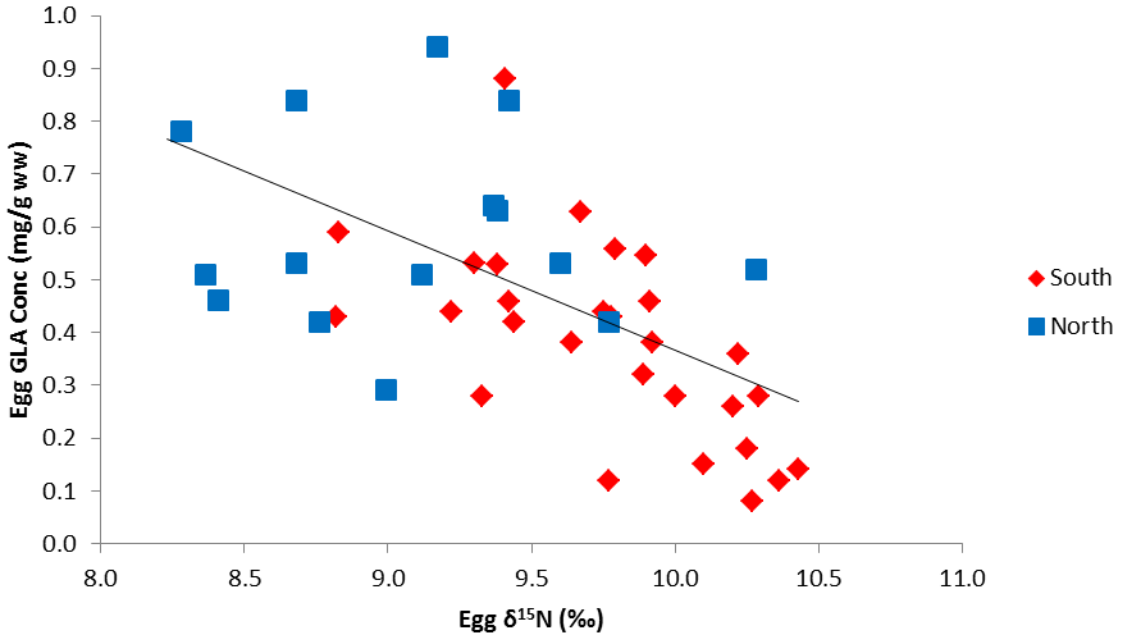

Figure 2.10: The relationship between the concentration of γ-linolenic acid (GLA) and $\delta^{15}N$ values in southern and northern Pukaskwa eggs, 2015 and 2016 (r = -0.52, $p < 0.001$).

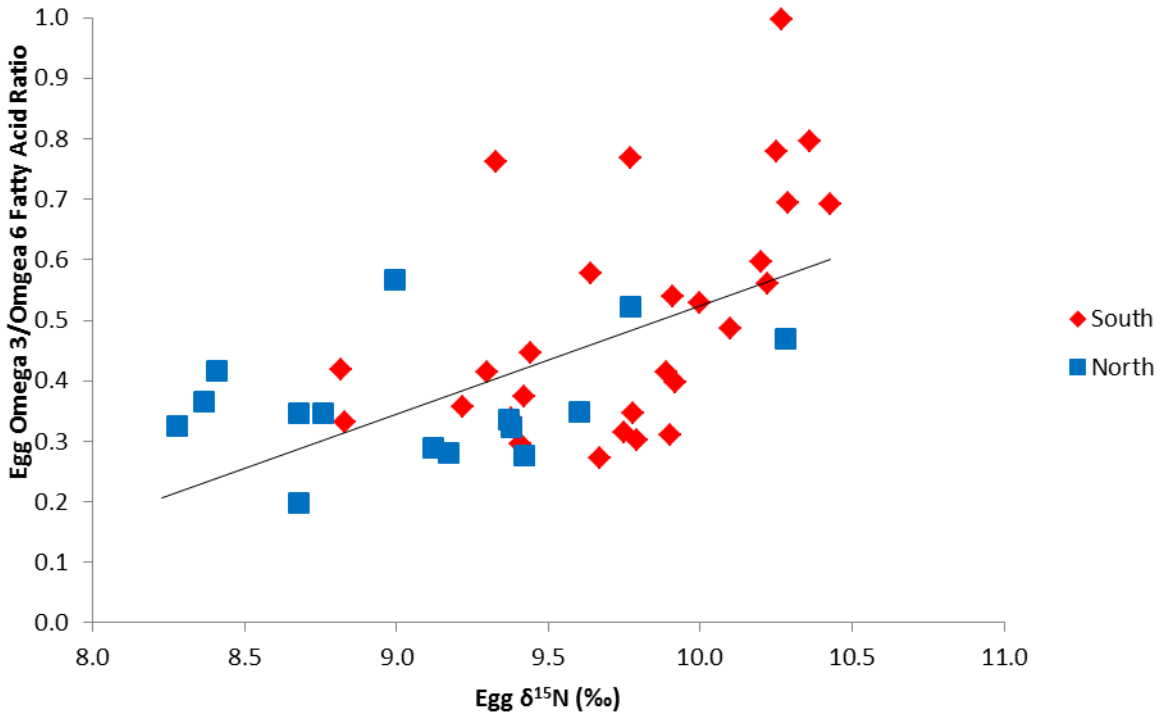

Figure 2.11: The relationship between the ratio of Omega 3/Omega 6 fatty acids and $\delta^{15}N$ values in southern and northern Pukaskwa eggs, 2015 and 2016 (r = 0.61, $p < 0.001$).

Discussion

Pellet Analysis

Pellet analysis provided a snap-shot of Herring Gull recent diet. Pellets from northern birds showed a much higher proportion of anthropogenic food incorporated into their diets compared to birds in southern PNP. Southern birds had a higher proportion of terrestrial foods, namely bird remains, in their diets compared to northern birds. Evidence from the literature suggests that gulls will pursue, exhaust, and then consume passerines as they migrate over water (Macdonald and Mason, 1973). This could explain the high incidence of bird feathers in pellets from south PNP. There was no statistically significant difference in the proportion of aquatic food in the diets of birds nesting in south and north PNP. A potential reason for the lack of difference between northern and southern pellets from PNP could be caused by the innate bias in pellet analysis. Pellet analysis is known to over-represent and under-represent species based on the size and durability of the prey species remains (Real, 1996; Resano-Mayer *et al,* 2013). For example, small fish are often under-represented as their remains are soft and easy to digest while animals such as mammals and birds are over-represented in pellets as their remains are undigestible (Mersmann *et al,* 1992). Since aquatic species are under-represented in pellets this may explain why there was no difference in the proportion of aquatic food found in northern and southern Herring Gull pellets. Southern gulls may be consuming more aquatic food than northern gulls but due to the nature of fish remains the difference in aquatic food consumption is not apparent in the pellets.

Stable Isotopes

Stable isotopes can potentially provide insights into gull trophic position ($\delta^{15}N$) and diet composition ($\delta^{13}C$). Here, $\delta^{13}C$ values in eggs were not useful in distinguishing between diets of northern and southern PNP Herring Gulls. On the surface this appeared to suggest that both populations are feeding on similar food sources. However, interpretation of $\delta^{13}C$ values may not be straight-forward as aquatic food sources can vary in their $\delta^{13}C$ values depending on where they come from in a waterbody. For example, lake $\delta^{13}C$ values have been shown to vary from -30.5‰ for profundal primary consumers to -23.8‰ in littoral primary consumers (Vander Zanden and Rasmussen, 1999). Therefore, even aquatic food resources can exhibit a wide range of $\delta^{13}C$ values. Terrestrial plants can also vary in their $\delta^{13}C$ values depending on what photosynthetic pathway they use. Plants that use the C4 pathway, such as corn, have less negative $\delta^{13}C$ values while plants that use the C3 pathway tend to have more negative $\delta^{13}C$. This is due to the fact that C3 plants and C4 plants fractionate carbon isotopes in different ways (Van der Merwe, 1982). When comparing egg $\delta^{13}C$ values in Herring Gulls to those in Double-Crested Cormorants it was evident that both northern and southern Herring Gulls from PNP had less negative $\delta^{13}C$ values which is consistent with their relying to a greater degree on terrestrial food. However, as previously mentioned, variability in source $\delta^{13}C$ values may have obfuscated dietary differences between Herring Gulls in north and south PNP.

Egg stable nitrogen isotope data offered more useful insights into dietary differences between north and south PNP gulls. Egg $\delta^{15}N$ values were statistically different between northern and southern Herring Gulls in PNP. Southern birds had a higher mean $\delta^{15}N$ value by roughly 0.7‰. Although this corresponds with only a fractional difference in trophic position (an ~3.4‰ increase would be expected from one trophic level to the next), it does indicate that

southern birds were feeding at a higher trophic level than northern birds. This result is consistent with the idea that southern birds were feeding to a greater extent on fish as fish occupy a higher trophic position than other foods that gulls consume (Hebert *et al,* 1999). When $\delta^{15}N$ values in eggs of Herring Gulls were compared to those in cormorant eggs it was evident that the northern and southern Pukaskwa Herring Gulls were feeding at a lower trophic level than cormorants. This was not unexpected given the gulls' greater reliance on terrestrial food. However, the difference seen in mean $\delta^{15}N$ values between cormorants and gulls was greater in the north (3.1‰) than in the south (2.4‰). Based upon pellet analysis, northern PNP gulls fed to a greater extent on anthropogenic foods and this is consistent with the egg $\delta^{15}N$ data. Human-related food items would be expected to have lower $\delta^{15}N$ values (Hebert *et al,* 1999).

Fatty Acids

Fatty acid composition of consumer tissues reflects their diet (Napolitano 1999, Iverson *et al*, 2004). When birds feed on different food sources this shows up in their fatty acid signatures as levels of some fatty acids increase while others decrease. Comparing eggs from northern and southern PNP gulls highlighted a few key differences between the two regions. Eggs of southern birds had higher levels of omega 3 fatty acids, such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). Omega 3 fatty acids come from aquatic food sources such as fish, aquatic plants and diatoms (Meyers *et al,* 2003; Tur *et al,* 2012). Since the omega 3 fatty acids were higher in concentration and percent composition in southern Herring Gulls than northern Herring Gulls it indicated that southern birds were feeding on more aquatic food sources overall. This was supported by the higher levels of myristic acid in southern Herring Gull eggs. Myristic acid comes primarily from fish oils and

fish that feed on phytoplankton (Arts *et al*, 1999). The fatty acids that are higher in northern birds tend to come from terrestrial food sources and are mostly omega 6 fatty acids (Meyer *et al,* 2003). Omega 6 fatty acids such as γ-linolenic acid (GLA) are found primarily in the terrestrial food web (Meyer *et al,* 2003). γ-Linolenic acid can be found in terrestrial plants including agricultural plants such as flax (Arts *et al,* 1999). Furthermore, eggs of southern birds had higher omega 3/omega 6 fatty acid ratios than northern eggs. This indicated that the diet of the northern birds was higher in terrestrial foods while the diets of southern birds were higher in aquatic food. Herring Gulls from southern PNP had mean omega 3/omega 6 fatty acid ratios of 0.5. This ratio falls within the range of 0.5-3.8 which is the typical ratio seen in freshwater fish (Arts *et al,* 1999). We can see by comparing the Herring Gull omega 3/omega 6 fatty acid ratio to the ratio observed in cormorants that even birds in south PNP were feeding on more terrestrial food than the obligate aquatic feeding cormorant. This indicated that although southern Herring Gulls incorporated more aquatic food into their diets than northern Herring Gulls, they were still being forced to rely on foods of terrestrial origin, primarily birds, as indicated by pellets. This reliance on terrestrial food in both northern and southern PNP Herring Gulls suggested low availability of aquatic food, namely prey fish. When gull egg omega 3/omega 6 fatty acid ratios were compared with egg $\delta^{15}N$ values an interesting relationship appeared. Gulls that had higher omega 3/omega 6 fatty acid ratios had higher $\delta^{15}N$ values. This indicated that birds eating more aquatic food also were elevated in their trophic position. Furthermore, eggs of southern birds tended to have higher omega 3/omega 6 fatty acid ratios and higher $\delta^{15}N$ values. Hence, it was likely that southern birds had more fish in their diets. Possible reasons for these differences in diet were the proximity of the northern Herring Gull colony to anthropogenic food sources and low availability of prey fish. These results appear to contradict the $\delta^{13}C$ results discussed above.

However, interpretation of $\delta^{13}C$ values can be complicated by the degree to which a variety of factors may influence them. For example, even within aquatic systems $\delta^{13}C$ values can vary according to where samples were collected in the waterbody, e.g. littoral, benthic, pelagic (Post, 2002). Hence, $\delta^{13}C$ values are likely a much poorer, i.e. less specific, indicator of the degree to which aquatic versus terrestrial foods were utilized by gulls.

Levels of trans-fats, i.e. elaidic acid, in northern and southern birds were not different. However, γ-linolenic acid levels were greater in northern PNP gull eggs and levels of arachidonic acid (ARA) were somewhat elevated in the northern eggs. A significant source for both of these fatty acids may be anthropogenic food. GLA is found in a variety of anthropogenic foods such as oats, barley, meat and eggs (Horrobin, 1992). Arachidonic acid is a fatty acid that can come from aquatic food sources; however, these food sources tend to be marine in nature such as Atlantic Salmon (*Salmo salar*) (Bigogno *et al,* 2002) and as such are not available to Herring Gulls in PNP. However, arachidonic acid is also abundant in anthropogenic food sources such as chicken, eggs and beef (Bosire *et al*, 2009). These results support the idea that northern Herring Gulls may have been using local anthropogenic food sources, such as garbage dumps, as a way to supplement their diets.

Anthropogenic food sources can act to subsidize the natural diets of birds helping to maintain their populations (Duhem *et al,* 2008). Such foods may provide birds with energy but past studies provide equivocal evidence regarding the pros and cons of anthropogenic food subsidies in maintaining gull populations. Some studies have shown that at Herring Gull colonies where birds rely on anthropogenic food sources they tend to be bigger and colony sizes are larger (Belant *et al,* 1998; Auman *et al,* 2007). However, anthropogenic foods may be of lower quality than natural foods such as fish. When higher quality foods such as fish are available, Herring

Gulls tend to prefer them over anthropogenic food (Belant *et al,* 1993). Negative impacts associated with the consumption of anthropogenic foods have been reported to include lower reproductive success and lower chick survival (Pierotti and Annett, 1991; Smith and Carlile 1993). Eggs from northern PNP gulls had lower levels of omega 3 polyunsaturated fatty acids (n-3 PUFAs), i.e. EPA, DPA and DHA, that are important in determining offspring quality in birds (Twining *et al,* 2016b). These PUFAs play an important role in neural development and in the proper functioning of the immune system (Twining *et al,* 2016b). However, it is hard to say whether the lower levels of n-3 PUFAs in northern Herring Gulls are low enough to harm chick development. Anthropogenic food subsidies may also be negatively impacting the northern Herring Gulls as anthropogenic food subsidies can be sources of harmful diseases, such as salmonellae (Durrant and Beatson, 1981; Monaghan *et al,* 1985, Ortiz and Smith, 1994). Herring Gull populations in the north appear to be declining at a slower rate than in the south. Hence, use of anthropogenic food resources may represent the lesser of two evils. In south PNP, birds can only rely on natural food resources and are being constrained by food availability. In the north, food quality may be somewhat compromised but "junk food" is likely better than no food. In both regions of the park it is evident that the availability of natural prey fish may be an important factor constraining gull diets. The impact this may be having on gull reproduction, behaviour, and physiology is examined in the next chapter.

Chapter 3 Reproductive and Physiological Endpoints in Pukaskwa National Park Herring Gulls and Relation to Food Resources

Introduction

Herring Gull populations in Pukaskwa National Park (PNP) have declined significantly through time (Figure 1.0). In the 1970s, the population peaked at just under 1000 breeding pairs but in 2015 the population had declined to about 300 pairs. When these data were divided by region, i.e. north and south PNP, the extent of the decline was greater in southern (82% decline) than in northern (41% decline) PNP (Figure 1.2). Previous studies have shown that changes in food abundance and shifts in food sources can lead to lower population levels and emigration of birds from breeding colonies (Chapdelaine and Rail, 1997). In Chapter 2, I present evidence of dietary differences between north and south PNP gulls. Here, I investigate reproductive and physiological endpoints in PNP gulls. I compare these endpoints between gulls nesting in south and north PNP and discuss how they may be influenced by diet. The reproductive endpoints I examine include: clutch size, egg mass and volume, egg energy density, nest attentiveness, and spatial distribution of nesting gulls. I also examine physiological stress in gulls by measuring corticosterone in their eggs. Since Herring Gulls rely on local food sources during egg formation and incubation these reproductive and physiological endpoints will be influenced by the availability of local food resources (Hobson *et al,* 1997).

Reproductive Endpoints

Clutch size reflects food availability to laying female gulls (Ankney and MacInnes, 1978). A typical Herring Gull nest contains three eggs. However, when food availability is low the female may not have the resources to produce a three egg clutch. This can result in lower clutch sizes of either one or two eggs. As clutch size decreases the number of potential offspring also declines which can result in decreased recruitment of chicks into the population.

Furthermore, low clutch sizes increase the impact of egg loss through factors such as predation. In one or two egg clutches such losses have more significant consequences in terms of detrimentally affecting reproductive success.

Egg size, i.e. egg mass, egg volume, can also provide insights into food availability for breeding gulls. A shift in food availability, either an increase or decrease, or a shift from one food source to a less ideal food source can cause a change in egg size and/or egg mass. Gulls with access to more food create larger eggs (Moss and Watson, 1984; Hiom *et al*, 1991). Larger egg size has been associated with increased chick survival (Hiom *et al*, 1991; Williams, 1994).

Egg energy density is a measure of the energy content of an egg and can be expressed on a per egg or per gram of egg mass basis. By measuring the energy density of an egg it is possible to get an idea of how much energy the maternal bird was able to invest in her eggs. This is a valuable endpoint as eggs with lower energy density are considered to be of lesser quality (Moss and Watson, 1984).

Nest attentiveness reflects the amount of time that gulls spend on their nests. Both male and female Herring Gulls incubate their eggs. They take turns doing this with one bird almost always being present on the nest. This ensures that the eggs are kept at an optimal temperature for development and adult presence deters potential predators such as conspecifics, other bird species, e.g. corvids, or mammals. As food availability decreases, gulls may be forced to spend more time foraging with resultant increases in the time they spend off their nests. Increased time off the nest may make eggs and chicks more vulnerable to predation.

The spatial distribution of nesting Herring Gulls may also provide information regarding food availability. Although philopatric to nesting sites, gulls will move to areas that offer more

abundant food resources. Hence, changes in the spatial distribution of nesting gulls can offer insights into such changes in food availability.

Physiological Endpoint

Corticosterone is a hormone produced by birds in response to environmental stress. Corticosterone levels in birds are a non-specific indicator of stress and can be affected by various stressors including: human disturbance, threat of predation, extreme temperatures or food shortages (Marra and Holberton, 1998). Here, I examine the possibility of measuring corticosterone in egg contents and eggshell. Corticosterone in egg components should reflect the female's exposure to all stressors during the period of egg formation. All of the reproductive and physiological endpoints described here can be influenced by food availability (Hayward *et al,* 2005).

Historical Data

The Great Lakes Herring Gull Monitoring Program (GLHGMP) is a program created by Environment and Climate Change Canada in the 1970s. The program annually collects Herring Gull eggs from each of the Laurentian Great Lakes in order to monitor a variety of chemical contaminants, biochemical indicators of diet, and egg size/energy content. Agawa Rocks is one of the sites monitored as part of this program and is located near PNP (approximately 150 km south-east) (Figure 1.3). Data regarding diet and egg size/energy content are available from this location dating back to 1978. I use these data to provide a temporal context to aid in the interpretation of my 2015 and 2016 results from PNP.

Gaining an understanding of how aquatic food availability has changed through time is important for interpreting historical changes in Herring Gull diets. Prey fish, such as Rainbow Smelt (*Osmerus mordax*), are an important food source for Herring Gulls. Using prey fish data from the United States Geological Survey (USGS) I examine how prey fish abundance has changed through time and how it differs between south and north PNP.

Methods

Reproductive Endpoints

In collaboration with Parks Canada Agency staff I conducted egg collections in PNP by boat in May, 2015 and 2016. For each nest sampled, clutch size was recorded and all eggs were measured (maximum length and maximum breadth) using digital calipers that measured to 0.01 mm. These measurements were used to calculate egg volume using the equation:

$$\text{volume } (cm^3) = 0.489*(\text{length}*\text{breadth}^2)/1000 \text{ (Ryder, 1975).}$$

In 2015, one egg was chosen randomly from each of a total of 28 nests (13 south PNP, 15 north PNP). In 2016, 30 eggs were collected randomly from a total of 30 nests in PNP (15 from both south and north PNP). Nests that had three eggs were preferred for egg collection over two egg nests while two egg nests were preferred over one egg nests. When available, eggs were collected from multiple egg nests to minimize impacts on reproduction. Once collected, eggs were labelled, and placed in a padded Pelican case for transport to the Tissue Preparation Laboratory at the National Wildlife Research Centre. Eggs were processed according to methods described in Chapter 2. Data from 2015 and 2016 were combined based upon the lack of inter-

year statistical differences in reproductive endpoints within northern and southern regions of the park.

Energy density data was calculated by obtaining the percent lipid content and percent moisture content from the fatty acid analysis described in Chapter 2. The two percentages were combined and the remaining percentage in the egg was deemed to be percent protein content. The percentage of each of the three categories was then multiplied by the egg weight in order to get lipid weight, water weight and protein weight. The lipid mass was then multiplied by 9.50 kcal/g and the protein mass was multiplied by 5.65 kcal/g to obtain energy content data (Paterson *et al,* 2014). A conversion factor of 4187 J/kcal was used to express data as kJ/g and the mass of the egg was used to obtain kJ/egg estimates.

Nest attentiveness was evaluated using remote infrared cameras (Reconyx PC900, WI, USA). A total of six nests were monitored (four in south PNP, two in north PNP). Cameras were set up so that the nest was in their field of view and they were programmed to take one photograph per minute (24 hours/day = 1440 photos/day). Infrared cameras allowed photos to be taken day and night without the use of a flash, hence, they did not disturb nesting birds. Nest attentiveness analysis was completed by looking at each photo to determine if one of the parents was attending the nest. Nests without an attending parent were considered vacant while nests with an incubating bird or a bird within 0.5m of the nest were considered occupied. A nest was considered disturbed if predated or if nest material was stolen from the nest while the incubating parents were gone. Images used in this analysis included those up to the time of hatch.

The Pukaskwa National Park Herring Gull nest survey was completed by Parks Canada Agency staff intermittently from 1977 to 2015. All islands with nesting gulls were surveyed by boat with biologists landing on islands and counting nests. A rock near each nest was marked

with spray paint to avoid counting the same nest twice. I categorized nest locations from each survey according to latitude. I used these results to visually examine changes in the spatial distribution of nesting Herring Gulls across all of PNP.

Physiological Endpoint

Corticosterone analysis was completed on the contents (yolk and albumen) of eggs collected in 2015 and 2016 while another analysis method utilizing eggshell was conducted on the 2016 samples only. For the egg contents analysis 1g of egg contents was retrieved from -80°C and left to thaw overnight in a refrigerator (4°C). Once thawed, a displacement pipette was used to place 0.025ml of egg contents into a 2ml centrifuge tube. The weight of the egg contents was recorded using a Mettler Toledo MX5 microbalance. Then 200µl of de-ionized water was added to each tube. The tubes were sealed and vortexed briefly. After being vortexed, the tubes were placed in a sonicator at room temperature and left for 15 minutes. They were then shaken for 60 minutes. After being shaken, 1.3ml of 100% methanol was added to each tube and the tubes were left overnight in a refrigerator. Next, the tubes were shaken for 90 minutes at 50°C before being centrifuged at 13000 rpm for 10 minutes at 4°C using a Beckman J2-21N/E centrifuge. The supernatant was transferred to a new 2ml centrifuge tube and evaporated to dryness on the Vacufuge at 30°C. Once dry, the samples were reconstituted with 300µl of assay buffer. The assay buffer consisted of 10ml buffer with 90 ml deionised water. Samples were vortexed briefly before being placed in a VWR Symphony sonicator at 30°C for 15 minutes. The samples were centrifuged again using an Eppendorf Centrifuge 5417 R at 13000 rpm for 5 minutes. The samples were then ready for corticosterone analysis using an enzyme-linked immunosorbent assay (ELISA).The first step in this analysis involved the creation of standards to

run with the samples. Five corticosterone standards were used with concentrations of 20ng/ml, 4ng/ml, 0.8 ng/ml, 0.16ng/ml and 0.032 ng/ml. Control samples with known corticosterone concentrations were also run with each ELISA analysis. The control standard consisted of Alpco, a rat corticosterone solution. 10µl of the Alpco standard was mixed with 490µl of the assay buffer. The ELISA was initiated by bringing all samples and reagents to room temperature. Assay buffer, standards, Alpco control samples, and egg samples were run together in the ELISA on 96-well plates. Using a repeater pipette, 50µl of blue conjugate were added followed by 50µl of yellow antibody. The plate was then left to incubate at room temperature on a Titer Plate Shaker Banstead set to low for two hours. The plate was covered to protect the samples from light. After two hours, the plate was washed three times using Thermo Scientific Wellwash and wash buffer. The wash buffer consisted of 5ml of concentrate and 95ml of deionised water. After washing, the plate was tapped dry. Two hundred µl of para-nitrophenylphosphate substrate (pNPP) was added to every well. pNPP substrate was used to measure the activity of various phosphatases. The plate was then protected from light and left to incubate for one hour at room temperature. After 1 hour, 50µl of stop solution was added to every well using a repeater pipette. The plate was then read using a Beckman Coulter DTX 880 Multimode Detector at 405nm.

The data were imported into Excel where absorption values obtained from the blank were subtracted from all other wells. These new values were imported into Graph Pad Prism 6 where results were transformed using the Log(X) function. These transformed results were used to create a standard curve using the results for the standards and their known concentrations. The remaining concentration values were then interpolated by Graph Pad using the standard curve. These values were then back-transformed using a 10^x transformation. These values were

imported into Excel where the amount of corticosterone in egg contents (in ng/g) was calculated using the transformed interpolated values.

Measuring corticosterone in eggshell required a different protocol for sample processing but the ELISA method was identical to that described above. To prepare the shell sample, approximately 1-1.2g of shell was ground so that it fit within two 1.8ml microcentrifuge tubes. These tubes then had a metallic ball added in order to ball mill the samples into a uniform size. Ball-milling was done using a Retsch Mixer Mill MM400 for 4 minutes at 20 cycles/second. Samples were then combined into one tube. Approximately 1g of shell material was weighed and placed into a 10ml plastic centrifuge tube. Fifty ml of 100% methanol were added and the tubes were capped. The tubes were left in the sonicater for 30 minutes and then left overnight to shake in a Temp Master at 50°C at 90 rpm. The following day the tubes were removed and placed on a VWR W-150 Waver at speed 10 for three hours. The tubes were then centrifuged in a Beckman centrifuge at 5000 rpm for 10 minutes. The supernatant was transferred to a glass culture tube. One ml of 100% methanol was added to the original pellet and vortexed briefly. The sample was then centrifuged again at 5000 rpm for 5 minutes. The supernatant was transferred to a glass culture tube. The culture tube was then dried on the Vacufuge at 30°C. Once dried, the samples were reconstituted with 300µl of assay buffer. They were vortexed and sonicated for 30 minutes. They were then left overnight at room temperature. The samples were filtered through a 0.45µm PFTE 13mm filter. The ELISA test was completed as described above. Eggshell corticosterone concentrations were reported in pg/g.

Historical Data

Data regarding egg mass, egg volume, and egg energy density were obtained from the GLHGMP (Agawa Rocks). These data were generated using the same methods as for PNP. No clutch size data were available from Agawa Rocks. Based upon the results of Chapter 2, I also compiled historical data regarding dietary tracers in eggs. These included egg $\delta^{15}N$, $\delta^{13}C$, and omega 3/omega 6 fatty acid ratios. Protocols for the measurement of these endpoints were the same as outlined in Chapter 2. However, at Agawa Rocks, one pooled sample was analyzed per year. Each pool consisted of 10-13 eggs.

Prey fish population data were obtained from the United States Geological Survey. These small fish are an important food item for Herring Gulls. Prey fish biomass (kg/ha) was used to assess temporal trends in prey fish abundance. Prey fish population data were obtained annually starting in 1989 for Agawa Rocks, 1991 for southern PNP and 1992 for northern PNP. Prey fish communities were sampled using a Yankee bottom trawl (11.9m headrope and 6.4mm mesh cod end). The trawl started inshore at 10-15 m depth and was towed offshore until a depth of 80-100 m was reached. The trawling was conducted in daylight hours. A subsample of 50 fish was selected for each species and their length and weight were recorded. Alewife (*Alosa pseudoharengus)*, Rainbow Smelt, Ninepsine Stickleback (*Pungitius pungitius*), Trout-perch (*Percopsis omiscomaycus*), Cisco (*Coregonus artedi*), Lake Whitefish (*Coregonus clupeaformis)*, Bloater (*Coregonus hoyi*), Shortjaw Cisco (*Coregonus zenithicus*), Pygmy Whitefish (*Prosopium coulterii)*, Round Whitefish (*Prosopium cylindraceum*), Longnose Sucker (*Catostomus castostomus*), White Sucker (*Castostomuc commersonii*), Slimy Sculpin (*Cottus cognatus)*, Spoonhead Sculpin (*Cottus ricei)* and Deepwater Sculpin (*Myoxocephalus thompsonii)* were counted as prey fish species. Prey fish abundance data were taken from

trawling locations situated within the typical feeding range of gulls nesting at colonies in northern PNP, southern PNP, and Agawa Rocks. Mean annual prey fish abundance was calculated by combining data from all trawling locations that fell within typical feeding ranges around those sites.

All statistical tests were performed using Statistica 13 Academic package. Results for southern and northern PNP were compared using either Chi-square tests, t-tests or Mann-Whitney U tests. Comparisons among PNP north, PNP south, and historical data from Agawa Rocks were done using ANOVA (or Welch's ANOVA when group variances were not homogeneous) followed by Dunnett's post-hoc test. For these comparisons, Agawa pre-1989 data were used as a reference against which other groups were compared. For all tests, $\alpha = 0.05$ was used. Graphs were produced using Microsoft Excel and Statistica 13.

Results

Reproductive Endpoints

Data from 2015 and 2016 were combined based upon the lack of inter-year statistical differences in reproductive endpoints within northern and southern regions of the park (see Appendix, Tables A3, A6). There was a significant difference in clutch size between gulls nesting in northern and southern PNP. Northern PNP birds had larger clutches than southern PNP (Pearson Chi-square, $df = 4$, $p < 0.001$) (Figure 3.0).

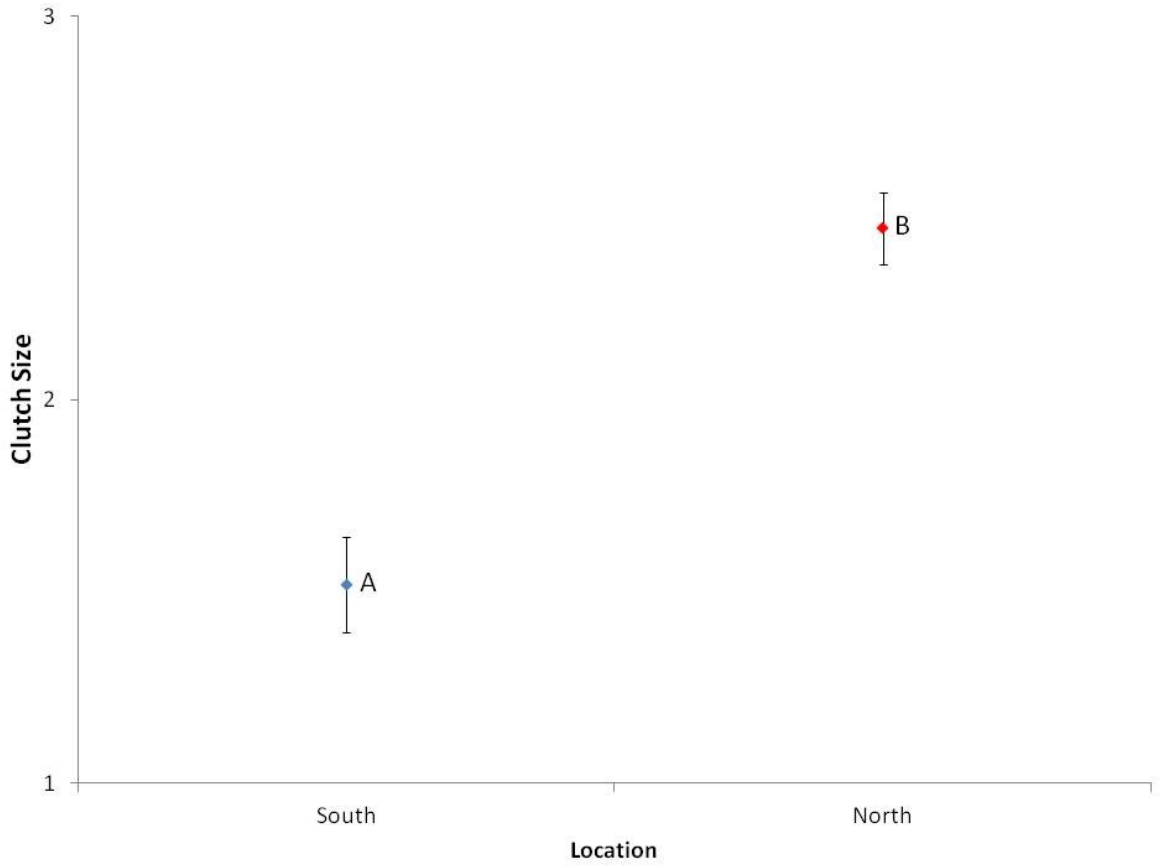

Figure 3.0: Mean (± standard error) clutch size in south and north Pukaskwa National Park (2015 and 2016). Letters beside means indicate statistically significant differences. Means that share letters are not different.

Egg mass ($t(56) = 1.1$, $p = 0.29$), egg volume (Mann-Whitney U test $Z = 1.0$, $df = 56$, $p = 0.33$), and egg energy density (kJ/egg, Mann-Whitney U test $Z = 0.9$, $df = 56$, $p = 0.39$; kJ/g, $t(56) = -1.2$, $p = 0.22$) were not statistically different between gulls nesting in northern and southern PNP (Figures 3.1-3.4).

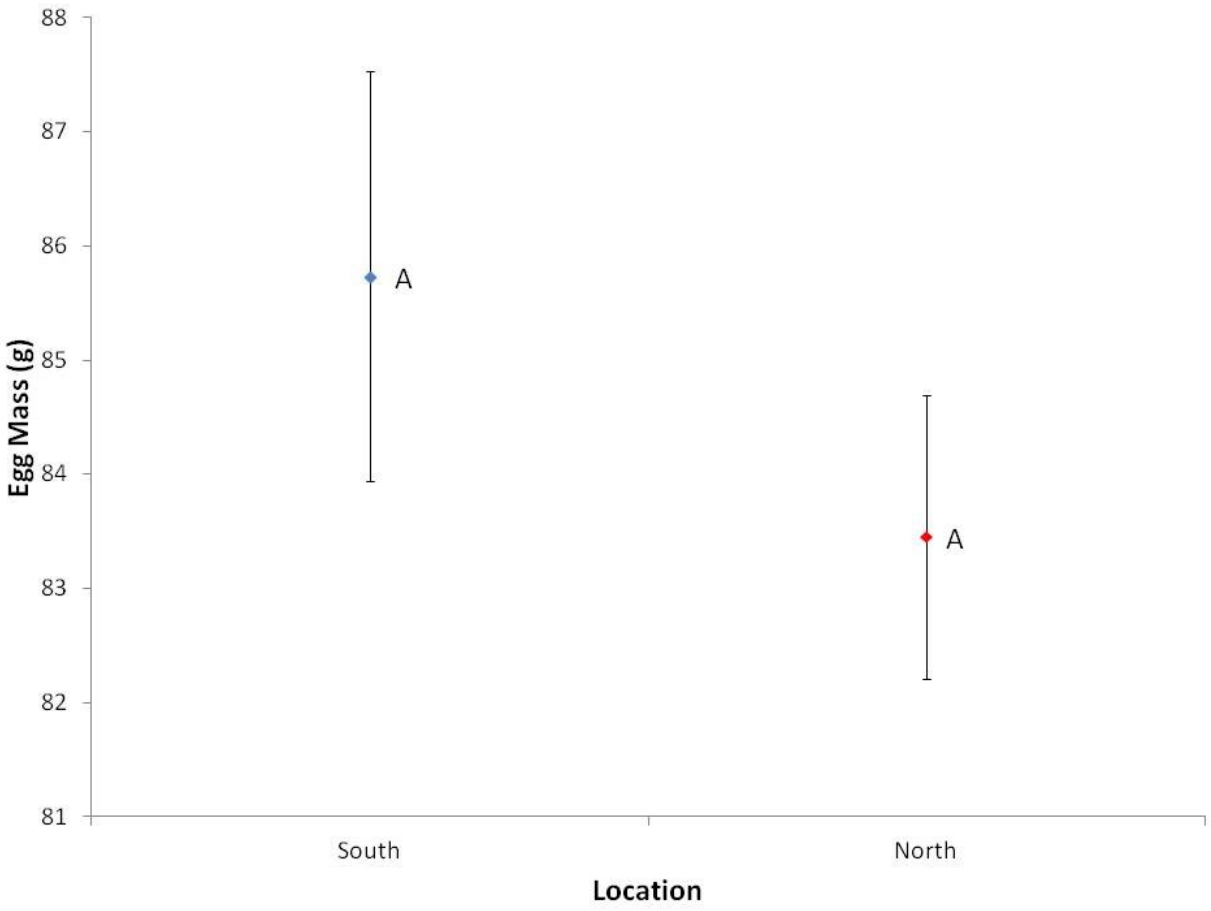

Figure 3.1: Mean (± standard error) egg mass in south and north Pukaskwa National Park (2015 and 2016). Letters beside means indicate statistically significant differences. Means that share letters are not different.

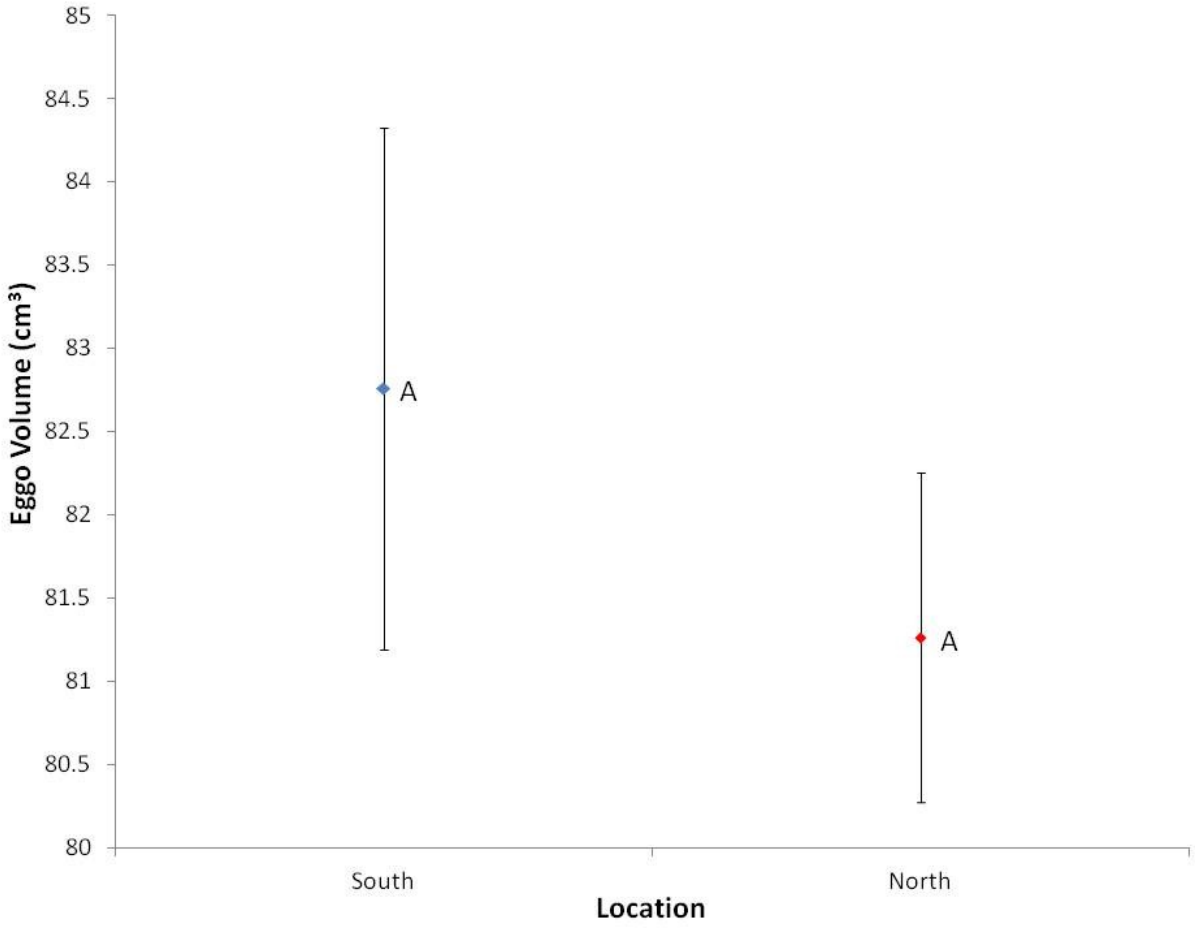

Figure 3.2: Mean (± standard error) egg volume in south and north Pukaskwa National Park (2015 and 2016). Letters beside means indicate statistically significant differences. Means that share letters are not different.

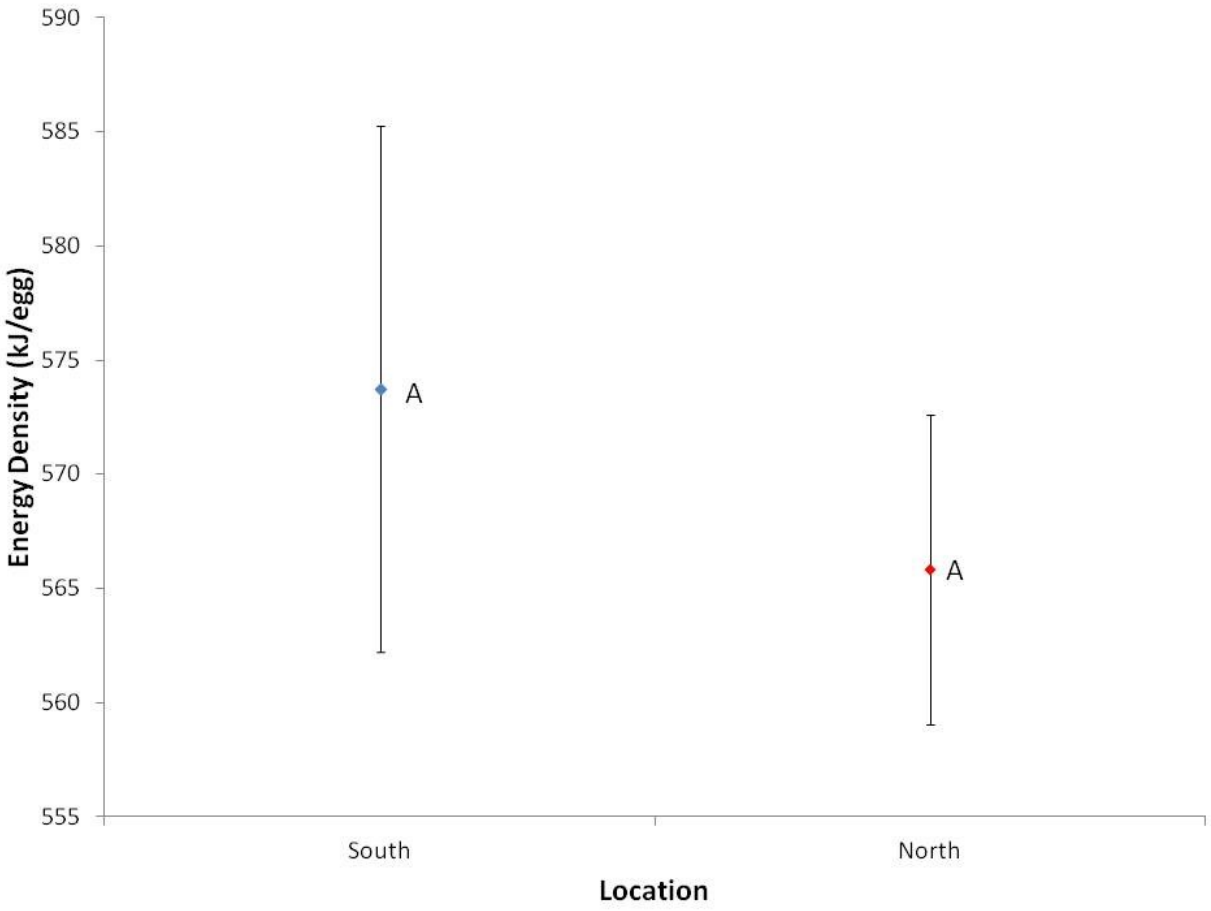

Figure 3.3: Mean (± standard error) egg energy density (kJ/egg) in south and north Pukaskwa National Park (2015 and 2016). Letters beside means indicate statistically significant differences. Means that share letters are not different.

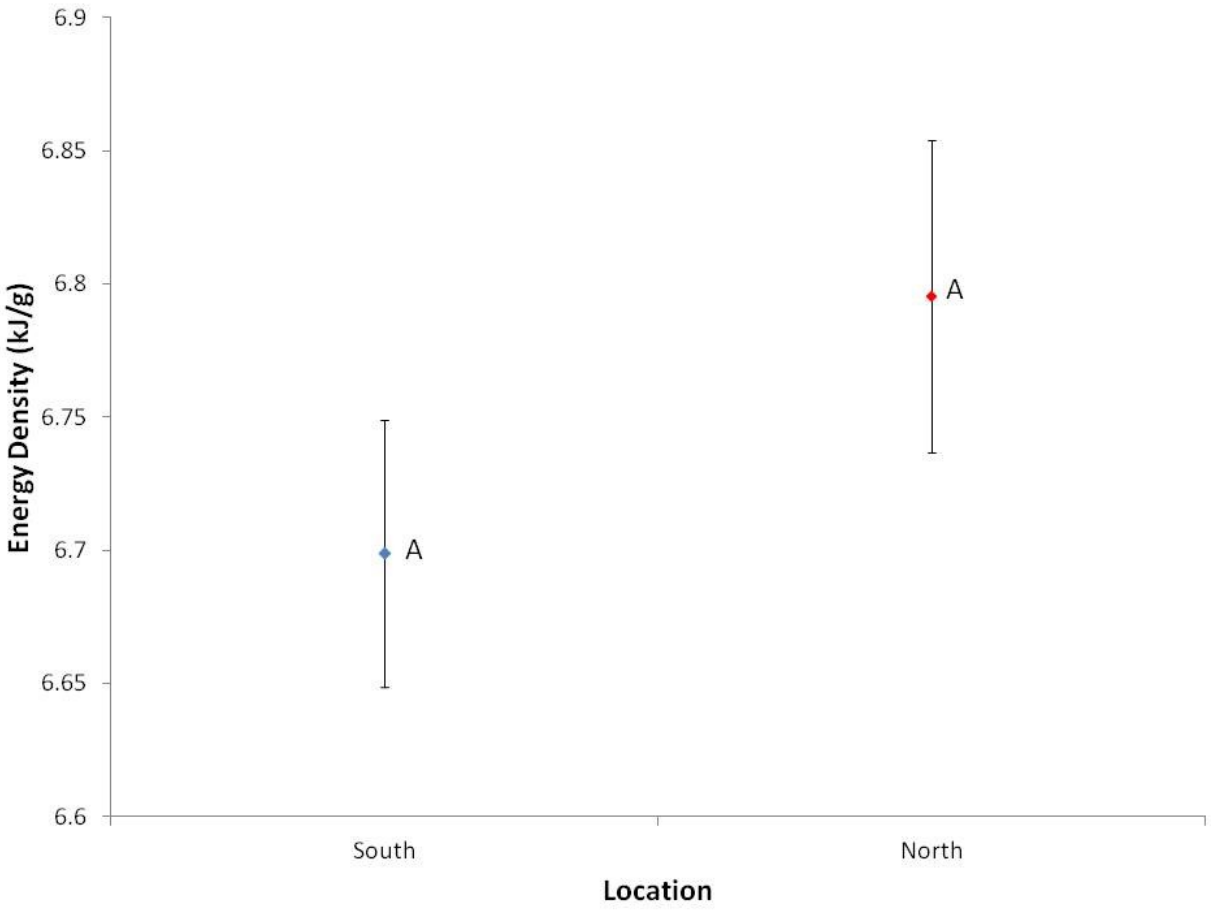

Figure 3.4: Mean (± standard error) egg energy density (kJ/g) in south and north Pukaskwa National Park (2015 and 2016). Letters beside means indicate statistically significant differences. Means that share letters are not different.

Nest Attentiveness

Given the low number of nests monitored using remote cameras only qualitative data could be obtained regarding nest attentiveness. However, for monitored nests, photos provided excellent information regarding nest attentiveness. Over 120,000 images were included in the analysis presented here. Those data indicated that southern PNP gulls exhibited a greater propensity to spend time off their nests particularly for longer periods (Figure 3.5).

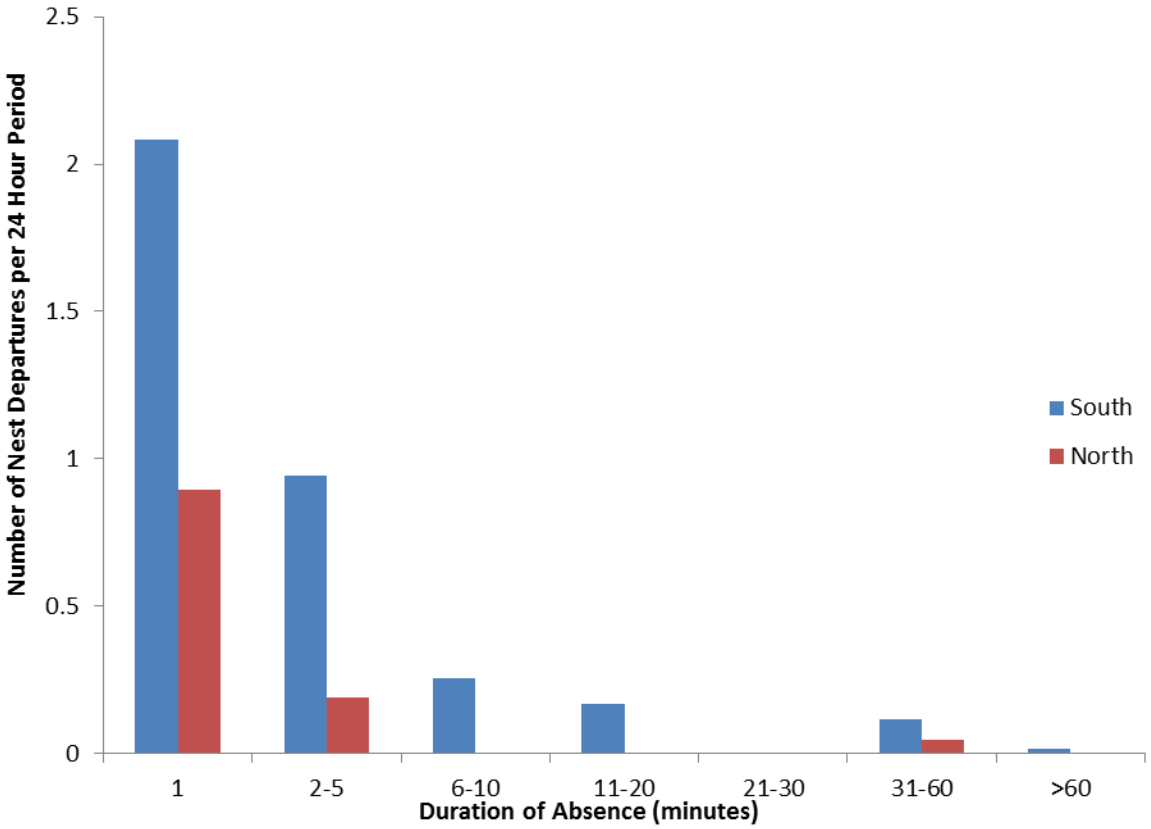

Figure 3.5: Nest attentiveness of Herring Gulls nesting in northern and southern Pukaskwa National Park, 2016.

When nests were categorized as disturbed versus undisturbed (combined across north and south PNP) it was also evident that birds which spent more time off their nests were also more likely to have their nests disturbed or predated (Figure 3.6).

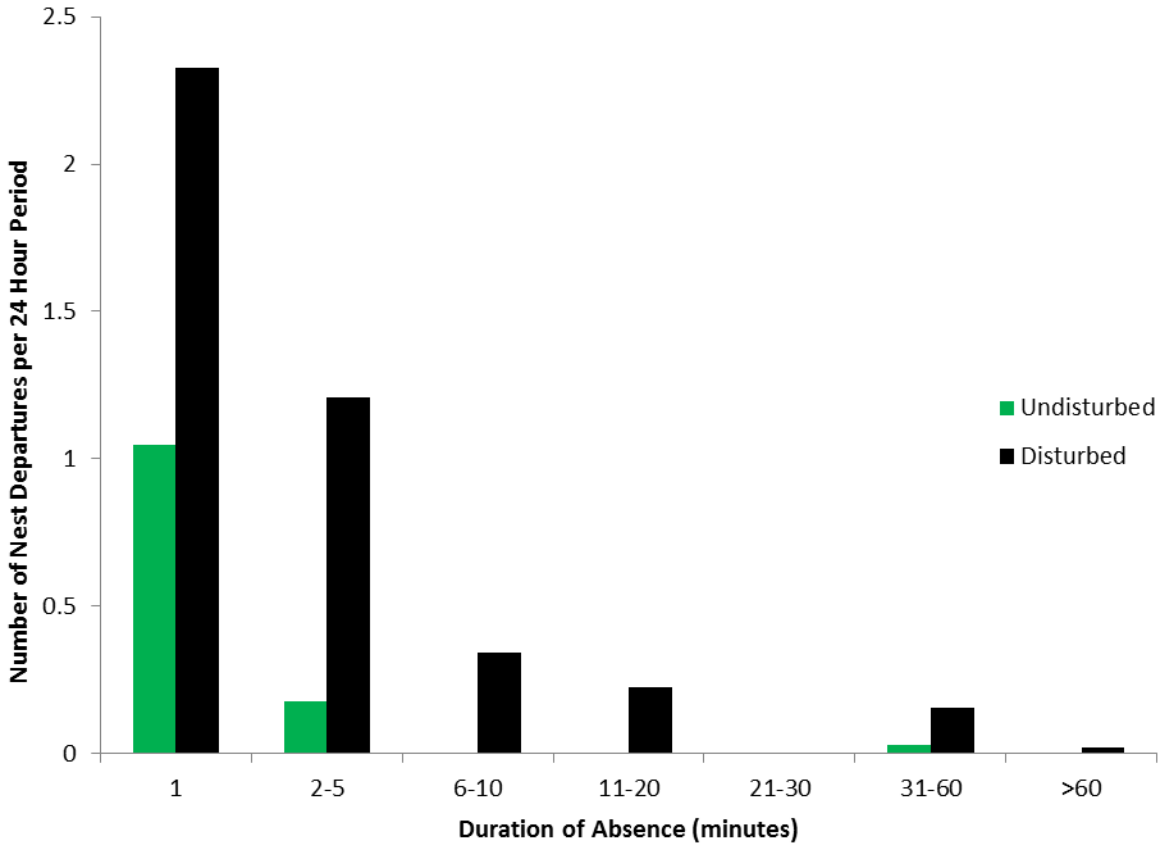

Figure 3.6: Nest attentiveness of Herring Gulls whose nests were disturbed versus undisturbed, Pukaskwa National Park, 2016.

In Pukaskwa National Park, Herring Gull populations, as evaluated through nest counts, have been decreasing since counts began in 1977 (Figures 1.0, 1.2). Breaking these census data down by year revealed changes in the spatial distribution of nesting Herring Gulls through time (Figures 3.7-3.8).

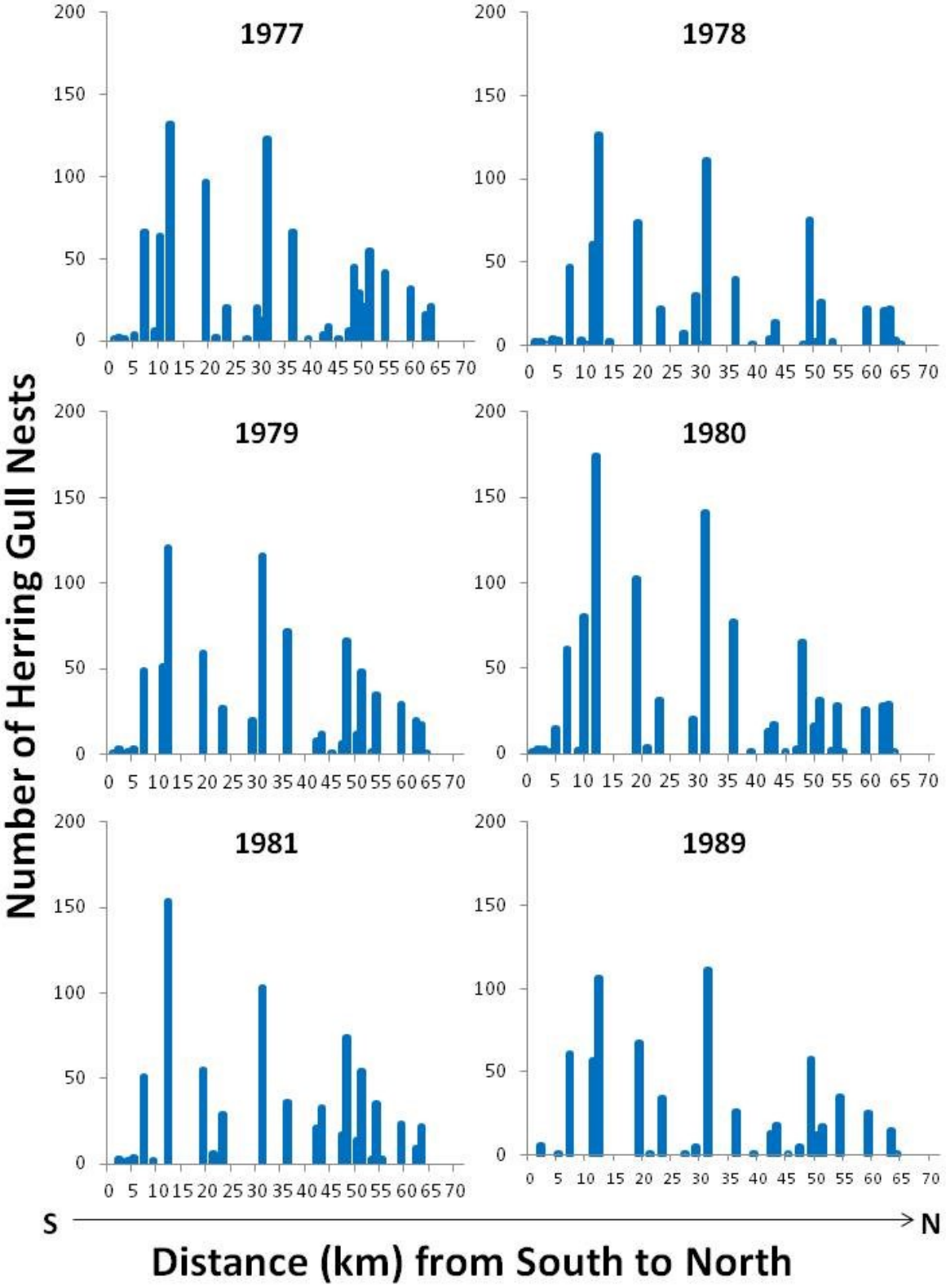

Figure 3.7: Distribution of Herring Gull nests from south to north Pukaskwa National Park, 1977-1989

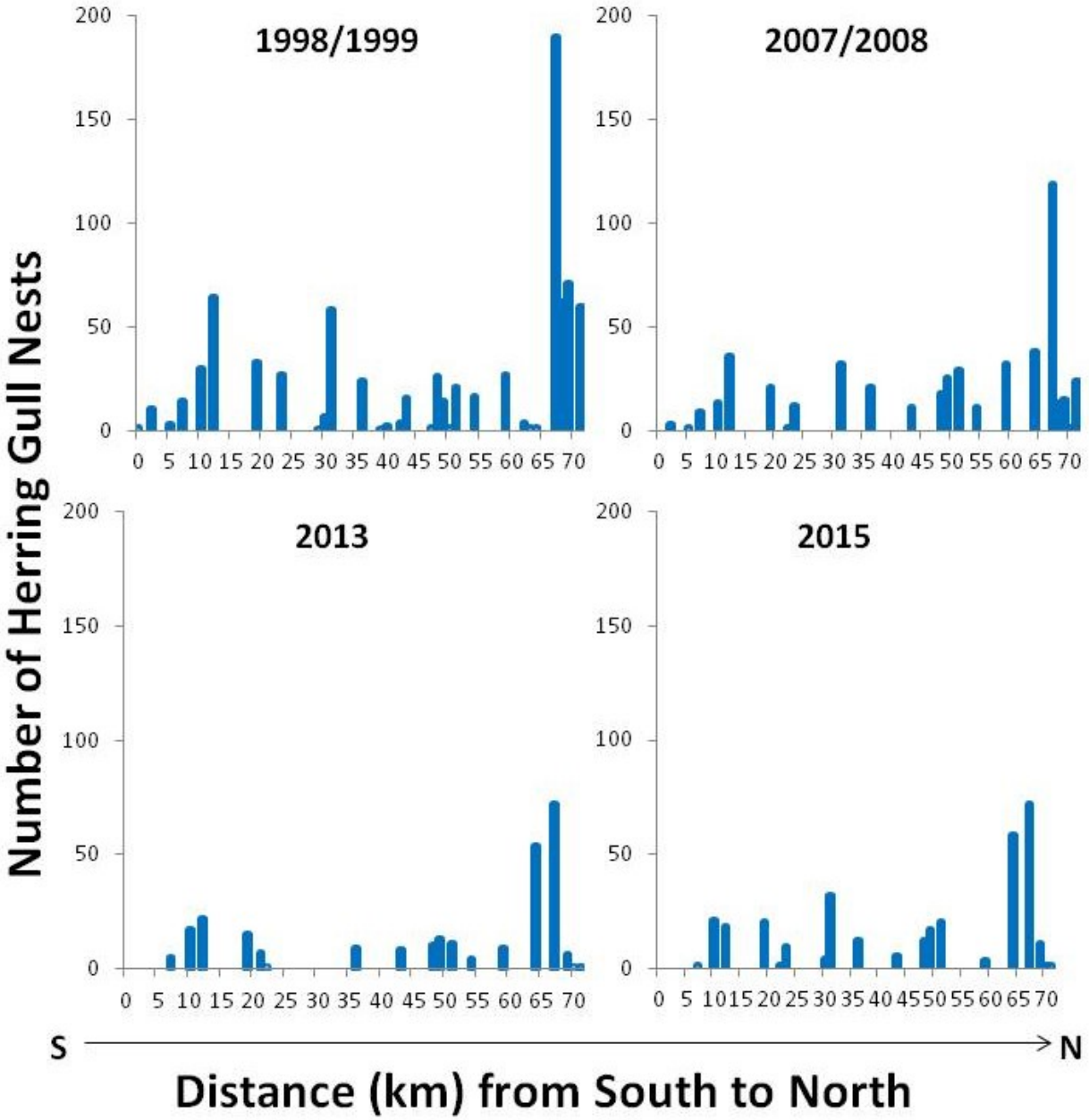

Figure 3.8: Distribution of Herring Gull nests from south to north Pukaskwa National Park, 1998/1999-2015

From 1977 to 1989, Herring Gull nest counts were higher in south PNP versus north PNP (Figure 3.7). However, between 1989 and 1998/1999, nest distribution shifted from southern PNP to northern PNP (Figure 3.7-3.9). Sometime between 1989 and 1998/1999 most of the

nesting population was found in northern PNP. However, through time, overall nest numbers in both regions decreased dramatically (Figures 3.7, 3.8).

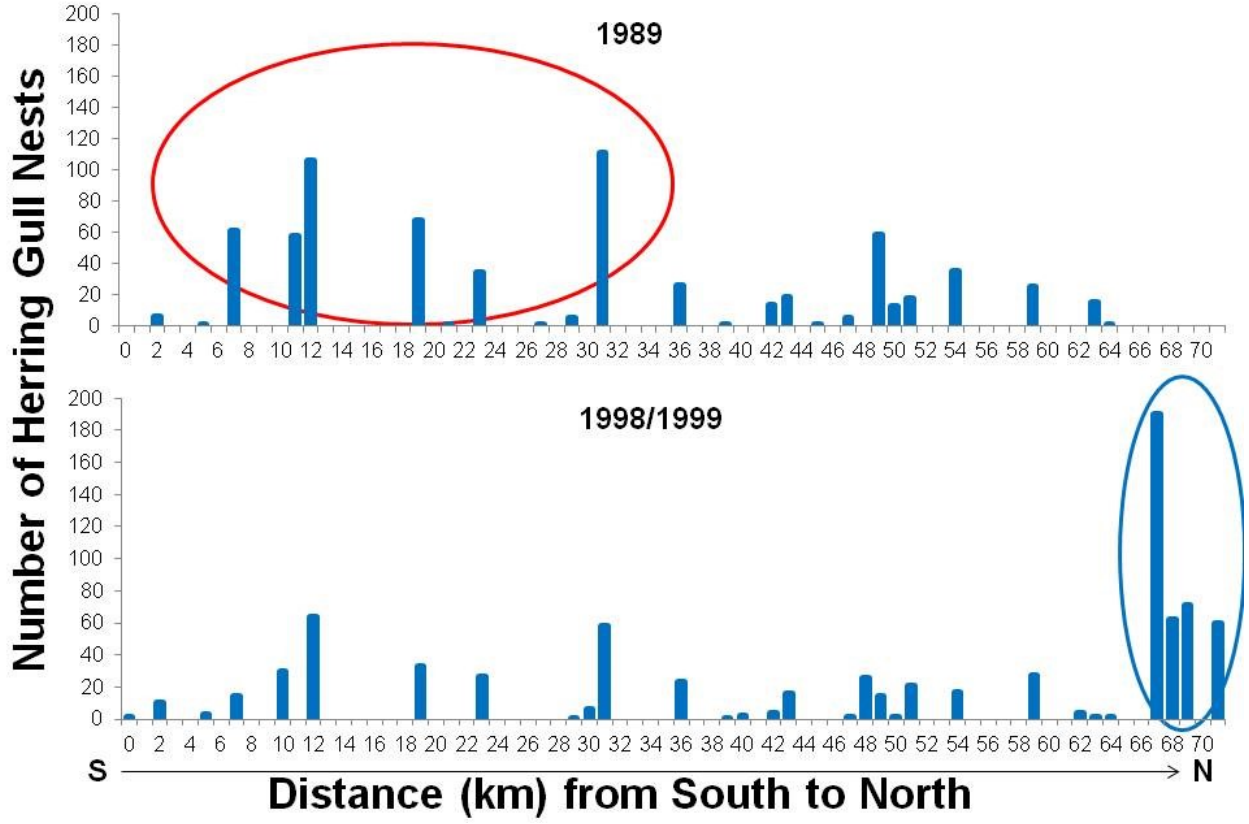

Figure 3.9: Herring Gull nests from south to north Pukaskwa National Park, 1989 versus 1998/1999.

Physiological Endpoint

Mean corticosterone concentrations in egg contents were not significantly different between eggs collected from northern and southern PNP (Table 3.0). Mean eggshell corticosterone levels also showed no regional differences (Table 3.0).

Table 3.0: Corticosterone concentrations in egg contents (ng/g) (2015-16) and eggshell (pg/g) (2016) from northern and southern Pukaskwa National Park.

	Mean South	Mean North	p	Std.Dev. South	Std.Dev. North	t	df
Egg Contents	5.4	6.6	0.24	2.8	4.7	-1.2	56
Eggshell	498.1	499.6	0.99	195.6	308.1	-0.02	28

Egg contents corticosterone values were correlated with eggshell values in samples from south PNP but not north PNP (Figure 3.10).

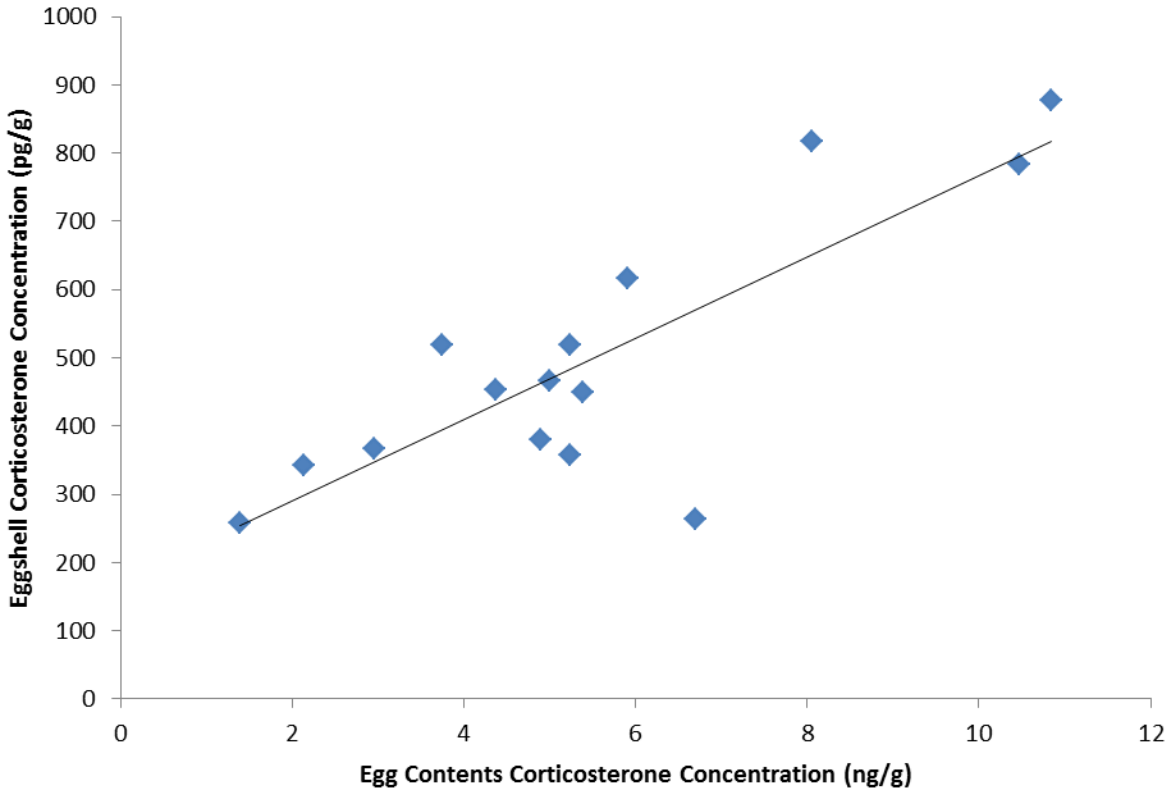

Figure 3.10: The relationship between egg contents and eggshell corticosterone concentration in Herring Gull eggs from south PNP, 2016 ($r = 0.82$, $p < 0.001$).

One-egg clutches from south PNP had greater corticosterone concentrations than 2-egg clutches (egg contents $t(24) = 2.5$, $p = 0.02$; eggshell $Z = 3.1$, $df = 13$, $p = 0.002$; Mann-Whitney U test) (Table 3.1). There were no differences in corticosterone concentrations between two and three-egg clutches in north PNP (egg contents $t(28) = -0.2$, $p = 0.82$; eggshell $Z = 1.1$, $df = 13$, $p = 0.27$; Mann-Whitney U test) (Table 3.2).

Table 3.1: Corticosterone concentrations in egg contents (ng/g) (2015-16) and eggshell (pg/g) (2016) comparing one egg clutches (OEC) and two egg clutches (TwEC) from southern Pukaskwa National Park

	Mean OEC	Mean TwEC	p	Std.Dev. OEC	Std.Dev. TwEC
Egg Contents	6.4	3.9	0.02	3	1.9
Eggshell*	611.3	328.2	0.002	170.5	53.7

t-test used for all comparisons except where indicated by * - Mann-Whitney U Test

Table 3.2: Corticosterone concentrations in egg contents (ng/g) (2015-16) and eggshell (pg/g) (2016) comparing two egg clutches (TwEC) and three egg clutches (ThEC) from northern Pukaskwa National Park.

	Mean TwEC	Mean ThEC	p	Std.Dev. TwEC	Std.Dev. ThEC
Egg Contents	6.4	6.8	0.82	4.8	4.7
Eggshell*	468.1	535.5	0.27	137.5	443.5

t-test used for all comparisons except where indicated by * - Mann-Whitney U Test

Historical Data

Based upon findings examining temporal changes in the distribution of Herring Gull nests in PNP (see above), historical data from Agawa Rocks were categorized into three periods: pre-1989, 1989-1998, and post 1998. The pre-1989 period at Agawa Rocks was defined as a reference period because Herring Gull populations at that time were high and distributed across PNP, particularly in south PNP where they would have been reliant on natural foods. Reproductive and dietary endpoints for northern PNP, southern PNP, Agawa Rocks 1989-1998

and Agawa Rocks post-1998 were compared to values obtained during the pre-1989 reference period at Agawa Rocks.

Reproductive Endpoints

Northern PNP was the only category with lower egg mass than the Agawa reference period (Welch's ANOVA, $F_{4,85} = 8.8$, $p < 0.001$, Dunnett's test) (Figure 3.11). Welch's ANOVA indicated significant differences in egg volume across categories ($F_{4,83} = 3.65$, $p = 0.02$) but Dunnett's post-hoc comparisons only indicated an almost significant difference between the Agawa reference and PNP north ($p = 0.12$) (Figure 3.12).

There were significant differences in egg energy density values in kJ/egg (Welch's ANOVA, $F_{4,82} = 15.8$, $p < 0.001$) and kJ/g (ANOVA, $F_{4,82} = 6.0$, $p < 0.001$). For energy density values expressed as kJ/egg, northern PNP , southern PNP, and Agawa post-1989 were significantly lower than the Agawa pre-1989 reference (Dunnett's test, $p < 0.05$) (Figure 3.13). Southern and northern PNP had lower energy densities (kJ/g) than the Agawa pre-1989 reference (Dunnett's test, $p < 0.05$) (Figure 3.14).

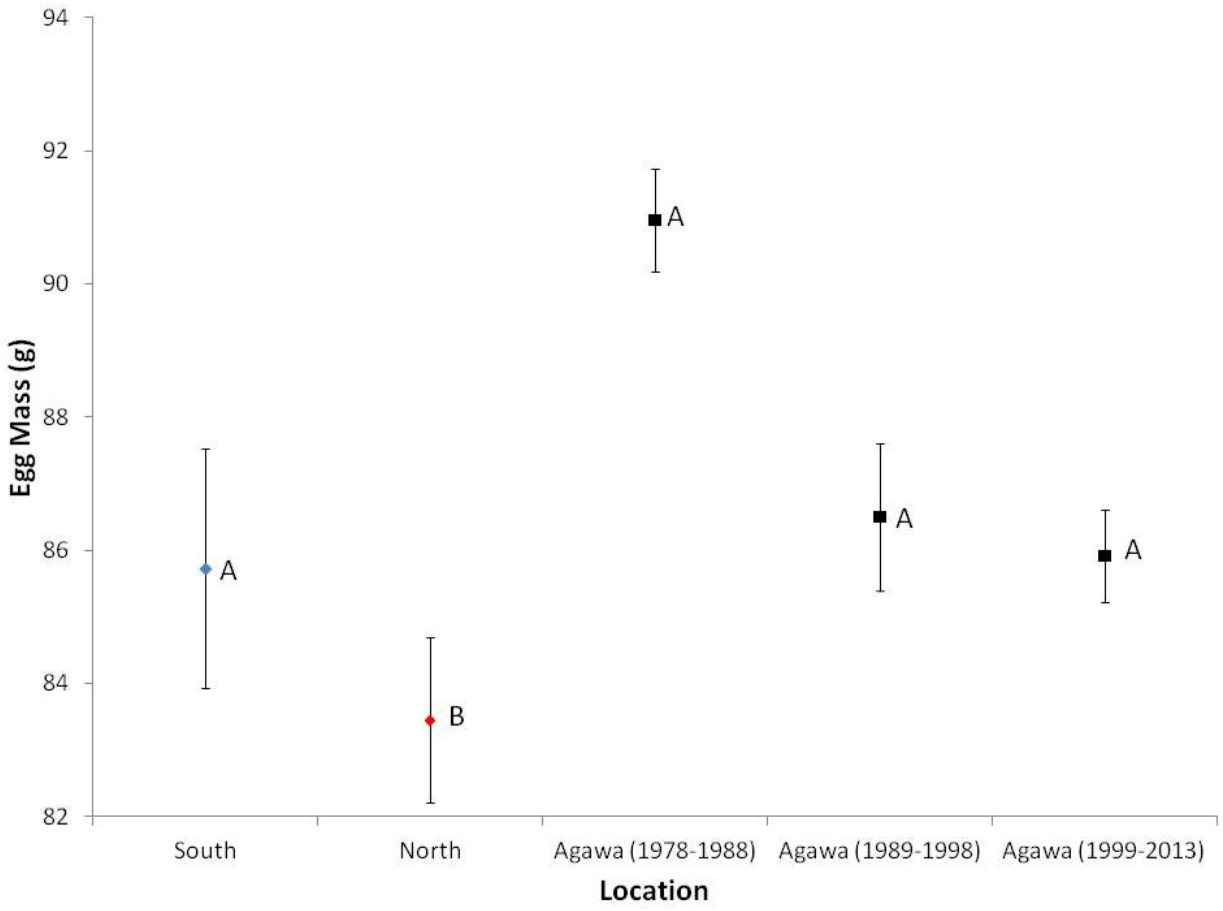

Figure 3.11: Mean (± standard error) egg mass at south and north Pukaskwa National Park (2015 and 2016) and at Agawa Rocks during different periods. Letters beside means indicate statistically significant differences from the reference period (Agawa, 1978-1988) (Welch's ANOVA followed by Dunnett's test). Means that share letters are not different.

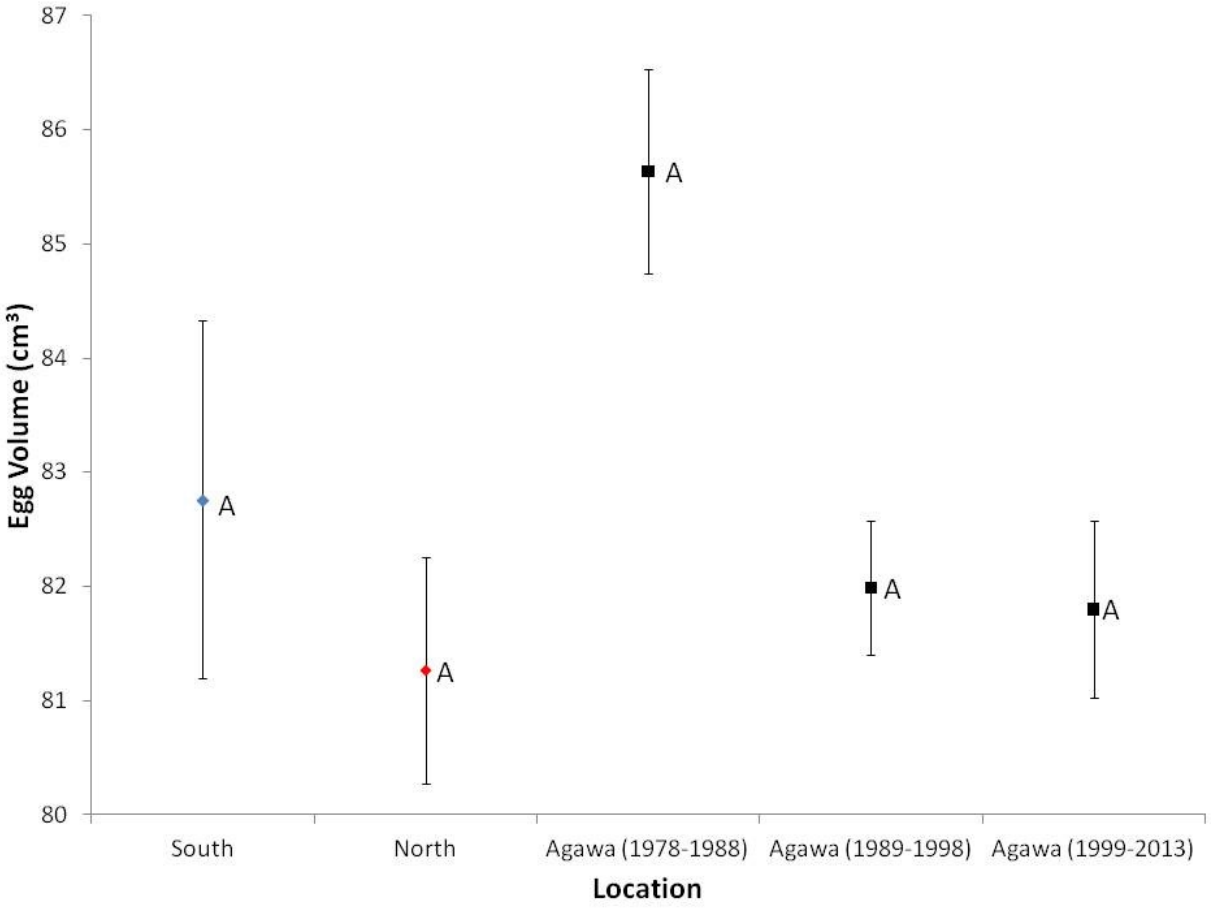

Figure 3.12: Mean (± standard error) egg volume at south and north Pukaskwa National Park (2015 and 2016) and at Agawa Rocks during different periods. Letters beside means indicate statistically significant differences from the reference period (Agawa, 1978-1988) (Welch's ANOVA followed by Dunnett's test). Means that share letters are not different.

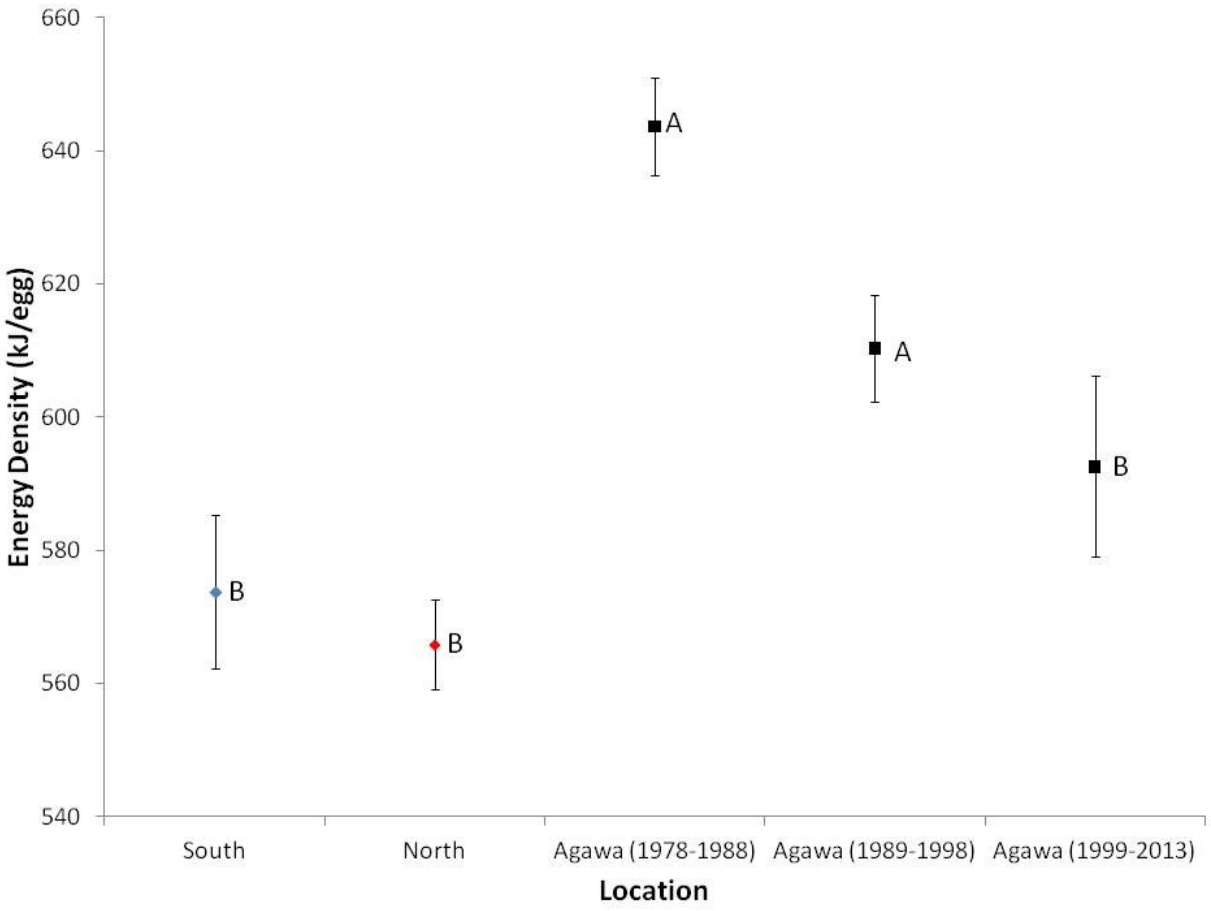

Figure 3.13: Mean (± standard error) egg energy density (kJ/egg) at south and north Pukaskwa National Park (2015 and 2016) and at Agawa Rocks during different periods. Letters beside means indicate statistically significant differences from the reference period (Agawa, 1978-1988) (Welch's ANOVA followed by Dunnett's test). Means that share letters are not different.

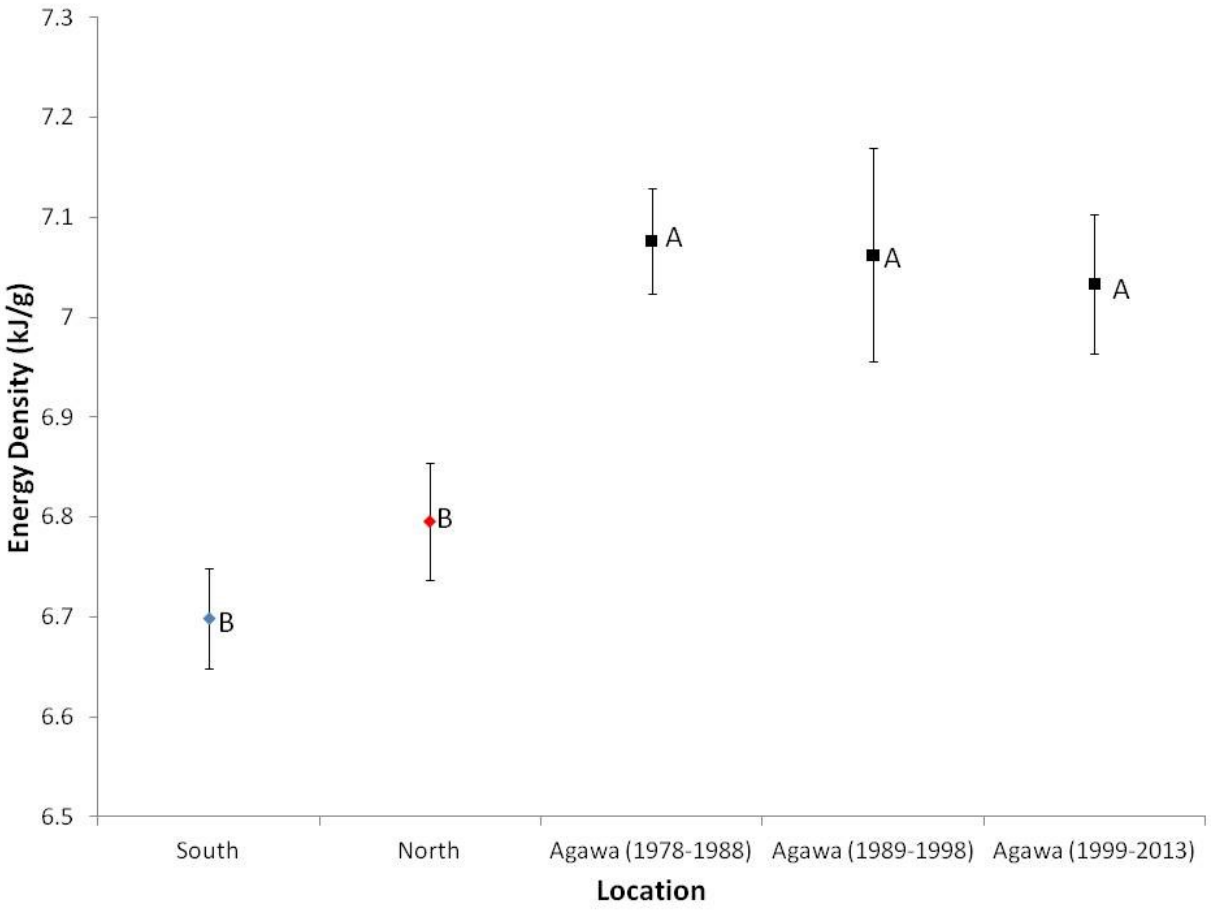

Figure 3.14: Mean (± standard error) egg energy density (kJ/g) at south and north Pukaskwa National Park (2015 and 2016) and at Agawa Rocks during different periods. Letters beside means indicate statistically significant differences from the reference period (Agawa, 1978-1988) (ANOVA followed by Dunnett's test). Means that share letters are not different.

Dietary Endpoints

At Agawa Rocks, there was no trend in egg $\delta^{15}N$ values between 1978-1988 ($r = 0.36$, $p = 0.27$). However, during the period 1989 to 1998 egg $\delta^{15}N$ values decreased ($r = -0.78$, $p = 0.008$). After 1998, egg $\delta^{15}N$ values stabilized at this lower value ($r = -0.03$, $p = 0.92$) (Figure 3.15). Mean egg $\delta^{15}N$ values across south and north PNP and the three Agawa Rocks periods differed (Welch's ANOVA, $F_{4,88} = 22.3$, $p < 0.001$). Northern PNP, southern PNP, and Agawa

post-1998 were all lower than the pre-1989 reference at Agawa Rocks (Dunnett's test, $p < 0.05$) (Figure 3.16).

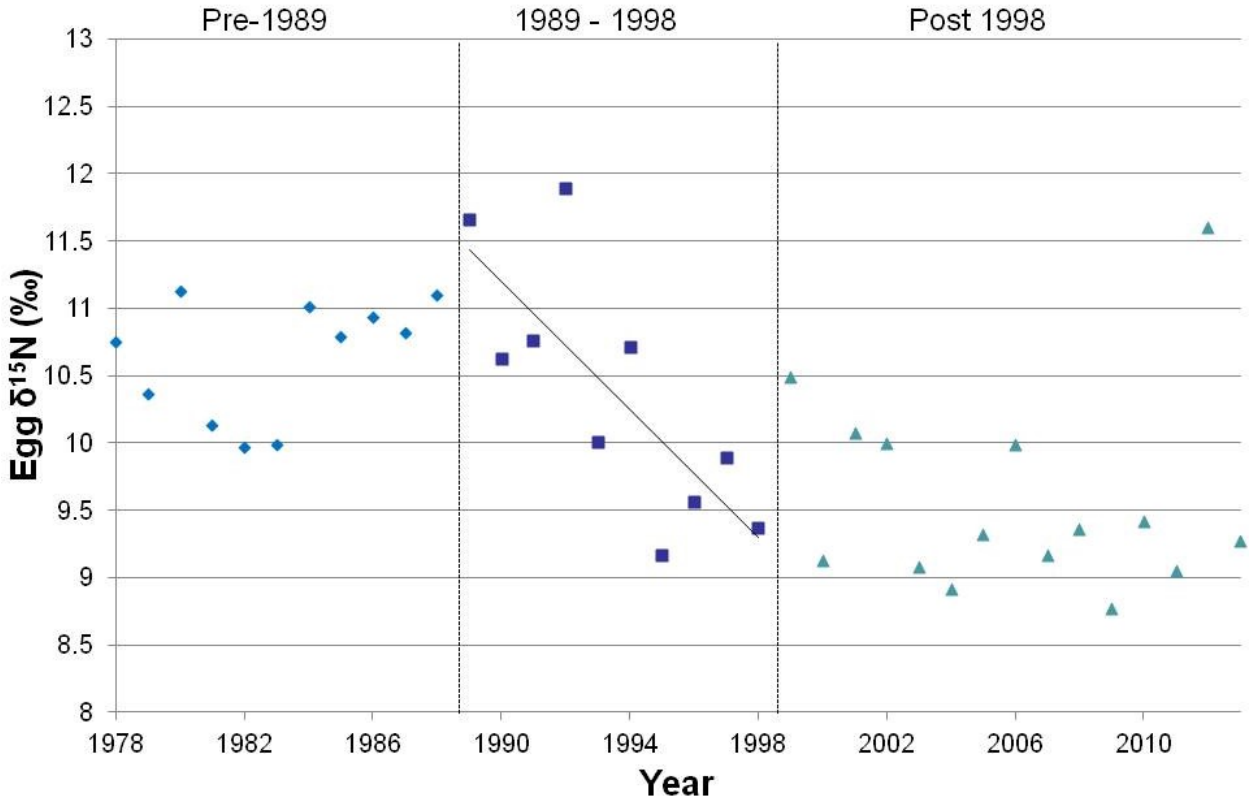

Figure 3.15: Temporal changes in egg $\delta^{15}N$ values at Agawa Rocks, 1978 to 2013. Data were categorized into three periods based upon spatial changes in the distribution of Herring Gulls nesting in Pukaskwa National Park.

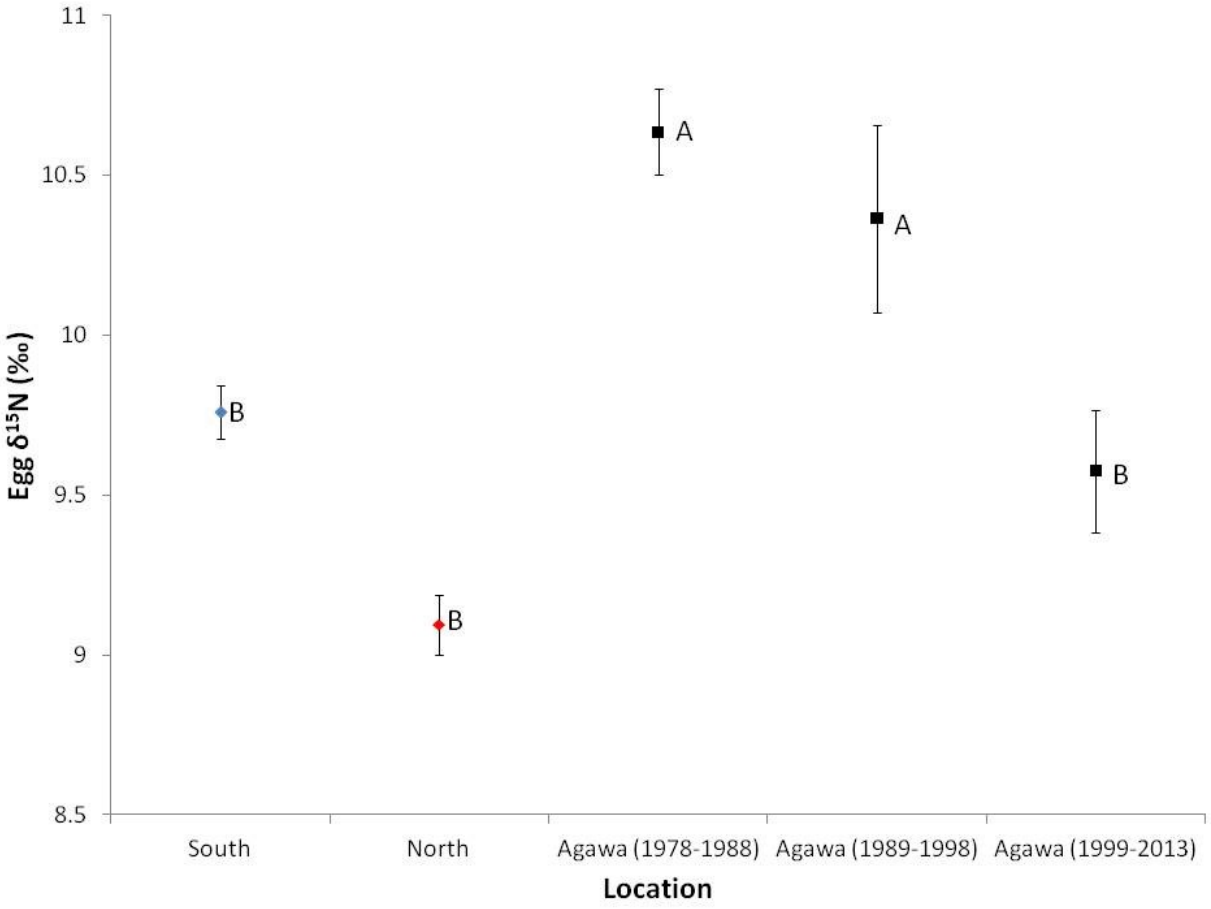

Figure 3.16: Mean (± standard error) egg $\delta^{15}N$ values at south and north Pukaskwa National Park (2015 and 2016) and at Agawa Rocks during different periods. Letters beside means indicate statistically significant differences from the reference period (Agawa, 1978-1988) (Welch's ANOVA followed by Dunnett's test). Means that share letters are not different.

Egg $\delta^{13}C$ trends were less clear with no significant trends during any period (pre-1989 r = -0.54, p = 0.09; 1989-1998 r = 0.53, p = 0.12, post-1998 r = 0.27, p = 0.34) (Figure 3.17). The post-1998 Agawa category was the only one showing significant differences in $\delta^{13}C$ values compared to the pre-1989 Agawa reference (Welch's ANOVA, $F_{4,88}$ = 3.3, p = 0.03 followed by Dunnett's test, $p < 0.05$) (Figure 3.18).

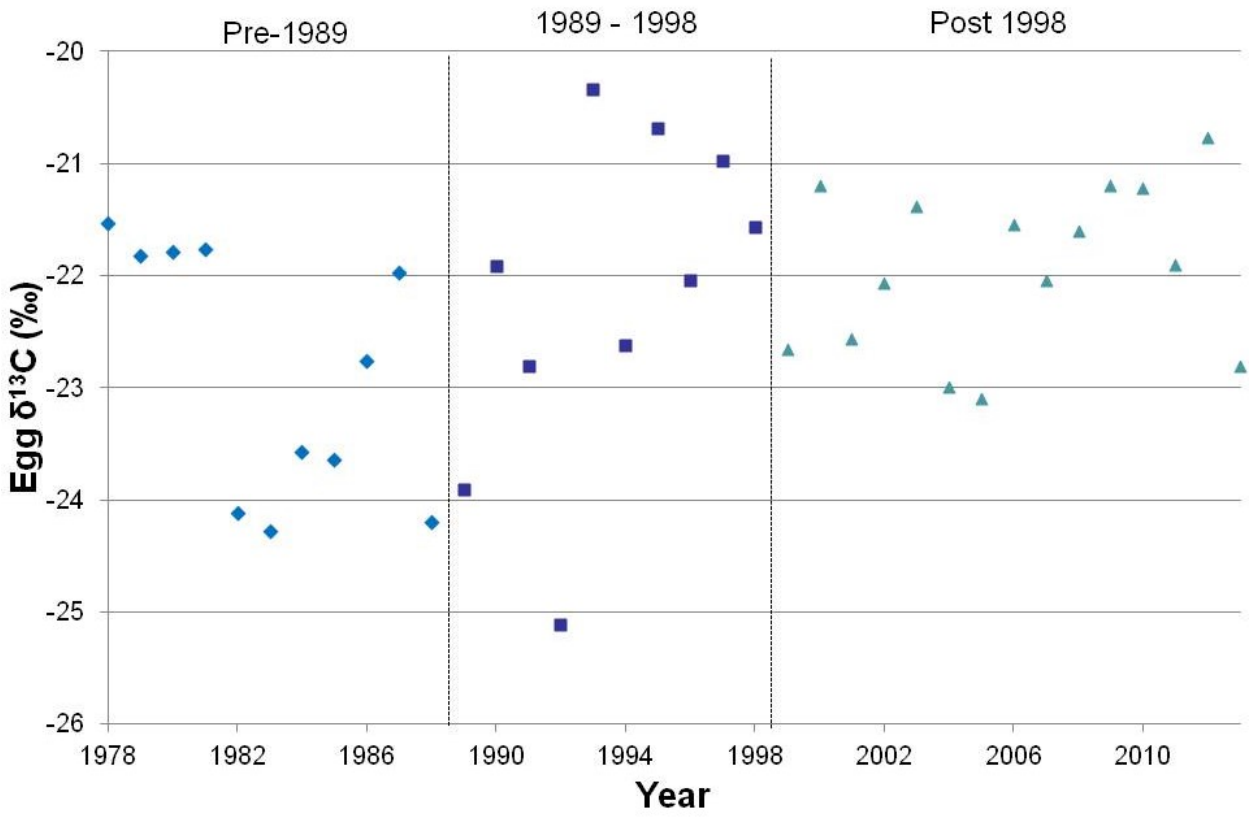

Figure 3.17: Temporal changes in egg $\delta^{13}C$ values at Agawa Rocks, 1978 to 2013. Data were categorized into three periods based upon spatial changes in the distribution of Herring Gulls nesting in Pukaskwa National Park.

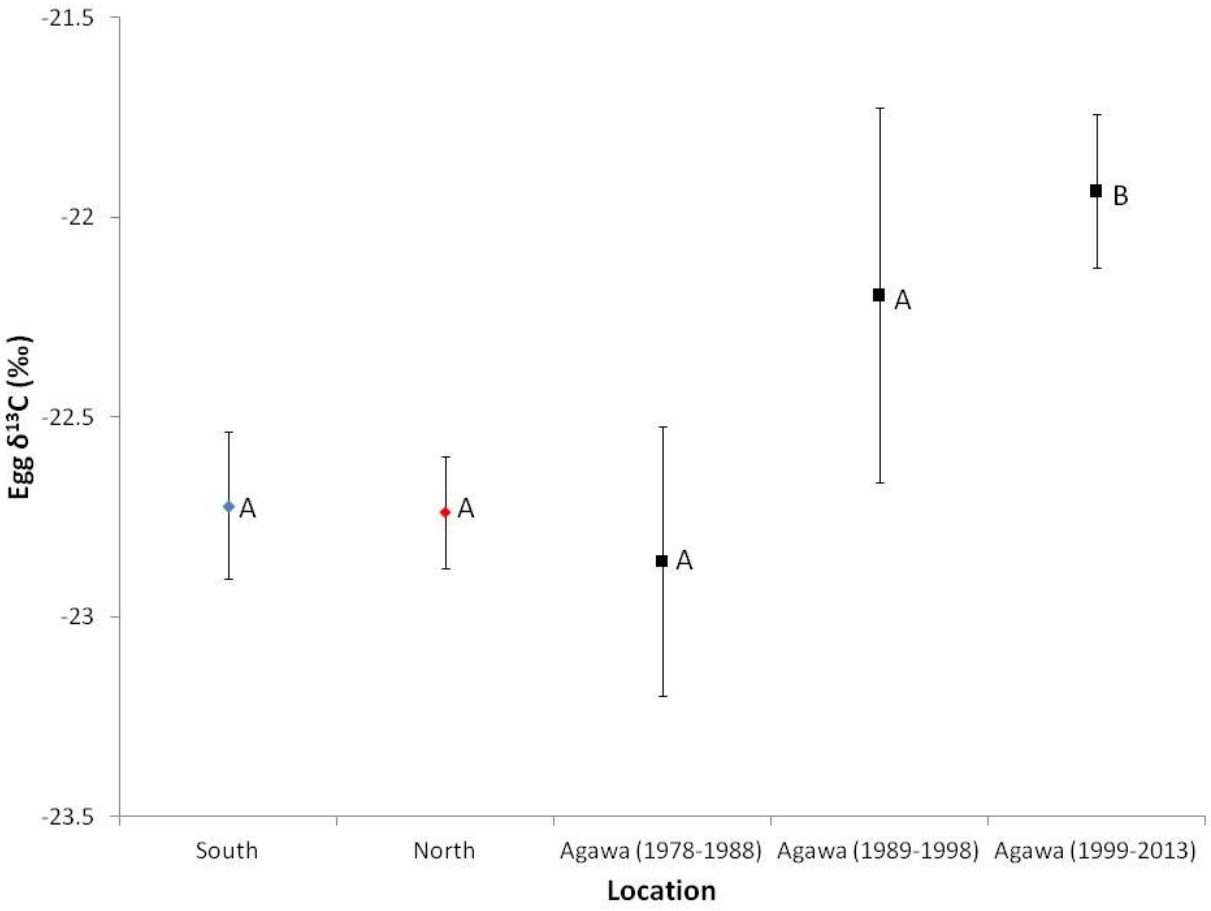

Figure 3.18: Mean (± standard error) egg $\delta^{13}C$ values at south and north Pukaskwa National Park (2015 and 2016) and at Agawa Rocks during different periods. Letters beside means indicate statistically significant differences from the reference group (Agawa, 1978-1988) (Welch's ANOVA followed by Dunnett's test). Means that share letters are not different.

There were no trends in egg omega 3/omega 6 fatty acid ratios at Agawa Rocks (pre-1989 r = -0.09, p = 0.83; 1989-1998 r = -0.28, p = 0.43, post-1998 r = -0.35, p = 0.18) (Figure 3.19). Mean egg omega 3/omega 6 fatty acid ratios differed across categories (Welch's ANOVA, $F_{4,86}$ = 16.0, $p < 0.001$) but Dunnett's test indicated that only northern PNP was significantly different from the pre-1989 Agawa reference ($p < 0.05$) (Figure 3.20)

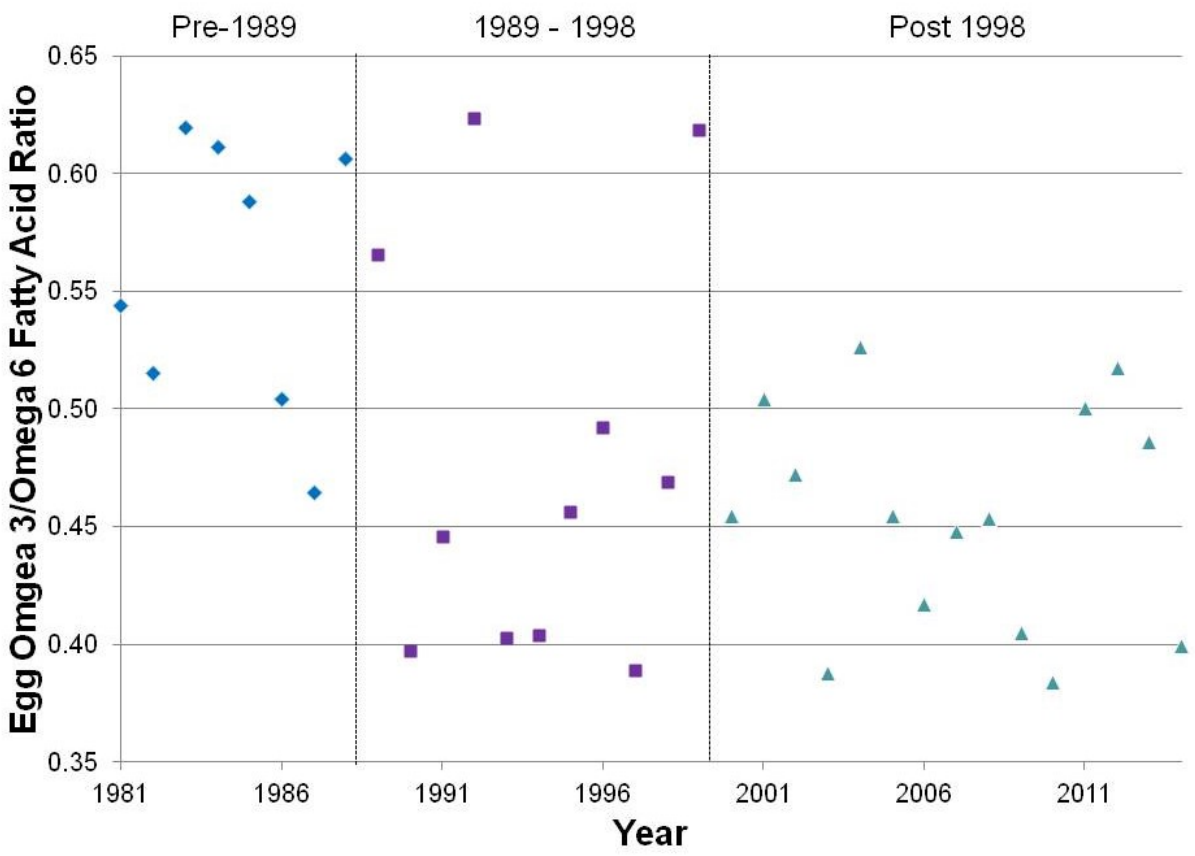

Figure 3.19: Temporal changes in egg omega 3/omega 6 fatty acid ratio at Agawa Rocks, 1981 to 2014. Data were categorized into three periods based upon spatial changes in the distribution of Herring Gulls nesting in Pukaskwa National Park.

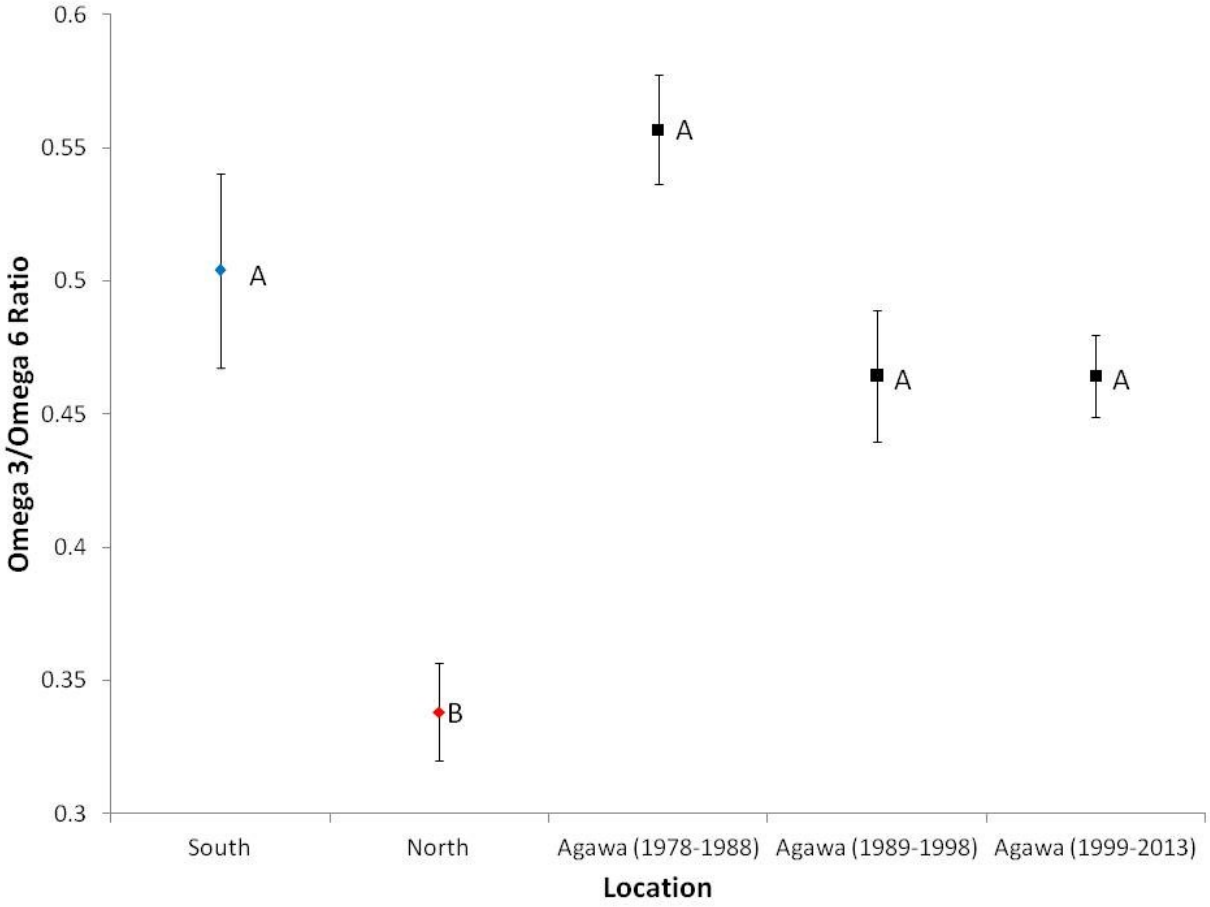

Figure 3.20: Mean (± standard error) egg omega 3/omega 6 fatty acid ratio at south and north Pukaskwa National Park (2015 and 2016) and at Agawa Rocks during different periods. Letters beside means indicate statistically significant differences from the reference period (Agawa, 1978-1988) (ANOVA followed by Dunnett's test). Means that share letters are not different.

Prey Fish Abundance

Prey fish abundance peaked at a similar time, i.e. 1998, in the areas adjacent to Agawa Rocks and southern PNP. After 1998, populations decreased with some of the lowest values observed in 2015 and 2016. Prey fish abundance in the northern PNP area was historically much lower than southern PNP or Agawa Rocks but peak abundance at that location was in 2014 (Figure 3.21)

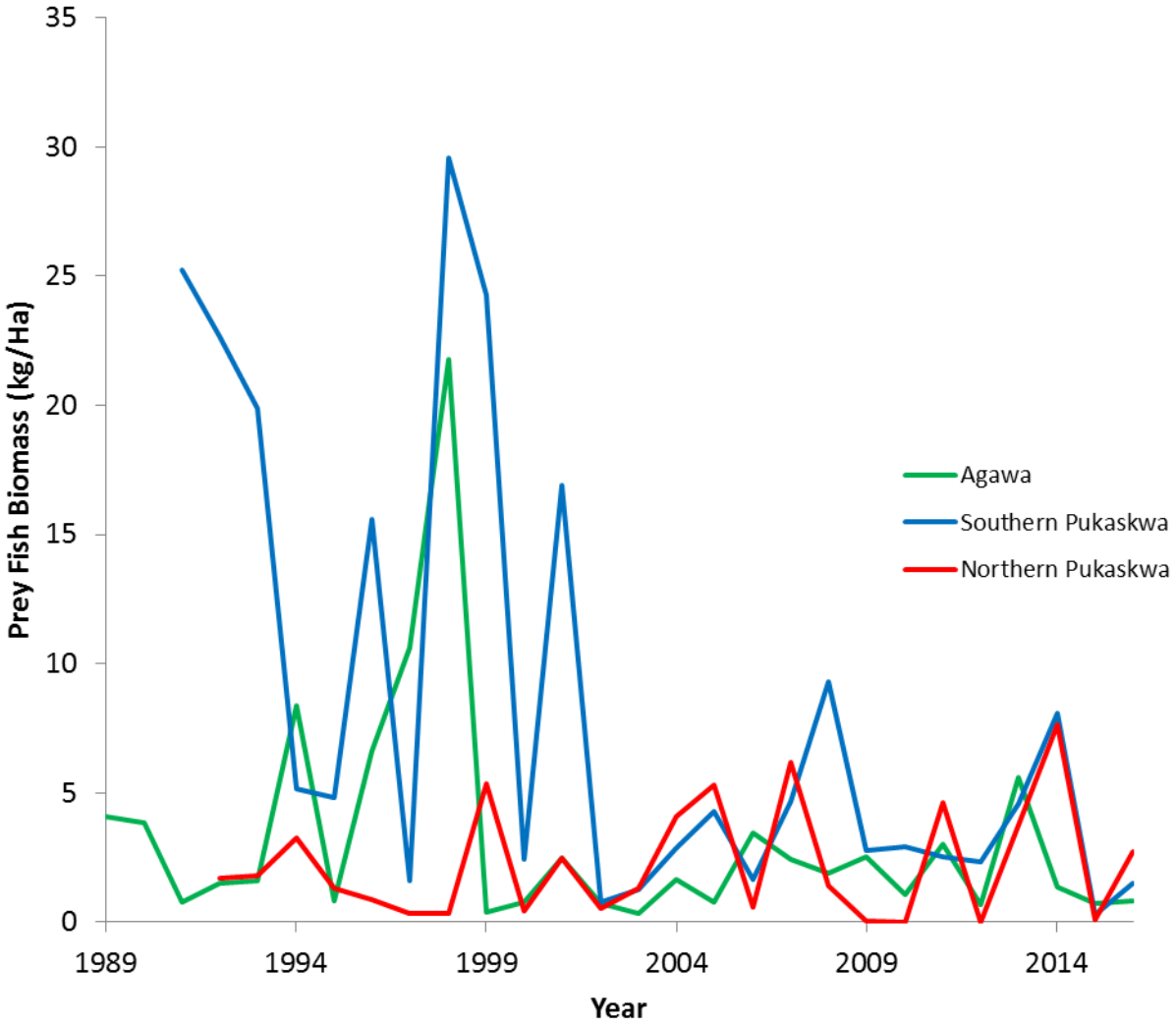

Figure 3.21: Temporal trends in prey fish biomass (kg/ha) for northern Pukaskwa National Park, southern Pukaskwa National Park, and Agawa Rocks. Source: United States Geological Survey.

Discussion

Reproductive Endpoints

Clutch size in Herring Gulls nesting in southern PNP was significantly smaller than for gulls breeding in northern PNP. The lower clutch size in southern Pukaskwa suggests that gulls nesting in the south may have been incapable of finding enough food to produce three-egg clutches (Ankney and MacInnes, 1978). This could have important ramifications as egg loss, through factors such as predation or inclement weather, could result in significantly reduced reproductive success. In fact, two of the four nests monitored in south PNP with remote cameras experienced conspecific predation while parents were absent from their nests. These predation events resulted in one of the nests being abandoned (one-egg clutch going to zero eggs) and the other rearing only one chick. Reduced reproductive success mediated through small clutch sizes and reduced chick recruitment may be contributing to the decline in gull populations in PNP, especially in the south. In northern Pukaskwa, predation did not appear to be significant as most nests contained three egg clutches and no predation events were recorded by remote cameras.

Egg mass, volume, and energy density were not different between gulls nesting in southern and northern PNP. This was somewhat surprising as the reduced clutch size seen in southern gulls may have reflected a shortage of food in the south. However, since egg mass, volume and energy density were not lower it suggests that southern Herring Gulls produced fewer eggs of equal quality to gulls nesting in north PNP.

Nest Attentiveness

Nest attentiveness provided insights into the degree to which Herring Gulls were absent from their nests. Food scarcity may force parental birds to spend more time off their nest in order to obtain adequate resources to meet their basic needs in addition to that required during reproduction, e.g. egg formation, chick provisioning. At PNP, gulls nesting in the north spent less time off their nests than birds nesting in the south. This may have reflected regional differences in food availability. In particular, in the north, birds may have had an easier time finding food by supplementing their diets with food from anthropogenic sources. Birds nesting in the south did not have this option and were limited to foraging for natural food sources. Parental absence increases the likelihood of nest disturbance, either through the stealing of nest material or through the predation of eggs or chicks. Southern nesting Herring Gulls spent more time off their nests and, as a result, 50% of nests (2/4) monitored by cameras in the south were predated (Figure 3.22) while no nest predation events were observed in the north. Nest predation could be contributing to the more significant population declines in south PNP.

Figure 3.22: Conspecific predation of Herring Gull egg in southern Pukaskwa National Park. Image was obtained by remote camera used to monitor nest attentiveness.

Corticosterone

Corticosterone is a stress hormone produced by birds exposed to environmental stressors such as predation or food shortages. Corticosterone levels were not different in eggs from northern and southern PNP indicating that there were no regional differences in Herring Gull stress levels. This suggested that birds nesting in both regions of PNP were incurring similar levels of stress. However, because corticosterone is a general indicator of stress, the factors contributing to similar stress levels in these birds could be different across regions. For example, birds living near urban environments or exposed to anthropogenic contaminants may have elevated corticosterone levels (Meillère *et al,* 2016). This could explain why northern PNP-nesting Herring Gulls had the same mean levels of corticosterone as southern PNP birds which could have had their stress levels affected by other factors, e.g. limited food availability.

Furthermore, the degree to which corticosterone is transferred from the laying female to the egg is not currently known. It is possible that future research will reveal that corticosterone is not linearly transferred from the mother to the egg. The importance of food limitations on imposing stress in south PNP birds was illustrated by differences in corticosterone levels in eggs from 1 versus 2-egg clutches in south PNP. Birds with 1-egg clutches may have been more severely limited in the resources they had available for egg formation and this could have contributed to eggshell and egg contents corticosterone levels. The reason no difference was detected in corticosterone levels between 2 and 3 egg clutches in northern PNP may have reflected the better foraging opportunities in that area. Greater availability of food in north PNP was reflected by the absence of one-egg clutches; all females were able to lay at least two eggs. Hence, north PNP egg corticosterone levels may have been affected by other factors than food availability.

Pukaskwa Population Trends

Herring Gull populations at PNP decreased significantly over time. My analysis of temporal changes in the distribution of gulls nesting in PNP revealed that originally the southern population of Herring Gulls was much higher than in the north but sometime between 1989 and 1998/99 Herring Gull populations shifted to the northern portion of PNP. This shift could have been caused by a variety of factors including decreased prey fish abundance, particularly in southern PNP. I investigated this further by using historic data from the GLHGMP and the USGS.

Historical Perspective

Reproductive Endpoints

Based upon the distributional shifts observed in breeding populations of Herring Gulls in PNP, I categorized the GLHGMP historical data for Agawa Rocks into three periods. Comparing PNP reproductive endpoints with data from Agawa Rocks indicated that the reproductive endpoints, i.e. egg mass and egg energy density, showed lower values at PNP compared to a reference period, Agawa pre-1989, when Herring Gull populations at PNP were higher. Furthermore, declines in egg energy density (kJ/egg) were also observed at Agawa Rocks with current values being similar to those observed at PNP. The timing of the shifts observed in egg mass and egg energy density (kJ/egg) coincided with shifts in the spatial distribution of PNP gulls. This suggested that sometime between 1989 and 1998 a large-scale change occurred that negatively impacted PNP and Agawa Rocks Herring Gull populations as evidenced by the reproductive endpoints examined here. Reduced egg mass may negatively impact reproductive success as it may lead to smaller chicks that may be delayed in their development for the first few weeks post-hatch compared to chicks from larger sized eggs (Schifferli, 1973; Ankney, 1980; Williams, 1980). This, in turn, could lead to increased rates of chick mortality leading to lower juvenile recruitment into gull populations (Williams, 1980). Although statistically significant differences were not observed in egg volume between north and south PNP nor among periods at Agawa Rocks, there was a general trend of decreasing egg volume through time. Egg volume has been positively associated with food availability (Moss and Watson, 1984; Hiom *et al*, 1991) and additional data should be collected to further evaluate trends in this potentially important reproductive endpoint.

Declines in egg energy density may also effect the health of offspring and reflect upon the quality of the maternal gull. As energy density decreases, the quality of the egg decreases which leads to smaller and less developed offspring at hatch (Martin, 1987).

Dietary Endpoints

Stable Isotopes

Using historical data collected through the GLHGMP it was evident that leading up to the 1989-1998/1999 PNP population shift that the nitrogen isotope composition of eggs at Agawa Rocks underwent a significant change with egg $\delta^{15}N$ values declining during the 1989-1998 period. This reflected a decline in gull trophic position. This shift suggests that, during the time period in PNP when Herring Gull populations in the south were declining and populations in the north were increasing, Herring Gulls from Agawa Rocks started feeding at a lower trophic level. Since that time, trophic position of Agawa Rocks gulls has remained at this low trophic position, similar to what is currently observed for gulls at south PNP. This suggests that during the period 1989-1998/1999 a change in diet occurred that corresponded with a decline in higher trophic level prey, likely prey fish, which occupy higher trophic positions than alternative foods that gulls may consume (Hebert *et al,* 1999). The decrease in fish abundance may have forced Herring Gulls at Agawa Rocks and PNP to switch to other food sources where possible, i.e. anthropogenic sources in north PNP.

Fatty Acids

Mean omega 3/omega 6 fatty acid values in eggs from southern PNP were not significantly different from any of the time periods at Agawa Rocks. Northern PNP eggs, however, had significantly lower omega 3/omega 6 fatty acid values than southern PNP (Chapter 2) or Agawa Rocks. Lower omega 3/omega 6 fatty acid ratios in northern PNP suggested that females laying those eggs were feeding on more terrestrial food than birds in other categories. This may have reflected the greater accessibility of anthropogenic food sources to northern PNP Herring Gulls. Access to anthropogenic foods could be a mixed blessing as it could provide birds with energy and some nutrients but natural foods, such as fish, are considered to be of higher quality (Belant *et al,* 1993). Furthermore, if levels of essential nutrients such as omega 3 fatty acids are limited in garbage then northern birds could experience negative impacts on chick development, immune system function, and compromised vision (Twining *et al*, 2016b). By consuming anthropogenic food, northern PNP gulls may also incur increased exposure to disease and chemical toxins (Durrant and Beatson, 1981; Monaghan *et al,* 1985; Inigo Elias, 1987; Ortiz and Smith, 1994; Fry, 1995). All of these factors could act together to negatively impact gull populations relying on anthropogenic food sources as a significant component of their diet.

Prey Fish Populations

To examine potential causes for the population shift and changes in reproductive and dietary endpoints I obtained data regarding prey fish biomass from the USGS and compiled it to get estimates of prey fish biomass for my three Herring Gull study areas: north and south PNP and Agawa Rocks. Trends in prey fish data coincided, in general, with shifts in the spatial

distribution of Herring Gull nests in PNP. That is, circa mid-1990s, prey fish biomass in south PNP declined, rebounded around 1998 but then immediately declined to a lower level where they have since remained. Trends at Agawa Rocks were similar. However, after 1998 prey fish abundance decreased significantly for Agawa Rocks and southern PNP, dropping by approximately 58%. This drop in prey fish abundance was reflected in the historical $\delta^{15}N$ and $\delta^{13}C$ values seen at Agawa rocks. Indeed, when Herring Gull diet shifts away from aquatic food sources there is a decrease in egg $\delta^{15}N$ (Hebert and Sprules, 2002), which was also seen at PNP. This could also be why Herring Gulls in northern PNP have lower omega 3/omega 6 fatty acid ratios than southern PNP and Agawa Rocks. Decreased prey fish abundance has forced Herring Gulls to find alternative food sources. This is relatively easy for northern PNP gulls as they have access to anthropogenic food subsidies. The decrease in prey fish abundance may also account for why egg $\delta^{13}C$ values at Agawa Rocks became less negative through time as gulls were forced to feed on alternative food sources.

In 2014 and 2015 there has been a small recent increase in prey fish abundance around all three sites. This increase in prey fish abundance may have influenced Herring Gull populations at PNP. In northern and southern PNP, Herring Gull nest numbers increased somewhat during the same time period. This could have reflected an increase in the proportion of birds attempting to breed as foraging conditions may have improved. Hence, gulls may have the ability to respond to annual changes in prey fish abundance.

Chapter 4 Conclusions

Herring Gulls are top-level predators and have been used to evaluate the state of ecosystems (Chapdelaine and Rail, 1997; Hebert *et al,* 2000). For example, they are used to monitor the health of the Great Lakes through Environment and Climate Change Canada's Great Lakes Herring Gull Monitoring Program (GLHGMP) and are used as an indicator of ecological integrity by the Parks Canada Agency at Pukaskwa National Park (PNP) on Lake Superior. In PNP, Herring Gull populations have decreased by approximately 70% since nest counts began in the 1970s but populations in the southern part of the park have declined to a much greater extent (~80%) than in the north (~40% decline). The goal of my research was to understand if food availability was playing a role in this population decline. My research consisted of two parts. First, I evaluated the Herring Gull diet using various measures: regurgitated pellets, egg stable isotope signatures, and egg fatty acid profiles. I examined spatial differences and temporal changes in these endpoints using data from north and south PNP as well as a nearby breeding colony at Agawa Rocks. Second, I looked at reproductive (clutch size, egg mass, egg volume, egg energy content, nest attentiveness, nest distribution) and physiological (stress hormone) endpoints to determine what impact diet may be having on Herring Gull populations. The key findings of my research are summarized below.

Pellet analysis showed greater proportions of anthropogenic food in the diets of Herring Gulls nesting in north PNP compared to the southern section of the park. Stable isotopes of carbon and nitrogen in eggs can provide information on food sources and trophic position, respectively. Here, egg $\delta^{13}C$ values were not useful in differentiating diets of gulls nesting in northern and southern PNP. However, egg $\delta^{15}N$ values were greater in south PNP than in the north. This indicated that Herring Gulls nesting in south PNP were feeding at a higher trophic level than in the north and this was consistent with the possibility that southern birds fed more on

higher trophic level aquatic prey, i.e. prey fish. Egg fatty acid analysis corroborated this idea in that gulls breeding in south PNP laid eggs with fatty acid profiles that indicated they were feeding to a greater extent on aquatic food sources than northern gulls. Taken together, the dietary endpoints described in Chapter 2 pointed to southern birds being more reliant on natural, primarily aquatic foods while northern nesting gulls incorporated more anthropogenic foods in their diets.

In Chapter 3, I examined reproductive and physiological endpoints associated with gulls nesting in north and south PNP. Clutch sizes were smaller in south PNP than in the north but other measures, i.e. egg mass, egg volume, egg energy content did not differ. Based upon evidence from remote cameras used to monitor nest attentiveness, gulls nesting in south PNP spent more time off their nests leading to a greater incidence of nest predation. My measure of physiological stress, i.e. corticosterone in egg contents and eggshell, did not reveal differences between south and north PNP. However, in south PNP, eggs from one-egg clutches contained higher corticosterone levels than those from two-egg clutches. Gulls laying one-egg clutches would have been severely constrained in resources available for egg production and egg corticosterone levels may have reflected the fact that females were working harder to find the necessary resources for egg formation. This could have led to their spending more time off their nests which was reflected in the nest attentiveness data. Reduced clutch size and increased nest predation in south PNP would have reduced reproductive success in that area and these factors could have contributed to the more severe southern population declines observed through time. In addition, my analysis of temporal changes in the distribution of Herring Gull nests in PNP revealed a shift in the distribution of nests from south PNP to north PNP sometime between 1989 and 1998/99. Factors contributing to this could have included poorer recruitment into the

southern population through reduced reproductive success and/or emigration of nesting birds from south PNP to north PNP in response to deteriorating environmental conditions required for successful nesting, e.g. declining food supplies. I investigated this latter possibility by putting the PNP results into a broader spatial and temporal context by looking at data from Agawa Rocks.

Temporal data from Agawa Rocks revealed a decline in egg $\delta^{15}N$ values during the 1989-1998 period, this coincided with the time when shifts in nest distributions occurred within PNP. Egg $\delta^{15}N$ values at PNP are now similar to the lower levels observed at Agawa Rocks. Egg fatty acid patterns also changed at Agawa Rocks during the 1989-1998 period with declines in omega 3/omega 6 fatty acid ratios being indicative of less aquatic food being incorporated into the diets of Herring Gulls. Egg energy content was also found to be lower at Agawa Rocks during the most recent period (1999-2013) compared to earlier periods (1978-88, 1989-1998). Recent estimates for egg energy content at Agawa Rocks are now similar to those in north and south PNP. These results indicated that a wide-scale change occurred in eastern Lake Superior sometime during the 1989-1998 period and this was reflected in various endpoints measured in gulls nesting in both PNP and at Agawa Rocks.

One possible explanation for the observed changes in Herring Gulls nesting at PNP and Agawa Rocks could be a reduction in the availability of aquatic food, e.g. declines in prey fish populations. I looked at this by examining trends in local prey fish abundance using data from the United States Geological Survey. Unfortunately, the prey fish abundance data only go back to 1989 making it difficult to compare prey fish trends with gull data, i.e. the fish data do not extend back to the period when greatest population/dietary changes were observed in Lake Superior Herring Gulls. Regardless, the existing prey fish data revealed that annual estimates of prey fish abundance varied greatly from year-to-year but in recent times prey fish abundance in

waters around south PNP and Agawa Rocks has been much lower than in the past. This decline in prey fish availability may, in part, be responsible for the spatial and temporal changes reported here in terms of Herring Gull diets and reproductive/physiological endpoints. It is likely that decreased aquatic food availability is negatively impacting Herring Gulls in PNP and, more broadly, across other parts of Lake Superior. Declines in prey fish availability may also be affecting other wildlife species as exemplified by the recent disappearance of breeding Double-crested Cormorants in PNP (see Chapter 2). Dietary differences in northern PNP gulls could have a negative impact on Herring Gull health. Eggs of northern PNP gulls contained lesser amounts of the important polyunsaturated omega 3 fatty acids: eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). These fatty acids are crucial in birds for neural development, vision, and immune system function (Twining *et al,* 2016b). Furthermore, the shift towards anthropogenic food may have negative impacts on Herring Gulls as the food may be of lower quality which can affect bird condition (Smith and Carlile, 1993; Annett and Pierotti, 1999). Anthropogenic food sources come with the added risk of ingestion of undigestible material, and possible increased exposure to chemical contaminants and pathogens. Plastics that are ingested by birds can lead to physiological problems such as blocking of the gastrointestinal tract which can hamper digestion of more nutritional foods (Azzerello and Van Vleet, 1987). It can also lead to direct mortality if ingested in sufficient quantities (Petry, da Silva Fonseca and Scherer, 2007). Furthermore, these undigestible synthetic materials may act as a medium for the transfer of pollutants, such as PCBs, to birds (Inigo Elias, 1987; Fry, 1995). Birds foraging in garbage dumps may also incur increased exposure to pathogens (Gamble *et al,* 2017).

Previous studies have shown that anthropogenic food subsidies can help to maintain bird populations (Duhem *et al,* 2008). This appears to be occurring in north PNP as gulls nesting in that region have access to anthropogenic food subsidies and their populations are declining at a slower rate than in south PNP. However, despite the fact that garbage subsidies may be providing birds with energy and nutrients they may also be hampering their ability to digest food, contaminating them with chemical toxins, and increasing their exposure to disease. In the long-term, all of these factors could have possible negative effects on bird fitness and ultimately, gull populations.

One avenue of future research is to further investigate the impact of anthropogenic food subsidies on Herring Gull health and populations. Anthropogenic food subsidies may be buoying the Herring Gull population in northern PNP but there may be negative side effects such as decreased food quality and increased exposure to disease and contaminants. These aspects could be investigated further. In addition, my work developing a method for the analysis of corticosterone in eggshell was sensitive enough to detect corticosterone at low pg/g concentrations. Corticosterone has been found to be stable in feathers (Bortolotti *et al,* 2009) but, to my knowledge, my study was the first to report it in eggshell. Having the ability to measure corticosterone in eggshell may open new research avenues such as measuring corticosterone in museum specimens or, possibly, in ancient eggshell samples. Through the analysis of such historical specimens it may be possible to determine how corticosterone levels from the past compare with recent samples providing a temporal perspective on how ecosystems may be changing.

The final goal of my research was to provide recommendations to the Parks Canada Agency regarding the use of Herring Gulls as indicators of ecological integrity in PNP. My

research revealed that Herring Gull populations in northern and southern PNP have different diets with southern birds being more reliant on natural foods. These dietary differences have likely been important in affecting reproductive endpoints, e.g. clutch size, nest attentiveness, which may be contributing to the more extreme population declines in the southern part of the park. In north PNP, gull populations are doing better as they exploit anthropogenic sources of food which may be acting to buffer population declines due to reductions in natural foods. Gaining a broader spatial and temporal perspective through the analysis of data from Agawa Rocks indicated that a general shift appeared to have occurred between 1989 and 1998/99 in terms of availability of aquatic food for Herring Gulls. Possible reasons for declines in aquatic food availability to gulls could have included: 1) a decrease in commercial fishing activities in the region that would have resulted in reduced fishery discards available for scavenging gulls (Figure 4.0) (http://www.glfc.org/great-lakes-databases.php) and/or 2) declines in prey fish availability that occurred across the eastern Lake Superior nesting locations examined here. Regardless of the mechanism, declines in aquatic food availability suggest that conditions suitable for sustaining Herring Gull populations have changed through time, i.e. presently there is a reduced carrying capacity for Herring Gulls in eastern Lake Superior compared to earlier times. The Parks Canada Agency evaluates the Herring Gull as an indicator of ecological integrity by comparing the current PNP nesting population size with that measured during the earliest censuses of the late 1970s. My results suggest that the 1970s population benchmark may not be a realistic one given the changes that have occurred across eastern Lake Superior (and possibly lake-wide). These broad-scale changes are outside the control of PNP management but they do influence the park ecosystem. Hence, information from PNP ecological integrity indicators must be interpreted in an ecosystem context that recognizes the dynamic nature of

ecological systems such as Lake Superior. In the particular case of using Herring Gulls as indicators of PNP ecological integrity, a more realistic approach might entail revising the benchmark against which current gull populations are assessed to reflect populations that are sustainable under current environmental conditions.

Figure 4.0: Annual commercial fishery harvest in Canadian waters of Lake Superior from 1977 to 2005 (round weight in thousands of kg). Source: Great Lakes Fishery Commission.

Here I suggest two potential options for the revision of the population benchmark: 1) use the average Herring Gull population after the shift in PNP nest distribution/Agawa Rocks diet indicators, i.e. post mid-1990s. This would provide a new baseline of approximately 350 breeding pairs in PNP. 2) adopt a threshold that reflects census data over the last three PNP nest surveys (2007/08, 2013, 2015). These data indicate a stabilization of the Herring Gull population over the last decade or so. This new baseline would be approximately 300 breeding pairs. Either of these baselines would likely provide a more realistic benchmark against which to assess the

health of Herring Gull populations in PNP given the current state of the Lake Superior ecosystem.

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Appendix

Table A1: Comparison of fatty acid concentrations (mg/g) in Herring Gull eggs from 2015 and 2016 for south PNP.

Variable	Mean 2015	Mean 2016	p	Std.Dev. 2015	Std.Dev. 2016
Myristic acid	1.17	1.57	0.005	0.35	0.33
Pentadecanoic acid	0.43	0.44	0.844	0.09	0.11
Palmitic acid	88.88	84.79	0.103	7.00	5.82
Palmitoleic acid	7.66	7.58	0.910	1.57	2.24
Heptadecanoic acid	2.06	2.43	0.079	0.46	0.60
Stearic acid	32.79	31.31	0.105	2.30	2.35
Elaidic acid	0.54	0.57	0.709	0.24	0.27
Oleic acid	149.78	144.68	0.305	13.27	12.46
Linoleic acid ‡	21.84	21.28	0.828	5.76	7.45
Arachidic acid*	0.24	0.12	0.000	0.08	0.03
γ-linolenic acid (GLA) ‡	0.39	0.37	0.773	0.17	0.20
Eicosenoic acid	1.00	1.07	0.377	0.23	0.18
α-linolenic acid (ALA) †	2.04	2.19	0.625	0.84	0.78
Cis-11,14-eicosadienoic acid ‡	0.54	0.64	0.274	0.27	0.23
Cis-8,11,14-eicosatrienoic acid (DGLA)‡	0.39	0.54	0.043	0.25	0.10
Cis-11,14,17 eicosatrienoic acid (ETE)†	0.45	0.29	0.007	0.11	0.17
Arachidonic acid (ARA) ‡	19.26	18.81	0.515	1.51	2.00
Eicosapentaenoic acid (EPA) †	1.50	2.04	0.140	0.87	1.01
Docosapentaenoic acid (DPA) †	2.05	2.31	0.397	0.74	0.82
Docosahexaenoic acid (DHA) †	12.61	14.24	0.075	2.53	2.10
Sum Omega 3	18.65	21.06	0.135	4.41	3.87
Sum Omega 6	42.41	41.83	0.851	6.75	9.00
Sum Omega 3/Sum Omega 6	0.46	0.54	0.285	0.17	0.21

† Omega 3 fatty acid

‡Omega 6 fatty acid

t-test used for all comparisons except where indicated by * - Mann-Whitney U Test

The Bonferroni-adjusted p-value for these tests is 0.05/102=0.0005. Only Arachidic acid is below that.

Table A2: Comparison of fatty acid percent composition in Herring Gull eggs from 2015 and 2016 for south PNP.

Variable	Mean 2015	Mean 2016	p	Std.Dev. 2015	Std.Dev. 2016
Myristic acid	0.34	0.46	0.003	0.10	0.11
Pentadecanoic acid	0.12	0.13	0.509	0.03	0.03
Palmitic acid	25.60	25.14	0.226	0.95	1.02
Palmitoleic acid	2.20	2.25	0.812	0.44	0.67
Heptadecanoic acid	0.60	0.72	0.029	0.11	0.15
Stearic acid	9.44	9.28	0.416	0.58	0.45
Elaidic acid	0.15	0.17	0.472	0.07	0.07
Oleic acid	43.41	42.84	0.390	1.75	1.72
Linoleic acid‡	6.59	6.25	0.623	1.44	2.06
Arachidic acid*	0.07	0.03	0.000	0.02	0.01
γ-linolenic acid (GLA) ‡	0.12	0.11	0.728	0.05	0.06
Eicosenoic acid	0.28	0.32	0.093	0.06	0.06
α -linolenic acid (ALA) †	0.57	0.65	0.358	0.23	0.24
Cis-11,14-eicosadienoic acid ‡*	0.15	0.19	0.088	0.07	0.07
Cis-8,11,14-eicosatrienoic acid (DGLA)‡	0.11	0.16	0.017	0.07	0.02
Cis-11,14,17 eicosatrienoic acid (ETE)†	0.13	0.09	0.022	0.03	0.05
Arachidonic acid (ARA) ‡	5.59	5.57	0.913	0.40	0.48
Eicosapentaenoic acid (EPA) †	0.39	0.62	0.045	0.23	0.32
Docosapentaenoic acid (DPA) †	0.56	0.69	0.146	0.19	0.27
Docosahexaenoic acid (DHA) †	3.56	4.22	0.016	0.80	0.55

† Omega 3 fatty acid

‡Omega 6 fatty acid

t-test used for all comparisons except where indicated by * - Mann-Whitney U Test

The Bonferroni-adjusted p-value for these tests is 0.05/102=0.0005. Only Arachidic acid is below that.

Table A3: Comparison of reproductive endpoints in Herring Gull eggs from 2015 and 2016 for southern PNP.

Variable	Mean 2015	Mean 2016	p	Std.Dev. 2015	Std.Dev. 2016
Delta 13Cvpdb	-22.56	-22.86	0.437	0.92	1.03
Delta 15Nair	9.88	9.66	0.193	0.31	0.51
Egg Volume (cm^3)	80.95	84.21	0.310	8.77	7.59
Egg Content Cort	5.19	5.49	0.783	3.00	2.69
Energy Density kJ/egg	536.00	606.42	0.001	60.88	38.93
Energy Density kJ/g	6.72	6.68	0.641	0.32	0.21
Egg Mass	79.76	90.91	0.001	9.00	6.51
Clutch Size°	1.69	1.40	0.781	0.75	0.51

M-L Chi Square test used instead of the t-test indicated by °

The Bonferroni-adjusted p-value for these tests is 0.05/102=0.0005. None of the p values are below 0.0005.

Table A4: Comparison of fatty acid concentrations (mg/g) in Herring Gull eggs from 2015 and 2016 for north PNP.

Variable	Mean 2015	Mean 2016	p	Std.Dev. 2015	Std.Dev. 2016
Myristic acid	0.93	1.30	0.001	0.32	0.19
Pentadecanoic acid*	0.38	0.34	0.590	0.09	0.05
Palmitic acid	88.92	83.91	0.040	6.78	5.94
Palmitoleic acid*	6.33	5.51	0.431	2.27	1.09
Heptadecanoic acid	1.98	2.24	0.062	0.36	0.40
Stearic acid	34.36	32.43	0.126	3.50	3.22
Elaidic acid	0.51	0.67	0.167	0.29	0.33
Oleic acid	152.96	153.80	0.877	12.72	16.57
Linoleic acid ‡	24.06	22.38	0.262	3.70	4.31
Arachidic acid*	0.19	0.12	0.002	0.07	0.03
γ-linolenic acid (GLA) ‡*	0.62	0.59	0.934	0.33	0.18
Eicosenoic acid	0.88	0.88	0.990	0.17	0.15
α-linolenic acid (ALA) †	1.50	1.39	0.636	0.68	0.49
Cis-11,14-eicosadienoic acid ‡	0.33	0.33	0.974	0.12	0.12
Cis-8,11,14-eicosatrienoic acid (DGLA)‡	0.37	0.68	0.000	0.16	0.14
Cis-11,14,17 eicosatrienoic acid (ETE)†	0.15	0.12	0.535	0.10	0.10
Arachidonic acid (ARA) ‡	19.99	20.71	0.400	2.27	2.38
Eicosapentaenoic acid (EPA) †*	0.68	0.79	0.983	0.32	0.56
Docosapentaenoic acid (DPA) †	1.69	1.53	0.278	0.46	0.29
Docosahexaenoic acid (DHA) †	9.95	11.97	0.034	2.63	2.33
Sum Omega 3	13.96	15.81	0.143	3.59	3.09
Sum Omega 6	45.37	44.72	0.739	5.42	5.12
Sum Omega 3/Sum Omega 6	0.32	0.36	0.227	0.10	0.10

† Omega 3 fatty acid

‡Omega 6 fatty acid

t-test used for all comparisons except where indicated by * - Mann-Whitney U Test

The Bonferroni-adjusted p-value for these tests is 0.05/102=0.0005. None of the p values are below 0.0005.

Table A5: Comparison of fatty acid percent composition in Herring Gull eggs from 2015 and 2016 for north PNP.

Variable	Mean 2015	Mean 2016	p	Std.Dev. 2015	Std.Dev. 2016
Myristic acid	0.27	0.38	0.001	0.10	0.06
Pentadecanoic acid	0.11	0.10	0.389	0.03	0.02
Palmitic acid	25.63	24.56	0.020	1.40	0.93
Palmitoleic acid*	1.83	1.62	0.507	0.67	0.31
Heptadecanoic acid	0.57	0.66	0.028	0.10	0.11
Stearic acid	9.91	9.49	0.192	0.98	0.76
Elaidic acid	0.15	0.20	0.121	0.08	0.10
Oleic acid	44.05	44.88	0.266	2.00	2.00
Linoleic acid ‡	6.95	6.57	0.414	1.14	1.34
Arachidic acid*	0.06	0.03	0.004	0.02	0.01
γ-linolenic acid (GLA) ‡*	0.18	0.17	0.836	0.09	0.05
Eicosenoic acid	0.26	0.26	0.834	0.05	0.05
α-linolenic acid (ALA) †	0.44	0.41	0.761	0.21	0.17
Cis-11,14-eicosadienoic acid ‡	0.10	0.10	0.806	0.04	0.04
Cis-8,11,14-eicosatrienoic acid (DGLA)‡	0.11	0.20	0.000	0.05	0.05
Cis-11,14,17 eicosatrienoic acid (ETE)†	0.04	0.04	0.656	0.03	0.03
Arachidonic acid (ARA) ‡	5.76	6.06	0.172	0.57	0.59
Eicosapentaenoic acid (EPA) †*	0.20	0.24	0.967	0.10	0.18
Docosapentaenoic acid (DPA) †	0.49	0.45	0.459	0.13	0.11
Docosahexaenoic acid (DHA) †	2.87	3.50	0.023	0.76	0.66

† Omega 3 fatty acid

‡Omega 6 fatty acid

t-test used for all comparisons except where indicated by * - Mann-Whitney U Test

The Bonferroni-adjusted p-value for these tests is 0.05/102=0.0005. None of the p values are below 0.0005.

Table A6: Comparison of reproductive endpoints in Herring Gull eggs from 2015 and 2016 for north PNP.

Variable	Mean 2015	Mean 2016	p	Std.Dev. 2015	Std.Dev. 2016
Delta 13Cvpdb	-22.48	-22.99	0.067	0.75	0.72
Delta 15Nair	9.09	9.10	0.929	0.57	0.48
Egg Volume (cm^3)	80.66	81.83	0.564	5.27	5.51
Egg Content Cort	8.00	5.11	0.091	5.48	3.33
Energy Density kJ/egg	545.67	586.00	0.002	31.47	31.40
Energy Density kJ/g	6.84	6.75	0.473	0.35	0.30
Egg Mass	79.95	86.95	0.003	5.67	6.08
Clutch Size°	2.47	2.47	1.000	0.52	0.52

M-L Chi Square test used instead of the t-test indicated by °

The Bonferroni-adjusted p-value for these tests is 0.05/102=0.0005. None of the p values are below 0.0005.

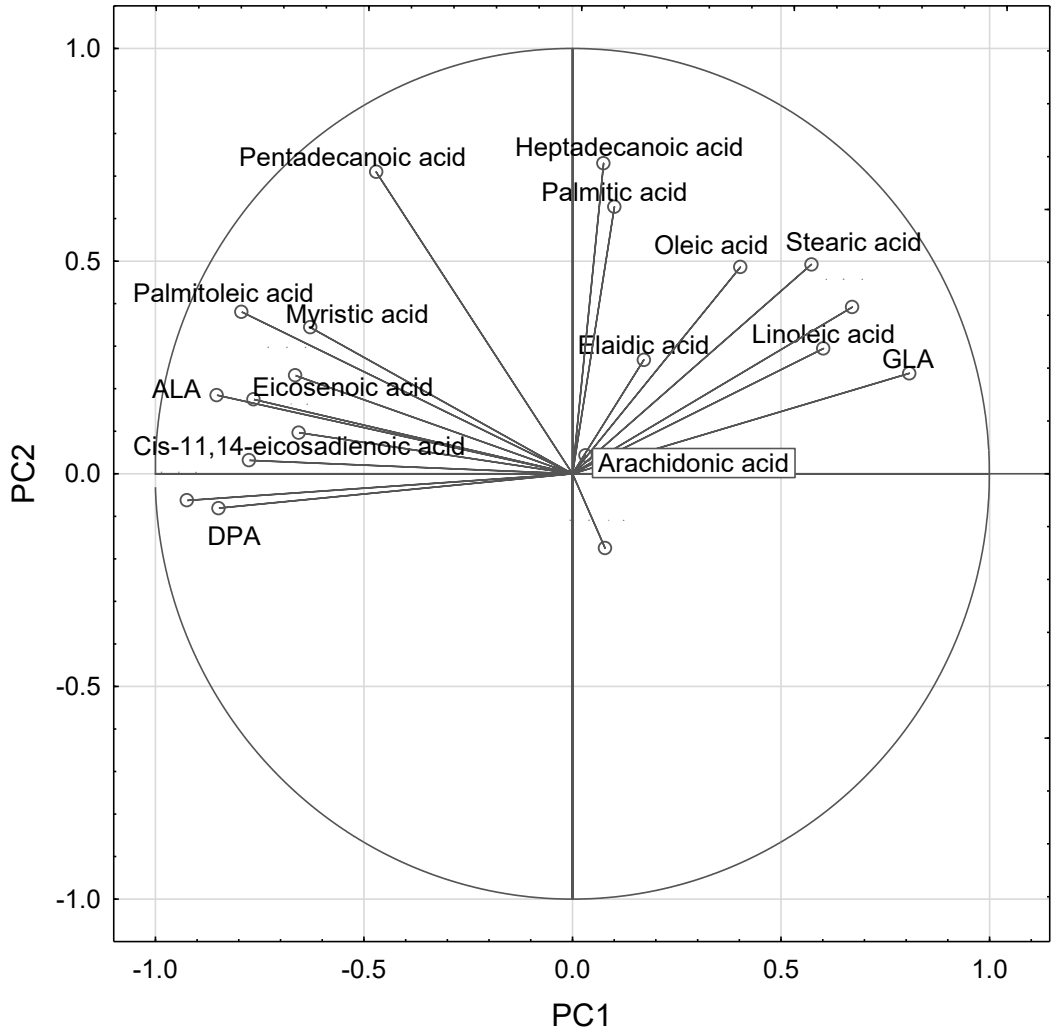

Figure A1: Variable loadings on principal component 1 (PC1) and PC2 from the principal components analysis of egg fatty acid concentration data. Data from 2015 and 2016 are included.

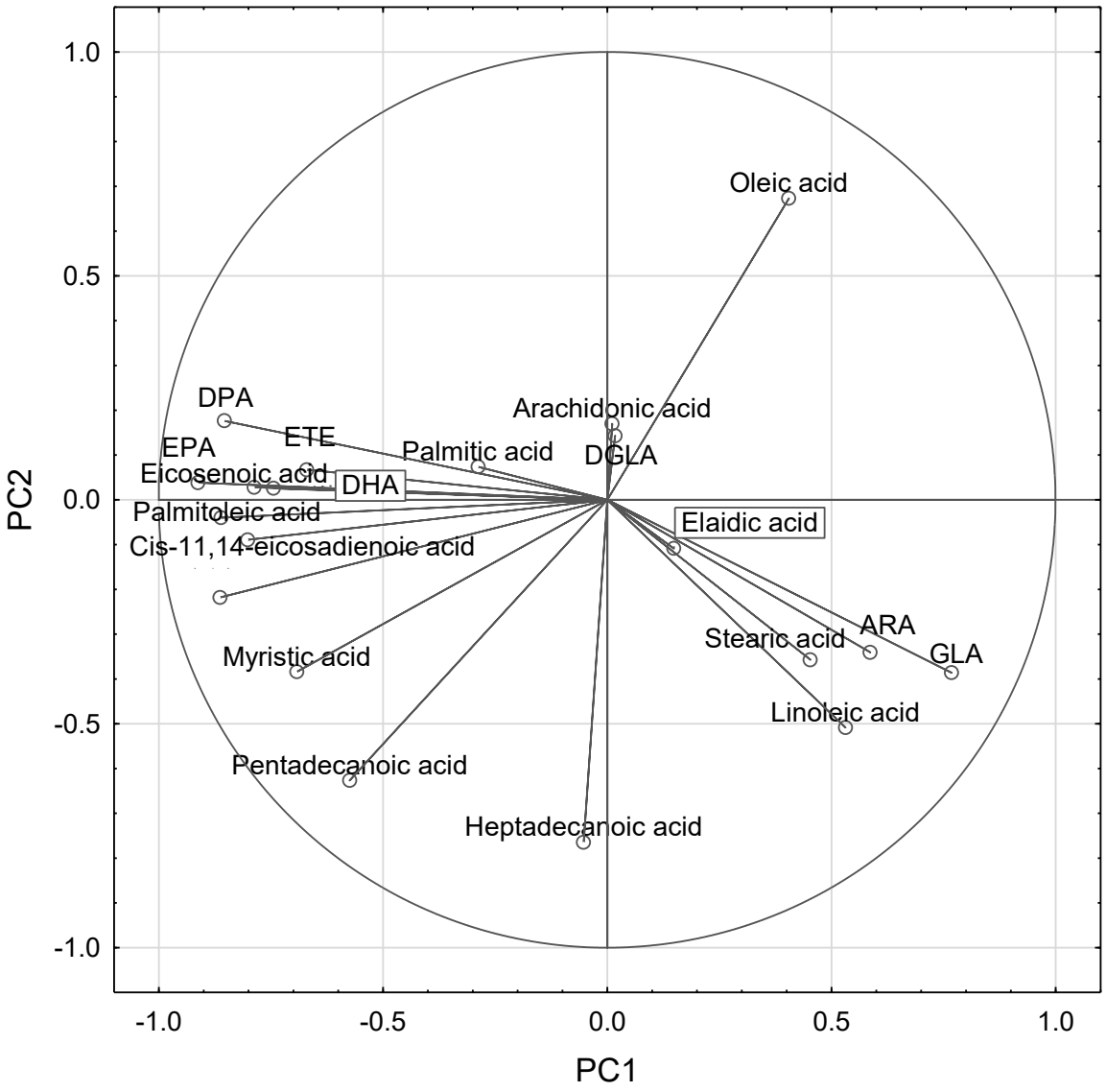

Figure A2: Variable loadings from the principal components analysis of egg fatty acid percent composition data. Data from 2015 and 2016 are included.