Seabirds of Newfoundland and Labrador: Using stable isotope techniques to investigate changing trophic position over ~120 years, and examining their influence on terrestrial coastal ecosystems

by

©Molly M. Morrissey

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ABSTRACT

Seabirds breeding around Newfoundland and Labrador (NL) are facing multiple destabilizing trends in recent decades, including major fisheries collapses and increasingly erratic temperature cycling in the north-west Atlantic Ocean. Evidence suggests that these seabirds are foraging on poorer quality prey, which could have consequences for terrestrial ecosystems near seabird colonies, as seabirds are one of the major nutrient biovectors in the world. In this thesis I employ stable isotope analysis (SIA) to investigate temporal and spatial questions related to NL seabird ecology.

In the first research chapter, I use stable isotopes of nitrogen ($\delta^{15}N$), as well as amino-acid specific stable isotopes of nitrogen ($\delta^{15}N_{AA}$), as a proxy for seabird trophic level. I measured $\delta^{15}N$ values in breast feathers of four common breeding NL seabirds (Atlantic puffin *Fratercula arctica*, Leach's storm-petrel *Hydrobates leucorhous*, common murre *Uria aalge*, and razorbill *Alca torda*) over a period of 120+ years (1899-2021). The results indicate that Atlantic puffins, razorbills, and common murres are feeding at lower trophic levels than they were historically, while Leach's storm-petrels' trophic position has remained steady.

In the second research chapter, I measured $\delta^{15}N$ values in zooplankton, phytoplankton, and sediments in freshwater ponds near a large seabird community at Cape St. Mary's Ecological Reserve (CSM), Newfoundland. Our results show that the pond nearest to the community had the highest $\delta^{15}N$ values in zooplankton, sediment, and phytoplankton, decreasing as pond distance increased. Overall, this thesis demonstrates that 1) NL seabirds are foraging at lower trophic levels than they were historically, and 2) seabird derived nutrient inputs are incorporated into freshwater food webs near colonies. This study provides data for both researchers and managers working in seabird conservation and considers broader terrestrial implications of declines in seabird populations.

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This project was conducted, analyzed, and written on the homelands of many Indigenous peoples and nations, in Canada and the United States. These lands are available to me as a result of unjust, violent colonial systems of power that are meant to primarily benefit non-Indigenous people like myself. In this land theft, we also see a deliberate erasing of knowledge, where one kind of way of knowing and living is favored over another. My scientific perspectives are influenced by the Eurocentric traditions I was trained in, from before elementary school through university and beyond. Western science is not wrong, but it is an incomplete view and devalues systems of Indigenous knowledge, to its detriment. I am continually fascinated and humbled by perspectives I am just beginning to understand.

The lands of Newfoundland and Labrador where I collected and analyzed data for two years are the homelands of many diverse populations of Indigenous people, past and present, including the Beothuk and Mi'kmaq on the Island of Ktaqmkuk (present day Newfoundland), and the Innu of Nitassinan, the Inuit of Nunatsiavut, and the Inuit of NunatuKavut (present day Labrador). I wrote my thesis in San Diego, California, which is the ancestral and present territory of the Kumeyaay people. I offer this land acknowledgment for myself and for all those reading, so that we may learn and teach with more thoughtfulness and acknowledgment of past and current harms.

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For my grandmothers

Mitzi & Marjorie

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LIST OF ABBREVIATIONS AND SYMBOLS

δ^{13}C – Delta carbon-13

δ^{15}N – Delta nitrogen-15

$\delta^{15}N_{AA}$ – Delta nitrogen-15 of amino acids

AIC – Akaike information criterion

AA – Amino acids

Ala – Alanine

Asx – Aspartic acid

CMN – Canadian Museum of Nature

CSM – Cape St. Mary's Ecological Reserve

CSIA-AA – Compound specific stable isotope analysis of amino acids

DCM – Dichloromethane

DOC – Dissolved organic carbon

EA – Elemental analysis

EDF – Estimated degrees of freedom

GAM – Generalized additive model

Glx – Glutamic acid

Gly – Glycine

Ile – Isoleucine

Leu – Leucine

Lys – Lysine

MUN – Memorial University of Newfoundland

NAO – North Atlantic oscillation

NL – Newfoundland and Labrador

Phe – Phenylalanine

Pro – Proline

ROM – Royal Ontario Museum

Ser – Serine

SIA – Stable isotope analysis

TDF – Trophic discrimination factors

TFAA – Trifluoroacetic anhydride

Thr – Threonine

TIC – Total inorganic carbon

TN – Total nitrogen

TP – Total phosphorus

Val – Valine

Chapter 1: General introduction and thesis overview

Seabirds are a diverse group of birds linked by their primary habitat and food source: the sea. They can be further classified by their specific marine habitat, including coastal, offshore, or pelagic (Harrison, 1983). Seabirds are found on every continent, and their global population is estimated at one billion (Otero et al., 2018). Yet, they are considered one of the most vulnerable groups of birds in Canada and worldwide, particularly at risk due to climate change, pollution, and unsustainable fishing practices (NABCI, 2019). The decline of seabirds brings with it the loss of the crucial ecosystem services, such as global nutrient cycling between sea and land (e.g., Doughty et al., 2016, Otero et al., 2018) and top-down trophic biological control of food webs (Signa et al., 2021).

1.1 Nutrient cycling and ecosystem engineering

Megafauna are crucial conduits in the global cycling of nutrients. Animal digestion allows nutrients locked in plant material to be quickly transformed into more easily used forms. These otherwise sessile plant nutrients are transported and deposited mainly via animal defecation (Doughty et al., 2016). Many traits are recognized to contribute to the effectiveness of a nutrient transporter, including migration patterns, body size and mass of the animal, and sociability of the species (Tavares et al., 2019). With regards to the movement of nutrients from the sea to land, seabirds are considered the main transporter bridging these two environments (Doughty et al., 2016; Anderson & Polis, 1999). They can be thought of as *mobile links*, defined as an animal able to connect two or more different patches with a barrier between them (Lundberg & Moberg, 2003). Worldwide, yearly estimations of seabird nitrogen (N) and phosphorus (P) excretions at breeding colonies are 591 million kg and 99 million kg, respectively. These values are estimated to be over six times higher if breeding and non-breeding birds, as well as breeding and non-breeding seasons, are considered (3.8 billion kg N y-1 and 631 million kg P y-1) (Otero et al., 2018). Commercial fishing activities transfer 3.7 billion kg N and 320 million kg P from sea to land every year, a number comparable to seabird nutrient transfer (Otero et al., 2018).

Seabirds are often an overlooked component of an ecosystem due to their migratory behavior during the non-breeding season, as well as their consistent aggregation at coastal colonies (Signa et al., 2021). However, their influence as ecosystem engineers at local scales cannot be overstated. Seabird mediated nutrient transfer has a significant bottom-up influence at terrestrial seabird colonies, starting with the soil. Soils, such as those in some penguin colonies in Antarctica, are so heavily infused with seabird nutritional additions that they are termed *ornithogenic soils* (Syroechkovsky, 1959). Additionally, increases of seabird refuse at seabird colonies correlates with an increase in the size and productivity of faunal communities (Adame et al., 2015; Duda et al., 2020). Conversely, the reduction of seabirds leads to decreased fertilization of soils and thus decreased vegetative growth, in some cases transforming a biome completely (Croll, 2005). The effects of seabirds on plant vegetation are highly variable; the influence of ornithogenic nutrients on vegetation growth and species richness can have both positive and negative impacts depending on factors like climatic region or season. For example, if a region is having a particularly dry year, bird guano can have a toxifying effect on soil and result in lower plant cover. The same region in a wet year could have plant cover increase by an order of magnitude in the presence of concentrated bird guano (Sánchez-Piñero & Polis, 2000).

Existing research is not clear on how nutrient enhancement affects zooplankton in freshwater habitats. In marine ecosystems, δ^{15}N values and trophic positions of zooplankton fluctuate with nutrient upwelling during El Niño and La Niña states, indicating that the zooplankton incorporate nutrient enhancements into their diets (Décima et al., 2013). *Daphnia* ephippia in freshwater ponds with high seabird influences have enriched $\delta^{15}N$ values, signaling seabird nutrient enhancements are being assimilated (Griffiths et al., 2010). However, other studies show that positive effects on zooplankton populations in seabird influenced ponds may result from the physical or chemical properties of the pond or to changes in the pond's benthic community, unrelated to nutrient assimilation within zooplankton (Signa et al., 2015; Van Geest et al., 2007).

1.2 Seabirds as marine health indicators

Seabirds are sensitive to changes in marine environments, thus considered robust biological indicators of environmental conditions (Piatt et al., 2007). Advantages of using seabirds over other biota as environmental indicators are their long lifespans, positions as top predator role in a food web (bio-magnification of nutrients), philopatry to the same colony year after year, and their large population sizes and wide dispersal (De La Peña-Lastra, 2020; Duda et al., 2020).

Most seabirds feed exclusively at sea, thus they are especially suitable marine indicators (Barrett et al., 2007; Davoren & Montevecchi, 2003; Montevecchi, 2007). Shifts in physical features of the ocean bring about changes in marine food webs that ultimately affect seabirds. For example, warming surface waters may cause fish to migrate deeper into cooler waters, disrupting the hunting of surface-feeding seabirds (Boyd et al., 2006). For specialist hunters, the declining availability of primary prey species results in parent birds exerting more energy and time foraging to provide for chicks, with potential deleterious consequences to chick growth and survival (Montevecchi et al., 2019). Generalist seabirds with a more flexible hunting strategy indicate changes in food web dynamics by shifting prey landings (Montevecchi, 2007). The population dynamics of seabirds are influenced by environmental changes, anthropogenic pressures, and changes in marine food web structure; thus, it is challenging to quantify the relative importance of multiple influencing factors on population fluctuations, although studies are attempting to parse through some of these factors (Regular et al., 2010). Tracking changes to seabird populations and dietary choices can inform, and be informed by, knowledge of marine food webs (Carscadden, 2002).

1.3 Stable isotope analysis

Stable isotope analysis (SIA) is a cost-effective tool used to investigate a range of ecological questions (Michener & Lajtha, 2007). SIA is frequently used to study diet (Jenkins et al., 2020; Gómez et al., 2018; Hedd & Montevecchi, 2006); animal migration (Vokhshoori et al., 2019; Polito et al., 2017; Wiley at al., 2013; Hedd et al., 2010), and climate shifts (Brault et al., 2018; Décima et al., 2013). Often, these studies inform multiple questions due to the interrelated nature of the subjects (i.e., diet informs migration, climate influences migration and diet, etc.). In this thesis, we used SIA to investigate temporal and spatial questions related to seabird ecology: 1) are seabird diets shifting in response to major food web disruptions (temporal or spatial)? and 2) how are seabird nutrient subsidies impacting freshwater ponds near colonies (spatial)?

For our study, we measure carbon and nitrogen isotopes in bulk tissues, and we measure nitrogen isotopes in amino acids for some samples. SIA is referred to as "bulk SIA" when we measure the weighted isotope average of all the components within a sample tissue. The ratios of ^{13}C to ^{12}C isotopes ($\delta^{13}C$) vary among primary producers. This variability persists into higher trophic levels, making carbon isotopes an indicator of food web origin (Whiteman et al., 2019). Nitrogen is frequently used for bulk stable analysis via $^{15}N/^{14}N$ ($\delta^{15}N$). Nitrogen isotopes undergo fractionation as they are processed by a consumer, therefore causing a difference in $\delta^{15}N$ values between producer and consumer. The lighter ^{14}N isotope is preferentially favored for protein transamination and deamination (Gaebler et al., 1966), thus is excreted more frequently, leading to the buildup of ^{15}N in tissues (Steele & Daniel, 1978). Each trophic level subsequently has higher $\delta^{15}N$ values than the preceding trophic level. These differences are known as trophic discrimination factors (TDF) (Minagawa & Wada, 1984; McClelland & Montoya, 2002). Carnivores typically have higher $\delta^{15}N$ values than herbivores by a factor of ~3.4 ‰ (Minawaga & Wada, 1984); however, 3.4‰ is an average value of dozens of organisms across taxa and trophic levels from a single 1984 study by Minawaga and Wada (range 1.3-5.3‰, mean 3.4‰ ± 1.1‰). A more recent review by Becker et al. (2007) of over a dozen seabird diet studies supports Minagawa and Wada (1984), finding a mean $\delta^{15}N$ increase of 3.6‰ per trophic level (range 3.0-4.6‰). Still, there is a great need for species-diet specific TDFs to better interpret isotopic diet studies (Bearhop et al., 2002; Becker et al., 2007; Cherel et al., 2014).

Stable isotopes are calculated with the following formula:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 1000$$

where X is the isotope of interest (e.g. $\delta^{13}C$) and R is the ratio of the isotope of interest to the natural form of the element (e.g. $^{13}C/^{12}C$). R of the sample is compared to R of the reference material (Vienna PeeDee Belmnite fossil, or VPDB, for carbon, and atmospheric N_2 for nitrogen). Isotope values are reported as parts-per-thousand, or per mille (‰).

Researchers are increasingly using compound-specific stable isotope analysis (CSIA) to answer questions more precisely about the dietary choices of an organism, and ecological changes at the base of a food web. This technique measures $\delta^{13}C$ and $\delta^{15}N$ values in single compounds, frequently amino acids (CSIA-AA). $\delta^{13}C_{AA}$ values in the essential amino acids are diagnostic of carbon origin and pass from producer to consumer with very little carbon fractionation, thus can be used to trace the origin of carbon in sample tissues (Larsen et al., 2013). Individual amino acids differ to the degree that their $\delta^{15}N$ values change as they move up trophic levels. (McClelland & Montoya, 2002). Amino acids are labeled either "source" or "trophic". Source amino acids are those with low nitrogen fractionation as they move up trophic levels relative to trophic amino acids, which record a larger increase of $\delta^{15}N$ values as they move up trophic levels. Phenylalanine (Phe) has been identified as an important diagnostic source amino acid, and glutamic acid (Glx) an important diagnostic trophic amino acid. (McClelland & Montoya, 2002; McMahon et al., 2019; O'Connell, 2017). It should be mentioned that glutamine (Gln) is converted to glutamic acid (Glu) during acid hydrolysis, resulting in a combined measurement of glu + gln, noted as "Glx". Similarly, asparagine (Asn) converts to aspartic acid (Asp) and is noted as "Asx".

1.4 Study species and area

My first research chapter explores stable isotope changes over time of four common Newfoundland and Labrador (NL) breeding seabirds: Atlantic puffin (*Fratercula arctica*, hereafter "puffins"), common murre (*Uria aalge,* hereafter "murres"), razorbill (*Alca torda*), all members of the Alcidae family (auks); and Leach's storm-petrel (*Hydrobates leucorhous*, formerly *Oceanodroma leucorhoa*, hereafter "petrels"), family Hydrobatidae. Breast feathers were sampled from museum collection birds for analysis. I collected breast feathers from adults captured at Newfoundland and Labrador breeding colonies during the breeding season from

1899-2021 (except for three murres collected outside of the breeding season) (Table 2.1). Breast feathers are mostly grown at-sea during the winter for puffins (Harris & Yule, 1977) and murres (Harris & Wanless, 1990). Fewer data exist for razorbills, but their molting strategy is thought to follow a pattern similar to common murres (Harris & Wanless, 1990). Unlike the three auks in this study, Leach's storm-petrels do not have a breeding, or *alternative*, plumage, thus follow a less complex molting strategy. Petrels complete their molt at sea before the breeding season (Cramp & Simmons, 1977).

The auks are all strong swimmers and employ pursuit-diving foraging strategies. Puffins, the smallest of the auks in this study (~300-550 g), generally feed on small to midsize schooling fish about 5-15 cm in length, diving down to 68 m to catch prey (Burger & Simpson, 1986). They are considered generalist feeders, although geographically distinct populations of puffins tend to favor a single species, with Newfoundland puffins fishing predominantly for capelin (Lowther et al., 2020). Murres are the largest (up to 1.125 kg) and deepest diving (often to 70 m to 180 m) of the auks in our study (Piatt & Nettleship, 1985), specializing in capelin (Montevecchi et al., 2019). Razorbills are ~500-900 g and dive up to 100 m, although most dives are typically much shallower (Lavers et al., 2020). Razorbill summer diets are predominantly sandlance (Pratt et al., 2017). Leach's storm-petrels are the smallest of the four study species (~ 50 g) and the smallest breeding bird in eastern North America (Hedd & Montevecchi, 2006). Unlike the auks, Leach's storm-petrels forage at the ocean surface and favor smaller fish, particularly myctophids (Hedd et al., 2009).

One issue when studying seabird diet is that typical diet studies are restricted to summer months (breeding season) when nesting seabirds are accessible to researchers. Thus, many studies are observing adult birds primarily focused on provisioning food for chicks, which is not necessarily what adults themselves are eating (Hedd et al., 2010; Baillie & Jones, 2004). Even less is known about adult winter diets, when many seabirds travel to geographically distant locations, inaccessible to researchers (Gómez et al., 2018). Advances in stable isotope techniques have allowed researchers to shed light on adult feeding patterns for some seabirds. My study aims to add to the dearth of seabird winter diet knowledge by analyzing the stable isotopes of seabird feathers grown on winter feeding areas.

In the second research chapter, I examine the effect of seabird nutrients on the biogeochemical dynamics in freshwater ponds where they are deposited. I collected zooplankton, water, sediment, and phytoplankton from ponds near a large seabird community ("Bird Rock") at Cape St. Mary's Ecological Reserve (CSM). CSM is located on the southwestern the tip of Avalon Peninsula on the island of Newfoundland. CSM is part of the eastern hyper-oceanic barrens, a type of ecoregion characterized by broad swaths of moss, scattered stunted fir trees (tuckamore), and extensive bogs and peaty soils (PAA, 2008). Tens of thousands of seabirds breed at CSM, providing an excellent opportunity to test the influence of seabird nutrient deposits on the numerous ponds in the reserve.

1.5 Northwest Atlantic Ocean

Both research chapters are situated in the north-west Atlantic Ocean, an area of significant ecological and economic importance (Kurlansky, 1997). Millions of tons of fish are commercially extracted from these productive ocean waters each year and millions of marine animals rely on the north-west Atlantic for their own survival (Kurlansky, 1997; Tam & Bundy, 2019).

Several currents influence the physical and geochemical properties of the northwest Atlantic along NL. The West Greenland Current brings water from eastern Greenland up to Baffin Bay, where the water mixes with colder Arctic waters. The water circulates and moves down the coast of Labrador to Newfoundland in the Labrador Current, bringing colder, nutrient-rich seawater southward (Drinkwater, 1996). These currents are influenced by the North Atlantic oscillation (NAO), a weather phenomenon describing fluctuating air pressures and westerlies movement across the North Atlantic (Hurrell, 1995). An NAO index is determined by measuring the difference between two areas in the northwest Atlantic with characteristically high and low sea-level air pressure: the Azores High and Icelandic Low, respectively (Drinkwater, 1996). Years with a high positive NAO index are marked by strong westerly winds and colder air and water temperatures in the north-west Atlantic, whereas weaker westerlies lessen the NAO index and are associated with warmer conditions (Drinkwater, 1996; Hurrell, 1995).

The north-west Atlantic in the 1950s and 1960s was characterized by a warming phase that peaked in the mid-1960s. This was followed by a significant cooling period, beginning in the 1970s and lasting through the early 1990s (Colbourne, 2004). The NAO index in the winters of 1983, 1989, and 1990 were the highest it had been since 1864 (Hurrell, 1995), with water temperatures off the NL bottom shelf reaching record lows in 1991 (Colbourne, 2004). The cold intermediate layer, an important indicator of marine conditions described as a layer of subzero water between the warmer surface and benthic layers, expanded from the 1970s through the late 1990s (Colbourne, 2004). This major oceanographic shift, coupled with extreme fishing pressures, has resulted in a radically different northwest Atlantic marine food web (Carscadden, 2002; Frank et al., 2005; Myers et al., 1997), which has persisted (Buren et al., 2014, 2019). It is estimated that biomass in the North Atlantic decreased by 90% in the twentieth century (Trites et al., 2006). The changes to the marine food web starting in the late 1980s off the coast of Newfoundland is described as a regime shift, a term meaning a significant, abrupt change in the abundance and species composition of biota within a habitat (Beaugrand, 2004).

The food web structure in the north-west Atlantic is best described as "wasp-waist", defined by an intermediate trophic level species controlling predator numbers through bottom-up interactions and prey numbers through top-down interactions (Buren et al., 2014). Capelin (*Mallotus villosus*), an ecologically and commercially important foraging fish, fill this role in the north-west Atlantic. At an intermediate trophic level, capelin transfer nutrients from lower-level primary producers and zooplankton to upper-level predators, including seabirds (Buren et al., 2014). Capelin spawn all along the Newfoundland coast in the summer, particularly in north-east bays and are a key prey to predatory fish, marine mammals, and seabirds (Mowbray, 2002).

Capelin experienced an abrupt population collapse in the early 1990s that coincided with the cooling waters (Buren et al., 2014). The other two Atlantic capelin populations, Iceland and Barents Sea, were able to recover swiftly and re-enter boom-bust cycles typical of capelin; however, the Newfoundland population has remained at a low abundance (Buren et al., 2019; Murphy et al., 2018). The collapse was marked by changes in diel vertical migration, with capelin aggregating in deeper waters after 1991 (Mowbray, 2002). Capelin spawning times were delayed by several weeks throughout the 1990s (Carscadden et al., 1997), and despite warming waters capelin are still spawning consistently later than they were pre-1991 (Murphy et al., 2018). Overall body sizes have similarly decreased since the collapse (Carscadden et al., 1997). Many seabirds depend on capelin for the bulk of their diet, and the decrease in capelin over the past three decades has caused some birds to increase foraging effort or pursue new prey, or both (Montevecchi et al., 2019; Rowe et al., 2000).

1.6 Thesis objectives

In this thesis I explore two questions related to NL breeding seabirds and their relationship to their environment. In the first research chapter (Chapter 2) my objective is to analyze stable isotope values of feathers sampled from Atlantic puffins, common murres, Leach's storm-petrels, and razorbills collected from 1899-2021. I measured $\delta^{13}C$ and $\delta^{15}N$ values from bulk feathers and $\delta^{15}N$ values of feather amino acids to examine changes in seabird trophic position and regional food web dynamics. We predict that Atlantic puffins and murres are foraging at lower trophic levels than they were historically because of less capelin availability. This change will be reflected in decreasing $\delta^{15}N$ values over time. Razorbills are less reliant on capelin, thus we expect to see smaller changes in $\delta^{15}N$ values. Leach's storm petrels employ a different foraging strategy than the auk species and forage in a different location in the winter. We expect no changes in Leach's storm petrel trophic position.

In the second research chapter (Chapter 3) I examine the spatial impact of seabird nutrient subsidies in 12 ponds near Cape St. Mary's Ecological Reserve (CSM). The ponds are situated along a transect moving north-east from the colony; the closest pond is 226 meters from the colony, and the furthest pond is 6.6 km from the colony. I utilize the bulk stable isotope content of several biotic and abiotic components of the ponds, including zooplankton, algae, and sediments, as well as measuring chemical analytes in each pond to study seabird nutrient subsidies in freshwater ponds at CSM. I predict 1) chemical analytes associated with seabirds, such as total nitrogen and total phosphorus, will be highest in the pond closest to the seabird colony and coast, with values tapering off with distance, 2) algae and sediment samples will have higher $\delta^{15}N$ values nearer to the colony and coast, 3) zooplankton are assimilating this enriched

$\delta^{15}N$ into their diets, which will be reflected in elevated $\delta^{15}N$ values nearer to the colony that decrease with distance, and 4) $\delta^{13}C$ values will be higher in algae, sediment and zooplankton nearer to the colony and coast than those further away.

Overall, this thesis aims to investigate changing trophic dynamics of common NL seabirds and examine nutrient dispersal at a NL seabird colony. The declines of seabird populations are well documented (NABCI, 2019), but what is less known is how those declines affect the chemical and biological characteristics of terrestrial seabird colonies. New advances in stable isotope techniques allow us to indirectly investigate these questions, by sampling seabird feathers over 120 years to analyze changes in diet related to known changes in fish populations, and by sampling algae, zooplankton and sediment at a seabird colony to explore the impact of seabird nutrient loadings. This study expands on prior NL seabird feather isotope analyses (Burke et al., 2014; Fairhurst et al., 2015) by including $\delta^{15}N_{AA}$ values of puffins and Leach's storm-petrels, and it is also the first investigation of seabird nutrient subsidies at Cape St. Mary's Ecological Reserve. Stable isotope analysis is a useful approach to answer questions about seabirds' roles in the Northwest Atlantic ecosystem, while also informing long-term trends in marine food webs.

1.7 Co-authorship statement

Chapter 2 and Chapter 3 of this thesis were coauthored with my supervisor, Kathryn Hargan. I lead the study design, data collection, data analysis, and writing for all chapters in this thesis. Kathryn Hargan provided significant guidance and funding. Chapter 2 was also co-authored by Alex Bond, April Hedd, and Sabina Wilhelm. Alex Bond provided feather data and gave feedback on data analysis, and April Hedd provided feather data. Sabina Wilhelm provided funding for $\delta^{15}N_{AA}$ analysis.

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Chapter 2: Trophic level decreases in Newfoundland and Labrador seabirds over 120 years indicated by bulk and compound-specific $\delta^{15}N$ analysis of feathers

2.1 Abstract

The seabirds breeding on Newfoundland and Labrador (NL) are facing multiple destabilizing forces in recent decades, including major fisheries collapses and increasingly erratic temperature cycling in the north-west Atlantic Ocean. This has resulted in a changing food web with smaller populations of top trophic level fishes and increasing biomass of smaller fish and invertebrates. In this study, we ask if and how trophic position has changed in four common NL seabirds (Atlantic puffin *Fratercula arctica*, Leach's storm-petrel *Hydrobates leucorhous*, common murre *Uria aalge*, and razorbill *Alca torda* from 1899 to 2021. We use stable isotopes analysis (SIA) of nitrogen ($\delta^{15}N$) to explore trophic changes. Compound-specific stable isotope analysis of amino acids (CSIA-AA) of puffin and petrel feather tissues indicate puffin trophic position has been decreasing since the 1940s-1960s while petrel trophic position has remained steady. Bulk $\delta^{15}N$ values for the murre and razorbill samples decrease over time, indicating decreasing trophic positions. Puffins, murres, and razorbills forage in the northwest Atlantic in the winter, whereas petrels migrate south to equatorial Atlantic. Our results indicate that puffins, murres, and razorbills are feeding on lower trophic level prey than they were historically in response to changing prey availability. Seabirds spending the winter in the northwest Atlantic appear to be adjusting to marine food web changes, however continued research is needed to examine if foraging at lower trophic levels is resulting in reduced fitness.

2.2 Introduction

The effects of anthropogenic pressures and an increasingly erratic climate have caused major disruptions to marine food webs at every corner of the globe, ultimately affecting the population dynamics of organisms within those food webs. To understand the drivers of this population disruption, we need to have knowledge of how a species behaves within its food web, including foraging habits (Rayner et al., 2021). Furthermore, having a knowledge of past food web dynamics can help predict future population trends. Seabirds are particularly sensitive to changes in oceanic conditions as some of the most vulnerable groups of birds (Dias et al., 2019). It is imperative to have a clear understanding of how they respond to environmental changes, especially recent anthropogenic disruptions.

Unfortunately, there are few long-term studies of seabird populations and behavior that capture changes stemming from the most disruptive of human activities, like industrial fishing. Complicating matters is that researchers only have access to seabirds during the breeding season; it can be difficult to study seabirds that spend much of the non-breeding season (winter) far offshore (Cherel et al., 2014). Stable isotope analyses (SIA) of seabird tissues stored in museums are increasingly being used to retrospectively study their dietary habits throughout history (Blight et al., 2015; Fairhurst et al., 2015; McMahon et al., 2019; Rayner et al., 2021). These types of analyses can serve as a proxy of environmental changes both spatially (Maynard & Davoren, 2020; Polito et al., 2017) and temporally (Montevecchi et al., 2019; Baillie & Jones, 2004). Modern surveys of food web changes can be bolstered by tracing nutrient movements in feathers or other preserved tissues, providing a historic food web baseline (Lotze & Worm, 2009).

The food webs in the northwest Atlantic Ocean surrounding Newfoundland and Labrador (NL) have shifted dramatically in the last century, due in part to increasing fishing pressure that eventually led to the official collapse of the millennia-old and once plentiful Atlantic cod (*Gadus morhua*) fishery in the early 1990s (Kurlansky, 1997; Myers et al., 1997). Another keystone species, capelin (*Mallotus villosus*), suffered a population collapse in the 1990s (Buren et al., 2014). Acoustic surveys from 1988-1990 record capelin abundance between 300 to over 600 million tonnes (Mt) to less than 200,000 tonnes from 1992 to the mid-2000s (DFO, 2018). There is a dearth of capelin survey data prior to the mid-1980s, however fisheries landing data recorded the peak off-shore capelin catch between 250,000 tonnes (DFO, 2018) to over 350,000 tonnes (Rose, 2003). Capelin are crucially important in the "wasp-waist" food web structure of the north-west Atlantic Ocean, described by an abundant intermediate taxon (capelin) exerting bottom-up and top-down control over the rest of the food web (Buren et al., 2014). The capelin collapse was preceded by an unusual cold-water event beginning in the late 1970s and lasting until the early 1990s, with the lowest sea-surface temperatures on record made in 1991 (Colbourne, 2004; Montevecchi & Myers, 1997), which is believed to have contributed to the capelin collapse (Carscadden et al., 1997). During this cold weather event there was an expansion of the cold intermediate layer beginning in the 1970s through the late 1990s; the cold intermediate layer is an important indicator of marine conditions described as a layer of subzero water between the warmer surface and benthic layers (Colbourne, 2004). Overall, it is estimated that biomass in the North Atlantic decreased by 90% in the 20th century (Trites et al., 2006).

There are indications that NL seabirds are being affected by these oceanographic shifts in the northwest Atlantic. Chick sizes of common murre *Uria aalge* have steadily decreased over the past three decades, correlating with reductions in the quality and availability of capelin

(Davoren & Montevecchi, 2003; Montevecchi et al., 2019). Winter dietary data for murres have historically come from stomach contents of murres killed in the annual murre hunt (both common murres and thick-billed murres *Uria lomvia*). In the 1950s, wintering common murres were feeding mostly on capelin and other fish (Tuck, 1961), which was again confirmed in the mid-1980s (Elliot et al., 1990). In the mid-1990s, a survey of 237 thick-billed and common murres recorded a higher percentage of stomachs containing crustaceans than fish, indicating murres may be pursuing more crustaceans than fish due to changes in fish availability (Rowe et al., 2000). As the least numerous seabird species in eastern Canada (Chapdelaine et al., 2001), less is known about razorbill *Alca torda* diet than other species. Surveys conducted both in the 1950s (Tuck, 1961) and 1990s (Huettmann et al., 2005) record wintering razorbills feeding mostly on krill and some fish, with little dietary preference changes over time. However, like murres, razorbill chick growth is reduced when capelin biomass is reduced (Lescure et al., 2023).Atlantic puffins *Fratercula arctica* chick growth is delayed when the parents do not deliver as much capelin (Baillie & Jones, 2004).

Puffins are considered generalist feeders, although geographically distinct populations of puffins tend to favor a single species, with NL puffins fishing predominantly for capelin (Gaston & Jones, 1998). The dramatic decrease in capelin populations after the 1990s may have resulted in puffins foraging for alternative prey. Additionally, the Leach's storm-petrel *Hydrobates leucorhoa* colony on Baccalieu Island, Newfoundland, home to over 50% of the world's population, has seen a population decline of approximately 40% since 1980 (Duda et al., 2020). It is unclear how much this population decline is due to oceanographic shifts in the northwest Atlantic. Leach's storm petrels travel down the Atlantic coast to South America or across the

Atlantic to the east coast of Africa in the winter (Cramp & Simmons, 1977; Harrison, 1983; Hedd & Montevecchi, 2006; Pollet et al., 2014).

In Newfoundland, stable isotope analyses have elucidated prey preferences between species occupying a similar ecological niche (Jenkins & Davoren, 2021) or within the same species at different colonies (Maynard & Davoren, 2020). Few studies have used stable isotope analysis to look at long-term changes in NL seabird trophic positions, notably before and after the major fishery collapses. Fairhurst et al. (2015) looked at the relationship between $\delta^{15}N$, $\delta^{13}C$ and corticosterone from feathers from Atlantic Canada Leach's storm-petrels over a 153-year period and found no changes in $\delta^{15}N$ values and corticosterone, and an increase in $\delta^{13}C$ values. Our present study expands on Fairhurst et al. (2015) by analyzing feather $\delta^{15}N$ and $\delta^{13}C$ values from Atlantic puffins, razorbills, and common murres in a similar time period.

The first diet studies of birds involved simple observations of feeding and provisioning events, while later studies used more invasive (and lethal) methods such as forced regurgitation and whole stomach sampling from sacrificed birds (reviewed in Hoenig, 2022). Stable isotope analysis (SIA), a well-established tool with many applications in ecological studies, is a robust alternative to invasive methods of dietary analysis (Boecklen et al., 2011; Michener & Lajtha, 2007; Whiteman et al., 2019). SIA of avian tissues (e.g., blood, feathers) serves as a proxy for trophic position, as organisms incorporate isotopes from prey into their own tissues, with some predictable isotopic fractionation from prey to consumer (Minagawa & Wada, 1984). Carbon and nitrogen are frequently used elements for SIA in ecological studies, measuring the ratios of the heavier isotope to the lighter one (^{12}C to ^{13}C, and ^{14}N to ^{15}N), and denoted by delta δ ($\delta^{13}C$ and $\delta^{15}N$). Broadly speaking, carbon analysis is used to determine and differentiate carbon sources in a food web (Harding et al., 2004; Larsen et al., 2013; Whiteman et al., 2019; Wood & Pietri, 2015), and nitrogen analyses provides information about trophic positions within a food web (Harding et al., 2004; O'Connell, 2017; Steele & Daniel, 1978; Vokhshoori et al., 2019; Whiteman et al., 2019; Zanden & Rasmussen, 2001).

A caveat to using "bulk" SIA of nitrogen, which measures the weighted isotope average of all the components within a sample tissue, is that we cannot know if the isotopic value changes over time are the result of changes in baseline $\delta^{15}N$ or changes in diet, or both. Researchers are increasingly using compound-specific stable isotope analysis of amino acids (CSIA-AA) to disentangle these two sometimes conflicting factors. Nitrogen isotopic signatures in amino acids ($\delta^{15}N_{AA}$) differ in how much their $\delta^{15}N$ values change as they move up trophic levels due to differing degrees of isotope fractionation during nitrogen transfer, particularly during transamination or deamination (McCathy et al., 2013; McClelland & Montoya, 2002; O'Connell). Amino acids are typically labeled as either "source" or "trophic"; trophic amino acids are enriched in ^{15}N by 5-7‰ in consumers relative to producers, whereas source amino acids show little change (Popp et al., 2007). The trophic amino acids are glutamic acid (Glx), alanine (Ala), aspartic acid (Asp), proline (Pro), leucine (Leu), and valine (Val). Phenylalanine (Phe) and lysine (Lys) (Popp et al., 2007).

There are differing opinions on which amino acids are considered source (McMahon & McCarthy, 2016; O'Connell et al., 2017), but there is a consensus on at least phenylalanine (Phe), lysine (Lys), methionine and tyrosine (methionine and tyrosine were not measured in our study). Serine (ser), glycine (Gly), and threonine (Thr) are sometimes considered source amino acids (McClellan & Montoya, 2002; Popp et al., 2007; O'Connell 2017), however for this study we follow McMahon and McCarthy (2016) and label these amino acids as neither trophic nor source, but "metabolic". Phenylalanine has been identified as an important diagnostic source amino acid, and glutamic acid an important diagnostic trophic amino acid (McClelland & Montoya, 2002; McMahon and McCarthy, 2016; O'Connell, 2017). The difference between the δ^{15}N values of the trophic (Glx) and source (Phe) amino acids provides a method to estimate relative trophic position regardless of source δ^{15}N (Sherwood et al., 2011). Trophic position cannot be reliably estimated from bulk δ^{15}N because it does not account for variable baseline δ^{15}N across and within different ecosystems (McMahon & McCarthy, 2016).

In this study, I use CSIA-AA and bulk SIA to analyze changes in trophic position over time for four NL breeding seabirds species: Atlantic puffin, common murre, and razorbill, all members of the Alcidae family (auks) and characterized as pursuit divers; and Leach's storm-petrel, family Hydrobatidae and an ocean surface forager. We chose puffins and petrels for our $\delta^{15}N_{AA}$ analysis because 1) we were able to collect the longest time series of the four species (1899-2021 for puffins and 1904-2019 for petrels), and 2) puffins and petrels feed in distinct wintering grounds, thus are likely to have different base δ^{15}N values.

We collected seabird breast feathers from several Canadian museums, as well as from personal collections of researchers. All bird specimens were collected at their colony during the breeding season; therefore, we assumed most feathers were grown during the fall and winter (Gaston & Jones, 1998; Harris & Wanless, 1990; Harris & Yule, 1977). Little is known about adult winter diets, when many seabirds travel to geographically distant locations, inaccessible to researchers (Gómez et al., 2018).

In our study, we investigate if and how NL seabird feather isotopes have changed over 120 years. We predict that Atlantic puffins and murres are foraging at lower trophic levels than they were historically because of less capelin availability. This change will be reflected in decreasing δ^{15}N values over time. Razorbills are less reliant on capelin, thus we expect to see smaller changes in δ^{15}N values. Leach's storm petrels employ a different foraging strategy than the auk species and forage in a different location in the winter. We expect no changes in Leach's storm petrel trophic position.

2.3 Materials and methods

2.3.1 Feather collection and cleaning

We chose to collect feathers from four common NL breeding bird species: Atlantic puffin, Leach's storm petrel, common murre, and razorbill. Institutional sources of feathers were identified using VertNet (http://www.vertnet.org/index.html), an online tool used to access biodiversity data (VertNet, 2016). We limited our search to institutions in Canada to make acquisitions easier. Our goal was to have five birds per species per decade (e.g., 1940-1949, 1980-1989), starting in the mid- to late-1800s through 202; however, numbers of birds sampled and archived over time occurred in batches, related largely to individual researchers, their sampling effort, and researcher likelihood to archive specimens. We identified birds at the Royal Ontario Museum (ROM) in Toronto, ON and the Canadian Museum of Nature (CMN) in Ottawa, ON. The birds were all collected during the breeding season for each species (approximately May-November). This ensured that the birds collected were NL breeders and not visitors from the Canadian Arctic or other countries (e.g., Europe). Four murre captured outside of the breeding season. These were the only murres we were able to collect later than 2000, so we kept them in our study; use caution when interpreting murre results. Additional birds were found at institutions not listed on VertNet, including The Rooms in St. John's, NL, Memorial University of Newfoundland (MUN) teaching labs, and personal collections of researchers at MUN.

To ensure we had a good representation of modern-day species, which proved difficult to find at museums, we received bulk isotope data from feathers previously processed by Dr. Alex Bond (Natural History Museum United Kingdom) and Dr. April Hedd (Environment and Climate Change Canada) for puffins and petrels in the 2010s. Those data were included in the dataset along with samples we processed. See Table 2.1 for a summary of bulk feather data collected by decade. We sampled 3 mg of breast feather for bulk SIA, and 10 mg for CSIA-AA.

For amino acid stable isotope analysis, we collected chicken feathers from Country Ribbon, a poultry processing facility in St. John's, NL, as a matrix standard match to seabird feathers. Chicken feathers are a close proxy for seabird feathers and poultry chickens have a known, homogenous diet, thus ensuring similar values for each isotope run.

Chicken feathers needed a preliminary wash to mitigate any potential salmonella contamination. Half the feathers were washed with a 10% bleach solution and half with a 70% methanol solution. We chose to use the chicken feathers that had been treated with methanol because an initial $\delta^{13}C_{AA}$ analysis showed that the methanol treated chicken feathers had less isotopic variation than the bleach treated feathers (see Table A.1 in Appendix A for chicken feather $\delta^{13}C_{AA}$ results). After chicken feathers were dry, they were cleaned following the same chloroform:methanol procedure listed below.

To clean the feathers, we placed them in individual glass test tubes and filled the tubes with a 0.87:0.13 chloroform:methanol solution to remove any oils from the feathers (Wiley et al., 2013). The tubes were then placed in a sonicator bath for six minutes to aid in the removal of any debris. The solvent was discarded, and the feathers were left in the tubes and placed in a fume hood overnight to dry. Dried feathers were stored in glass tubes covered with paraffin wrap until analysis.

2.3.2 Elemental analysis (EA) and bulk stable isotope analysis (SIA)

We sent samples for analysis to the Earth Resources Research and Analysis (TERRA) facility at MUN; duplicate samples were sent to the Ján Veizer Stable Isotope Lab at the University of Ottawa. An analytical scale (4 decimals) was used to weigh ~1 mg of feathers, which we cut with scissors and placed into 7x7 mm tin trays that were folded into cubes. The tins were run in a Carlos Erba elemental analyser coupled to a Delta V Plus isotope ratio mass spectrometer (EA-IRMS, Thermo Scientific) via a ConFlo III interface. The feathers were analyzed for elemental composition (%C and %N) and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). $\delta^{13}C$ is measured the following way: R = ratio of ^{13}C:^{12}C, where R of the sample is compared to R of a standard specific to the element of interest. The $\delta^{13}C$ value is calculated by:

$$[(R_{\text{sample}}/R_{\text{standard}})-1] \times 1000$$

The same formula is used for $\delta^{15}N$ (^{15}N:^{14}N), with a separate standard R for N. The accepted international standards for R for carbon and nitrogen comes from the $\delta^{13}C$ value derived from VPDB and the $\delta^{15}N$ value of atmospheric nitrogen.

Scale calibration is needed for every run of the EA-IRMS. We used EDTA #2 (Schimmelmann Research Lab, Indiana University: $\delta^{13}C$ -40.38, $\delta^{15}N$ -0.83), caffeine #2 (Schimmelmann Research Lab, Indiana University: $\delta^{13}C$ -14.79, $\delta^{15}N$ 20.17), casein (B2155, Elemental Microanalysis: $\delta^{13}C$ -27.03, $\delta^{15}N$ 5.97), and glutamic acid (in-house standard, $\delta^{13}C$ -26.74, $\delta^{15}N$ -2.77).

2.3.3 Amino acid stable isotope analysis

Prior to hydrolysis and derivatization of feathers, a solution of AAs was created as the standard to run with every batch of feathers. The amounts of AAs used in the standard solution closely duplicated the AA makeup of feathers determined by an initial $\delta^{15}C_{AA}$ analysis of chicken feathers run at TERRA (see Table A.2 in Appendix A for AA mixture used).

All glassware used in $\delta^{15}N_{AA}$, including pipettes for transferring chemicals, was ashed at 500°C for 4 hours in a muffle furnace prior to sample preparation. Samples were prepared for $\delta^{15}N_{AA}$ according to protocols developed by Dr. Owen Sherwood at Dalhousie University (Halifax, NS) based on methods established by Silfer (1991), with modifications. Approximately 10 mg of feather was weighed into a 7 mL borosilicate glass vial (~2-6 feathers per bird, depending on size of the feathers). Each round of analysis included six seabird samples, one chicken sample, and two AA standards (pipetted into separate vials after the seabird and chicken feathers were hydrolyzed). Each vial with feathers was filled with 3-4 mL of HCl and flushed for 20-30 seconds with a gentle stream of N_2 before quickly being capped with a PTFE-lined cap. I shook the vials until all the feathers were submerged under the HCl (approximately 5 seconds). Vials were placed on a heating block in an oven at 100°C for exactly 20 hours for hydrolysis. The caps were tightened after two minutes and four minutes.

After 20 hours, we removed samples and placed them in the fridge for $\geq$ 30 minutes. While samples were cooling, 300 µL of the AA standard was pipetted into two 7 mL vials; from this point on “samples” will include the two AA standards. Thirty µL of norleucine was added to each sample- norleucine is the universal internal standard (~34.5 mg norleucine/10 mL 0.1M HCl). The samples were dried down at 60°C under a gentle stream of N_2 (approximately 2-5 hours), and then 1 mL of 0.01M HCl was added to the dried hydrolyzed samples and placed in a freezer overnight.

To begin derivatization, samples were dried down at 60°C. I applied a layer of Teflon tape to each vial once it was dry. Acidified 2-propanol was created by slowly adding 3.5 mL of acetyl chloride to a 50 mL vial containing 17.5 mL of 2-propanol on ice; 2 mL of the resulting acidified 2-propanol was added to each vial. The vials were flushed with N_2 for 10-15 seconds, capped and put into the oven at 110°C for one hour, with caps tightened at five and 15 minutes.

After one hour, I took the samples out of the oven and put them into the fridge for ≥ 30 minutes. Samples were then dried at room temperature under a gentle stream of N_2. Once samples were dry (2-4 hours), I removed and replaced the Teflon tape. I added 0.25 mL dichloromethane (DCM) to each vial and dried down; this step was repeated twice. Once dry, 2.25 mL DCM and 0.75 mL trifluoroacetic anhydride (TFAA) were added to each vial. The vials were flushed with N_2 for 10-15 seconds, capped with a new cap, and vortexed for 10 seconds. I placed the vials in the oven at 100°C for 15 minutes, tightening the caps at two and five minutes. The samples were fully derivatized at this point and moved to the -20°C freezer. The derivatized samples were shipped on dry ice to Dalhousie University for gas chromatography.

Once at Dalhousie, the samples were prepared for gas chromatography (GC). Nine GC vials were labeled corresponding to the sample ID and weighed. The samples were removed from the freezer and vortexed for 10 seconds to re-combine the TFAA and DCM. Approximately 25 mL of each sample was inserted into the corresponding vial; 0.5 mL of standards is used. The samples and standards were suspended in 50 µL and ≥150 µL of ethyl acetate, respectively. All vials were flushed with N_2 for 10 seconds, capped, and vortexed for 30 seconds. The final weight of each vial was recorded. After the GC vials were prepared, a new layer of Teflon was applied to the 7 mL vials, and they were flushed and capped and stored in the -20°C freezer.

Samples were injected in triplicate or quadruplicate, bracketed by triplicate or quadruplicate injections of calibration standards, and analyzed for $\delta^{15}N$ using a Trace 1310 Gas Chromatograph coupled with a Delta V IRMS (Thermo Scientific). The following amino acids were measured: alanine (Ala), glycine (Gly), proline (Pro), valine (Val), leucine (Leu), isoleucine (Ile), asparagine + aspartic acid (Asx), threonine (Thr), serine (Ser), glutamine + glutamic acid (Glx), phenylalanine (Phe), lysine (Lys) and tyrosine (Tyr). The mean reproducibility of $\delta^{15}N$ for the internal standard norleucine (nle) was 0.5‰, with a range reproducibility among AAs from 0.2‰ (Pro) to 0.9‰ (Thr). Acid hydrolysis converts glutamine into glutamic acid and asparagine into aspartic acid, thus why they are reported together (Glx and Asx, respectively) (McMahon & McCarthy, 2016).

The relative abundance of individual AAs was also reported for all feather samples, reported as percent mole (%Mol). %Mol was quantified by calibration of mass 44 peak areas measured during $\delta^{15}N$ analysis using relative response factors between each AA and Nle internal standard (Chen et al., 2022). A derived bulk $\delta^{15}N$ value was calculated by adding up the concentration-weighted $\delta^{15}N$ of each AA.

2.3.4 Statistical analysis and data visualization

All bulk $\delta^{13}C$ data were corrected for the Suess Effect—the phenomena describing the increase of $\delta^{13}C$ depleted carbon entering the atmosphere due to fossil fuel burning. The formula I used to correct for the Suess Effect follows Vokhshoori et al. (2019): a decrease in atmospheric

$\delta^{13}C$ of 0.05‰ for every decade between 1860 and 1960, and a decrease of 0.16‰ in every decade from 1990 to 2021.

All analyses were performed in R (v4.2.2., R Core Team, 2022). When parametric assumptions were met, we fit $\delta^{13}C$, $\delta^{15}N$ and $\delta^{15}N_{AA}$ as a function of time to linear models using the `ggplot2` package (Wickham, 2016). The razorbill $\delta^{13}C$ and $\delta^{15}N$ data were non-parametric; out of the many approaches to modeling nonparametric regression models, we decided to use the generalized additive model (GAM), a nonparametric method that allows for local regression to fit smoothers over non-linear data (Hastie & Tibshirani, 2014). We used the `mcgv` package to create our GAMs (Wood, 2017). An unpaired two-sample t-test was used to quantify differences in puffin and petrel $\delta^{15}N_{AA}$ and %Mol concentrations of AA in $\delta^{15}N_{AA}$ samples between species.

Assumption testing

Prior to statistical analysis, we visualized data to determine the best fitting regression models, then checked for parametric normality. We decided to visualize diagnostic plots rather than use a definitive normality test, like Shapiro-Wilks: tests like Shapiro-Wilks tend to be misleading, having a bias towards reporting normality in small datasets and reporting non-normality in large datasets (Wharton, 2022). Diagnostic plots allow researchers to visualize the residuals of a dataset, learning more about the data and which model is the best fit. We visualized residual versus fit plots to test for non-linearity (checking for “bowl” shaped or “fanning” residuals), QQ-plots to check if residuals are normally distributed, and residuals versus leverage plots to check for any strong leverage points. R has built-in functionality that allows for regression diagnostic plots to be created without the extra steps of creating separate data frames for residuals and fitted values, using: `plot(model)`. See Appendix B for residual diagnostic plots.

An important assumption in statistical analyses is that the data are independent. This assumption is particularly at risk of being violated when one of the variables is time; "autocorrelation" describes the correlation of residuals in a time series (Anderson, 1954). To test for autocorrelation in our data, we plot the residuals on a lag plot. A data set without autocorrelation would display a residual lag plot with no discernible pattern, like a "cloud" of points. Any deviation from a "cloud" could indicate autocorrelation. Based on the residual lag plots, it looked like petrel bulk δ^{15}N and puffin bulk δ^{13}C may violate assumptions of independence. From here, we used the autocorrelation function `acf` to further explore the independence of the petrel δ^{15}N and puffin δ^{13}C data. The output for both petrel δ^{15}N and puffin δ^{13}C showed no autocorrelation, so we feel confident that the data do not violate the assumption of independence. Another test that can be done to examine autocorrelation is the Durbin-Watson test in the `lmtest` package in R (Zeileis & Hothorn, 2002). The Durbin-Watson test shows slight autocorrelation for both petrel δ^{15}N and puffin δ^{13}C data, however we feel it is small enough to not be of concern.

2.4 Results

2.4.1 Amino acid specific $\delta^{15}N$

Amino acid δ^{15}N was measured in puffins and petrels only. There was no linear trend for phenylalanine nor glutamic acid with either bird species (Table 2.2, Figures 2.1 and 2.2). Since we did not analyze the razorbill or murre feathers for $\delta^{15}N_{AA}$, we are assuming the phenylalanine results would be similar to the puffin results because all three species winter in the northwest Atlantic.

The puffin Glx δ^{15}N values have a similar curve as the puffin bulk δ^{15}N values, this time with the values peaking around 1960 and dropping in modern specimens. Those data were fit to a GAM (EDF = 2.26, deviance explained = 19.9%) (Figure 2.2).

Petrel and puffin glutamic acid and phenylalanine δ^{15}N diverge, with petrel glutamic acid δ^{15}N values higher than puffin glutamic acid (mean 23.2 and 21.5‰, respectively), and petrel phenylalanine δ^{15}N values lower than puffin phenylalanine (mean 4.3 and 6.5‰, respectively). An unpaired two-sample t-test confirmed differences in glutamic acid (p-value < 0.001) and phenylalanine (p-value = < 0.001) between species.

We compared the AA concentrations (%Mol) between puffins and petrels and found differences in all AAs except for phenylalanine (Table 2.3). However, a summation of all the trophic amino acids (glutamic acid, aspartic acid, alanine, proline, valine, leucine, and isoleucine) and the source amino acids (phenylalanine and lysine) revealed no overall differences between species (trophic p-value = 0.99, source p-value = 0.15).

2.4.2 Bulk δ^{15}N and δ^{13}C

Murres showed the strongest decreasing linear trend for δ^{15}N values ($r^2 = 0.32$, p-value = 0.004), followed by razorbills (deviance explained = 34.5%) (Figure 2.3). Note that we used the GAM method to create the razorbill model, so we report estimated degrees of freedom (EDF) and deviance explained. No linear trend was detected for petrels for δ^{15}N values, nor was a trend detected for any of the birds with δ^{13}C values. Puffin δ^{15}N data increase up until the 1980s and decrease in modern samples. We summarized those data using a GAM (deviance explained = 19.9%). Data are summarized in Table 2.4.

2.4.3 Relative trophic position estimates

We estimate the relative tropic position (TP) for puffins and petrels by subtracting $\delta^{15}N$ values of phenylalanine from $\delta^{15}N$ of glutamic acid (Figure 2.4). The results were binned into four discreet time periods: 1890-1929, 1930-1959, 1960-1989, and 1990-2021. ANOVA tests found differences in TP between time periods for puffins (0.40) but not petrels ($p = 0.77$). A post-hoc analysis shows a significant change in TP between 1899-1929 and 1930-1959 ($p = 0.03$). We do not estimate the relative TP of murres and razorbills because we do not have information about base $\delta^{15}N_{Phe}$; however, we interpret generally about relative TP changes using bulk $\delta^{15}N$ data because we assume $\delta^{15}N_{Phe}$ for murres and razorbills would be similar to puffins.

2.5 Discussion

In this study we investigated changing trophic positions of four common NL breeding seabirds over a period of 120 years, using $\delta^{15}N$ as a proxy for trophic position. The northwest Atlantic Ocean has been subjected to major oceanographic changes over the last couple centuries driven by anthropogenic pressures (e.g., increasing fishing pressures) and dramatic climate fluctuations (e.g., cold intermediate layer expanding), leading to food web shifts and fish population collapses. Our study surmises that changes in the marine northwest Atlantic food web will be detected in the stable isotope values of top marine predators, seabirds, because they will be forced to forage for lower quality and/or lower trophic level food. We measured $\delta^{15}N$ in bulk feather tissues of Atlantic puffins, Leach's storm-petrels, common murres and razorbills, and we measured $\delta^{15}N$ in individual amino acids only in feathers of puffins and petrels. Relative trophic position changes of puffins and petrels over time were determined by calculating the difference in $\delta^{15}N_{AA}$ of glutamic acid and phenylalanine. Atlantic puffins' relative TP increases significantly from 1899-1929 to 1930-1959, although caution should be applied when interpreting these results because there were only three samples in the 1899-1929 time bin. Puffin relative TP decreased from 1930-1959 through 2021, albeit not significantly. Petrel relative TP did not change over time.

We did not measure $\delta^{15}N$ in amino acids for the murre and razorbill samples, so we could not estimate relative TP. However, murres and razorbills stay in the northwest Atlantic during the winter (Chapdelaine et al., 2001; Hedd et al., 2011; Huettmann et al., 2005; McFarlane Tranquilla et al., 2013), similar to puffins (Fayet et al., 2017). The puffin $\delta^{15}N_{Phe}$ values do not change over time (although there was considerable variation), thus we cautiously interpret bulk $\delta^{15}N$ of murres and razorbills as a proxy for TP. Both murre and razorbill data record decreasing $\delta^{15}N$ over time, indicating TP is decreasing for both species.

There were no trends in $\delta^{13}C$ data over time for any of the species. Few long-term data exist to support or reject our findings. Farmer and Leonard (2011) recorded no change in $\delta^{13}C$ in great black-backed gulls, known to winter in the northwest Atlantic, over 110 years (1896-2006). There is evidence that baseline $\delta^{13}C$ values cycle in the northwest Atlantic over a period of several years, likely related to changes in sea surface temperature (Espinasse et al., 2022). It is possible that we did not capture these changes in our puffin, murre, and razorbill samples due to large yearly gaps in our data set. For the tropical Atlantic wintering petrels, Fairhurst et al. (2015) detected a small significant increase in $\delta^{13}C$ values in Leach's storm-petrel feathers from 1860 to 2012, in contrast to our results which showed no changes. Future research is needed to understand if and why δ^{13}C values change in seabird tissues wintering in different parts of the Atlantic Ocean.

Researchers have proposed several methods for estimating TP with stable isotope data. It is well established that stable isotopes, particularly nitrogen, show a step-wise enrichment from producer to consumer, known as a "trophic discriminating factors", or TDFs (Hobson et al., 1994; Minagawa & Wada, 1984; Zanden & Rasmussen, 2001). Bulk δ^{15}N data can be used to estimate TP with the following equation:

$$TP_{CONSUMER} = 2 + (\delta^{15}N_{CONSUMER} - \delta^{15}N_{PRIMARY\ CONSUMER})/TDF$$

where we assume the primary consumer is occupying the second trophic level. This equation relies on a knowledge of the base δ^{15}N within the study system (the primary consumer) and the TDF of the target species (see Hobson et al., 2002; Persaud et al., 2012; Zanden & Rasmussen, 2001). More complex calculations using CSIA-AA consider baseline δ^{15}N as well as TDFs, for example:

$$TP_{CONSUMER} = 1 + (\delta^{15}N_{Glx} - \delta^{15}N_{Phe} - TDF_1)/TDF_2$$

where TDF_1 is specific to the sample tissue being used (e.g. seabird feathers), and TDF_2 is a mean TDF value of multiple trophic steps within the study food web (Chikaraishi et al., 2009). A limitation with using these equations is the amount of knowledge of the study system required to make reasonable comparisons between food webs. When specific knowledge is unknown, we make substitutions, such as in the bulk equation where δ^{15}N of the primary consumer is used in lieu of the actual baseline δ^{15}N. Similarly, TDFs are very specific to consumer-prey interactions, and often require studies of a captive population fed a controlled diet (see Becker et al., 2007). For these reasons, we chose to use neither a bulk nor CSIA-AA based TP formula for our analysis. We did not know the baseline δ^{15}N for the murre and razorbill data, nor did we have specific TDFs for any of the four species (besides murres fed a controlled capelin diet, see Becker et al., 2007). Instead, we calculate the difference between glutamic acid and phenylalanine to serve as a relative trophic position indicator. The resulting values do not give us an accurate trophic level estimate, however we can still interpret trends over time.

One interpretation for the puffin, murre, and razorbill decreasing TP over time is that the birds are consuming more small fish and invertebrates than they were historically because of less availability of preferred fish species, mainly capelin. In general, the invertebrate prey species targeted by puffins have lower $\delta^{15}N$ values than the fish species targeted (see Appendix A.5) (Jenkins & Davoren, 2021; Sherwood & Rose, 2005). The collapses and continued low productivity of cod and capelin populations in the northeast Atlantic since the 1990s are well documented (Buren et al., 2014; Kurlansky, 1997; Mowbray, 2002; Myers et al., 1997; DFO, 2018). It is important to note that these are not the only two fish species that have suffered population collapses in the northeast Atlantic: haddock *Melanogrammus aeglefinus*, white hake *Urophycis tenuis*, pollock *Pollachius virens*, and many other commercially exploited species along the Atlantic coast collapsed in the years leading up to the 1990s (Frank et al., 2005). Factory trawling was introduced to the northwest Atlantic in the late 1940s and had a significant impact on fisheries. These vessels allowed for intensive exploitation of fishes and, combined with poor fishery management and destructive fishing practices, lead to the eventual collapse of many species (Lear, 1998; Myers et al., 1997). The loss of larger benthic fish species results in a dramatic shift in the species composition of a food web. Often, it leads to an increase in lower trophic level fishes and invertebrates, as was seen in along the NL shelf with increasing biomass of northern shrimp *Pandalus borealis* and northern snowcrab *Chionoecetes opilio* (Dawe et al., 2012; Frank et al., 2005).

Our evidence suggesting a weak decrease in TP in puffins since the 1930s is supported indirectly by limited diet studies. To our knowledge there have been no investigations of the winter diet of Atlantic puffins wintering around Newfoundland; however, studies of puffins captured in the northeast Atlantic in the winter record a taxonomically varied prey composition of both fish and invertebrates, including sandeels and sandlances *Ammodytes*, spp., capelin, other mesopelagic fish, Euphausiid krill, Polychaetes, and squid (Falk et al., 1992; Harris et al., 2015). These studies do not analyze diet compositions over time; however, they indicate a generalist feeding strategy that may allow puffins to forage for lower trophic level prey if their preferred species are in short supply. Additionally, NL puffins show a high degree of plasticity in prey provisioning for their chicks during the breeding season when capelin populations are low, focusing more efforts on foraging for lower trophic level fishes and invertebrates (Baille & Jones, 2004; Burke & Montevecchi, 2008). While prey provisions for chicks is not necessarily representative of what the adults eat themselves, it indicates puffins may have the ability to target alternative prey when preferred populations are low.

For our murre data, we interpret a significant decrease in bulk δ^{15}N values as indicating a decrease in TP since 1900 which may be caused by feeding on a higher percentage of invertebrates in modern murre populations than historic ones. Our interpretation is supported by limited diet studies of murres: in the 1950s and 1980s their predominant prey species were capelin and other fish (Elliot et al., 1990; Tuck, 1961), while in the 1990s their diet was made up of a higher percentage of crustaceans and squid than fish (Rowe et al., 2000). Similarly, the razorbill data record a downward trend in bulk δ^{15}N values since 1900 which we interpret as a decrease in TP. We were not able to find studies to corroborate these data: studies of razorbill winter diet in the 1950s (Tuck, 1961) and the late 1990s (Huettmann et al., 2005) record crustaceans as a major component, with fish occurring less frequently. To the best of our knowledge, these are the only recordings of razorbill winter diets, which do not indicate any changes from the 1950s to the 1990s.

Leach's storm petrel TP remained steady from 1904 to present day. To our knowledge, there are no studies of Leach's storm petrel winter dietary preferences. In contrast to the three auk species, the petrels' surface foraging strategies and wintering locations are completely distinct (Harrison, 1983). Newfoundland petrels travel much further in the winter, down the Atlantic coast to South America or across the Atlantic to the east coast of Africa (Cramp & Simmons, 1977; Harrison, 1983; Hedd & Montevecchi, 2006; Pollet et al., 2014). Although less studied than the North Atlantic Ocean, there is evidence that the tropical Atlantic has been similarly subjected to environmental disruption due to anthropogenic climate change, affecting fish and plankton biomass (Myers & Worm, 2003; Piontkovski & Castellani, 2009). Our isotope data suggest that petrels' winter trophic position have been consistent over 120 years, despite decreasing global populations. More research is needed to contextualize the decline of this endangered species (Pollet et al., 2021).

The declining $\delta^{15}N$ for puffins, murres, and razorbills reflects oceanographic and anthropogenic trends in the 20th century that have decreased fish populations and/or made fish more inaccessible to seabirds. North Atlantic fish biomass decreased by 90% from 1900 to 1999, due considerably to the industrialization of commercial fishing (Trites et al., 2006). The commercial exploitation of several higher trophic level fish species resulted in substantial increases in small pelagic fishes, benthic macroinvertebrates and pelagic zooplankton (Frank et al., 2005; Pedersen et al., 2017); it is likely that wintering seabirds are foraging on these lower trophic level prey in response to fish population collapses. In addition, capelin diel vertical migration behaviors have become increasingly erratic since 1991, with capelin staying in deeper waters (200+ meters) than they were prior to 1991 (Mowbray, 2002). This may make capelin more difficult to capture for the diving seabirds in our study and puts them totally out of reach for the shallow diving puffins (dives up to 68 m, Burger & Simpson, 1986).

2.6 Conclusion and considerations

Over a period of 120 years, we recorded a decrease in $\delta^{15}N$ values of common murres and razorbills which we interpret as a decrease in TP. The puffin data show an increase in TP from 1899 to the 1930s-1960s, then a weakly decreasing TP in modern samples. The petrel data show no change in TP. There were no patterns detected in $\delta^{13}C$ values over time for any species. The $\delta^{15}N$ patterns of the three auk species coincided with major oceanographic and fishery disruptions in the North Atlantic throughout the 20th century, resulting in decreased populations higher trophic level fishes and increased populations of smaller pelagic fishes and invertebrates. Leach's storm-petrels winter in tropical Atlantic waters and employ different foraging strategies than the auks, thus recording stable $\delta^{15}N$ throughout time.

Any conclusions gleaned from 1899 to ~1950 should be interpreted with caution due to sample sizes. In total, we were only able to collect specimens from 10 birds from 1890-1940; there were many decades where we had no representatives for a species. These decades are important for establishing seabird TP because it immediately precedes industrialized fishing in the northwest Atlantic. While we do see a decrease in TP for the three auk species since the 1940s, we do not know if the TP decrease began at the onset of industrialized fishing, or if it had been falling prior to the 1940s. A more robust collection of historic seabird tissues would help fill in these knowledge gaps.

Additionally, we only measure bulk $\delta^{15}N$ for our murre and razorbill specimens. We interpreted those data as a proxy for TP because we felt confident the baseline $\delta^{15}N$ would not show any temporal trends for those specimens. We based this assumption on the puffin $\delta^{15}N_{AA\text{-}Phe}$ data, since the three species forage in the northwest Atlantic in the winter. However, it is important to note that even though there was no trend in the puffin $\delta^{15}N_{AA\text{-}Phe}$ data, there was significant variation (3.4-9.3‰). Future studies should consider CSIA-AA to investigate trophic changes of razorbills and murres.

In conclusion, we establish that there are likely long-term shifts in seabird trophic positions in response to changing oceanic food webs in the north-west Atlantic, demonstrated by both bulk SIA and $\delta^{15}N_{AA.}$ Newfoundland seabirds are responding to changing marine food webs by shifting foraging targets, however surveys of chick growth and population trends show that is the birds are experiencing negative trade-offs.

Tables

Table 2.1. Summary of number of sampled seabird specimens collected by decade and species.

Decade/ Species	**Puffin**	**Petrel**	**Murre**	**Razorbill**
1890	3			
1900		1	2	1
1910				
1920		2		
1930		1		
1940	2	3	2	1
1950	5	1	2	
1960	6	7	8	8
1970	5	1	5	1
1980	4			2
1990		5	2	
2000	1	3	3	1
2010	11	10		
2020	2			
Total	**39**	**34**	**24**	**14**

Table 2.2. Summary of results of linear model testing of $\delta^{15}N_{AA}$ of phenylalanine (Phe) and glutamic acid (Glx) over time for puffins and petrels. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	Petrels				**Puffins**			
	n	**Slope**	**r^2**	**p-value**	**n**	**Slope**	**r^2**	**p-value**
Phe	21	-0.002	0.003	0.816	26	-0.013	0.066	0.204
Glu	22	-0.001	0.002	0.855	26	0.001	0.001	0.865

Table 2.3. Welch two-sample t-test results of differences between species of %Mol concentration of AAs in feathers analyzed for $\delta^{15}N_{AA}$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

^{T} = trophic amino acid, ^{S} = source amino acid, ^{M} = metabolic amino acid

	Puffin mean (%)	Petrel mean (%)	p-value
Glu^{T}	7.9	9.1	< 0.001***
Ala^{T}	7.3	6.0	< 0.001***
Asp^{T}	6.6	7.6	< 0.001***
Gly^{M}	16.0	15.2	0.008**
Ile^{T}	4.7	4.3	0.004**
Leu^{T}	9.3	8.1	< 0.001***
Lys^{S}	0.9	1.2	< 0.001***
Phe^{S}	2.2	2.3	0.161
Pro^{T}	13.6	14.8	< 0.001***
Ser^{M}	15.2	16.5	0.021*
Thr^{M}	6.7	6.3	< 0.001***
Val^{T}	9.0	9.5	< 0.001***

Table 2.4. Summary of results of bulk δ^{13}C and δ^{15}N data model testing. All petrel and murre data, and puffin δ^{13}C data were fit to a linear model. Razorbill data and puffin δ^{15}N were fit to a generalized additive model (GAM). Estimated degrees of freedom (EDF) reported for GAMs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	δ^{13}C				**δ^{15}N**			
	n	**Slope**	**r^2**	**p-value**	**n**	**Slope**	**r^2**	**p-value**
Petrel	34	-0.004	0.022	0.407	34	0.003	0.011	0.550
Puffin	39	-5.52×10^{-4}	0.001	0.892	--	--	--	--
Murre	24	-0.003	0.025	0.460	24	-0.015	0.316	0.004**
	n	**EDF**	**Deviance explained**		**n**	**EDF**	**Deviance explained**	
Puffin	--	--	--		39	2.3	19.9%	
Razorbill	14	1.7	19.5%		14	1.2	34.5%	

Figures

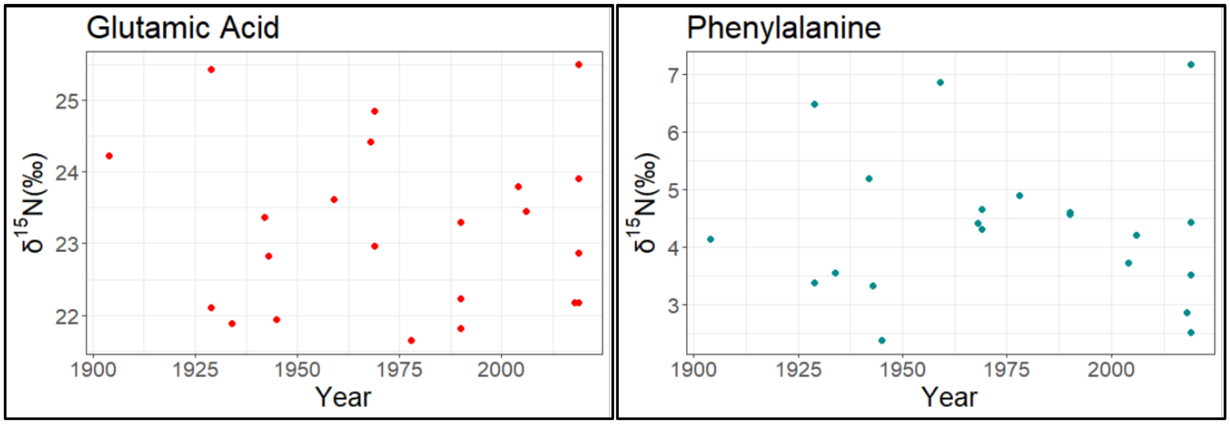

Figure 2.1. Petrel $\delta^{15}N$ values of glutamic acid and phenylalanine over time. No linear trends detected.

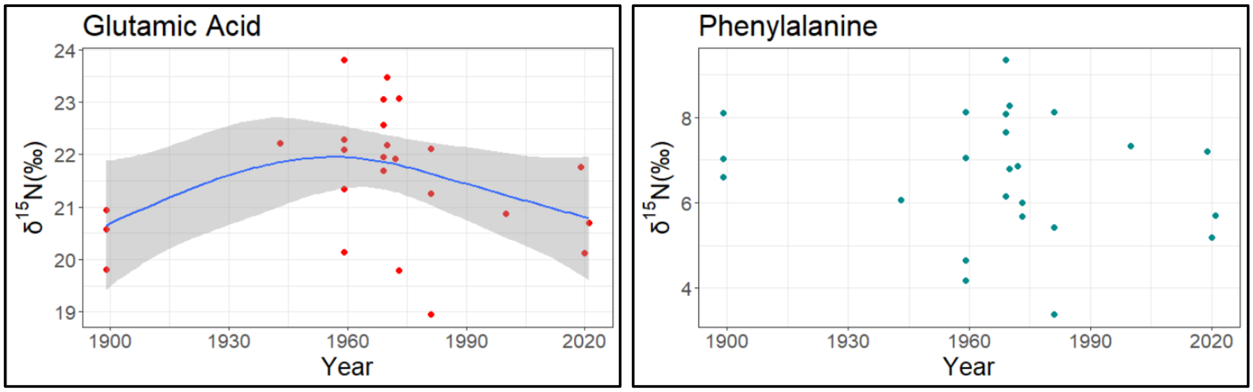

Figure 2.2. Puffin δ^{15}N values of glutamic acid and phenylalanine over time. No linear trend detected for phenylalanine, glutamic acid fit to a GAM, 95% confidence interval in grey.

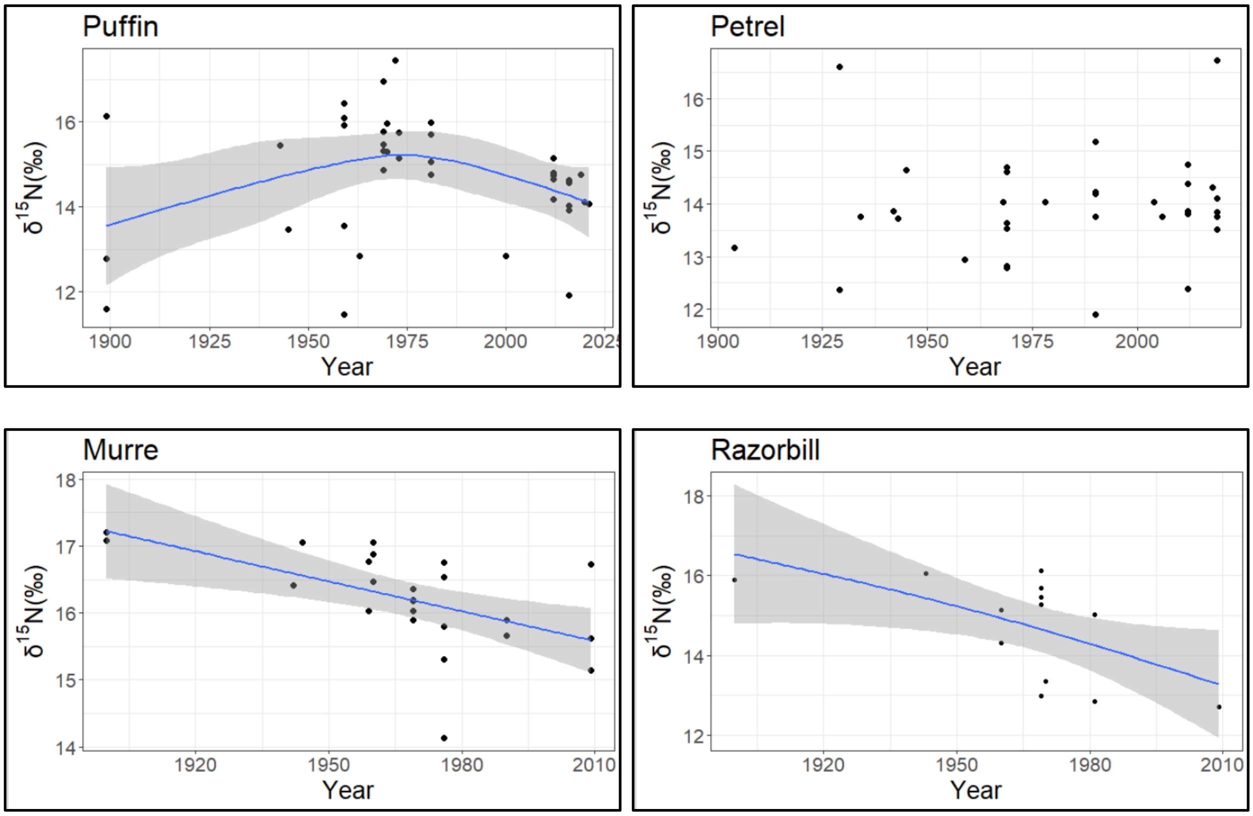

Figure 2.3. Puffin, petrel, murre and razorbill bulk $\delta^{15}N$ values over time. Puffin and razorbill data fit to a generalized additive model, and murre data were fit with a linear model. 95% confidence interval shaded in grey. No trend detected for petrel data.

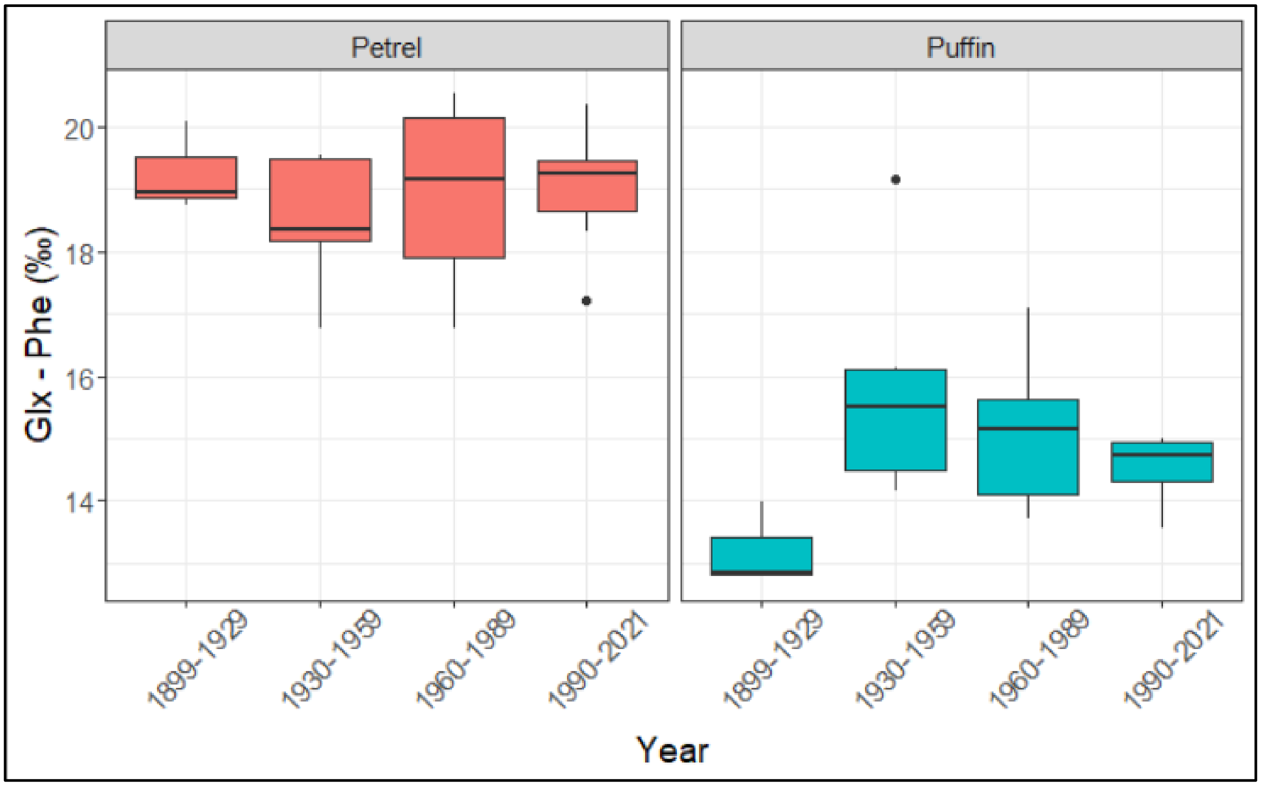

Figure 2.4. Relative trophic level estimates of puffins and petrels in four discreet time bins calculated by subtracting $\delta^{15}N_{Phe}$ values from the $\delta^{15}N_{Glx}$ values.

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Chapter 3: Seabird nutrient subsidies detected in freshwater ponds downwind of a large seabird colony at Cape St. Mary's, Newfoundland

3.1 Abstract

Seabirds are one of the most important nutrient biovectors in the world, moving billions of kilograms of nutrients from marine to terrestrial environments. Seabird colonies are heavily influenced by these marine-derived nutrients brought in by the seabirds, as the nutrients travel past colony boundaries by water movement/runoff and/or wind. As top predators in a marine environment, seabird tissues are enriched in $\delta^{15}N$, which is passed to terrestrial colonies via guano, feathers, eggshells, and carcasses. Here, we examine the isotopic enrichment of zooplankton, sediment, and phytoplankton in freshwater ponds near the numerous bird colonies in Cape St. Mary's Ecological Reserve, Newfoundland (CSM). CSM is a bog habitat characterized by poor soil nutrition, thus we expect seabird waste to add significant amounts of nutrients to nearby ponds. Our main questions are: 1) how far are seabird nutrients being incorporated into terrestrial pond food webs located outside of direct drainage from the colonies? and 2) is primary production increasing in ponds with elevated seabird nutrients? We selected 12 ponds positioned northeast of the colony to account for the prevailing southwest winds. The ponds were 226 meters to 6 km away from the bird community. Results show that the pond nearest to the community (226 meters) had the highest $\delta^{15}N$ values in zooplankton, sediment, and phytoplankton, decreasing in the second pond (414 meters) by an average of 5.2‰, before reaching what appears to be a lower, stable $\delta^{15}N$ value by Pond 11 (5.46 km). Our study also shows increased chlorophyll α and lower C:N values in ponds nearer to the colony, indicating increased primary production.. Future studies are needed to explore if seabird nutrients influence the species composition of zooplankton, phytoplankton, or terrestrial vegetation at Cape St. Mary's, as it does at other studied seabird colonies around the world.

3.2 Introduction

The natural feeding and breeding behaviors of seabirds make them critical players in global nutrient cycling. The Earth's approximately one billion seabirds (Otero et al., 2018), defined as birds whose normal habitat and source of food is the sea (Harrison, 1983), aggregate in dense colonies around the world every breeding season. During the breeding season, seabirds transport billions of kilograms of nutrients globally from marine to terrestrial environments primarily in the form of guano (De La Peña-Lastra, 2020; Doughty et al., 2016; Otero et al., 2018; Signa et al., 2021). These nutrients, particularly nitrogen and phosphorus, can dramatically alter the chemical makeup, landscape, and biological diversity of the ecosystem around a seabird colony (Clyde et al., 2021; Duda et al., 2020; Ellis, 2005; Huang et al., 2014). For example, soils affected by some penguin colonies in Antarctica are so heavily infused with seabird nutrients that they are termed *ornithogenic soils* (De la Peña Lastra, 2020). The nitrogen inputs in the well-studied Antarctic seabird colonies, in addition to nitrogen inputs from nearby marine mammal colonies, increase terrestrial biodiversity well beyond colony boundaries (>1,000 m), corresponding with colony sizes (Bokhorst et al., 2019). Increases in the size and productivity of faunal communities at seabird colonies positively correlates with increases in seabird refuse (Adame et al., 2015; Duda et al., 2020), although excess seabird nutrient input can have a toxifying effect that decreases plant productivity (Ellis et al., 2006; Sánchez-Piñero & Polis, 2000). Nutrient inputs are not limited to the land. Uric acid in guano enters the atmosphere as ammonia (NH_3), a greenhouse gas. In some remote or high-altitude areas, seabirds are the leading source of local NH_3 emissions (Blackall et al., 2007; Croft et al., 2016; Otero et al., 2018).

Nutrients from seabird colonies can be tracked because they differ from their terrestrial counterparts isotopically. Stable isotope analysis (SIA) is a well-established tool that has many applications in ecological studies (Boecklen et al., 2011; Michener & Lajtha, 2007; Whiteman et al., 2019). Both stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) provide insight into food sources of the sample tissues, answering different questions. Broadly speaking, stable carbon isotopes can be used to explore spatiality of food sources, which in turn is useful to track animal migrations (Hedd et al., 2010; Polito et al., 2017; Vokhshoori et al., 2019; Wiley at al., 2013), whereas stable nitrogen isotopes are more often used to examine trophic relationships (Adams & Sterner, 2000; Hedd & Montevecchi, 2006; McClelland & Montoya, 2002; McMahon & McCarthy, 2016; Minagawa & Wada, 1984).

Nitrogen-15 enrichment at increasing trophic levels is a nearly universally observed phenomena across all food webs. The lighter ^{14}N isotope is preferentially favored for protein transamination and deamination (Gaebler et al., 1966), thus is excreted more frequently, leading to the buildup of ^{15}N in tissues (Steele & Daniel, 1978). Seabird tissues are highly enriched in ^{15}N relative to top predators in terrestrial ecosystems. This likely occurs from a combination of two main factors: (1) the $\delta^{15}N$ of Arctic ocean particulate organic matter (POM) and algae is relatively enriched in ^{15}N relative to terrestrial vegetation (Wada et al., 1975), ranging between 5.1 and 6.8‰ (Hobson et al., 2002) thus elevating the ^{15}N of marine food webs relative to terrestrial food webs; and (2) oceanic food webs often have more trophic interactions relative to terrestrial food webs (Post, 2002; Potapov et al., 2019; Shurin et al., 2006). In general, there is an increase of ~3.4‰ of $\delta^{15}N$ per trophic level, which was established by Minagawa and Wada (1984), who examined diets across numerous taxa (range 1.3-5.3‰, mean 3.4 ± 1.1‰). A review by Becker et al. (2007) of over a dozen seabird diet studies supports Minagawa and Wada (1984), finding a mean $\delta^{15}N$ increase of 3.6‰ per trophic level (range 3.0-4.6‰).

Carbon isotope signatures form at the base of food webs based on the carbon source of the autotrophic organisms. Generally, the marine carbon source is bicarbonate, which is enriched in $\delta^{13}C$ relative to the terrestrial carbon source, CO_2 (Craig, 1953; Mackensen & Schmiedl, 2019). If marine carbon is transported into terrestrial habitats, such as freshwater ponds, we would expect to see enriched $\delta^{13}C$ values in abiotic and biotic samples.

Our study focuses on seabirds at the Cape St. Mary's (CSM) Ecological Reserve on the island of Newfoundland, Canada. CSM is the breeding home to tens of thousands of seabirds, most notably the conspicuous northern gannet (*Morus bassanus*). Nearly all the seabirds at CSM congregate at "Bird Rock", a ~100-meter-tall sandstone stack separated from the mainland by scant meters to a perilous drop to the ocean (Figure 3.1b.) (Environment and Climate Change Canada, n.d.).

Like all seabirds, the residents of CSM bring with them nutrient subsidies from offshore feeding grounds. This may be especially important to a marine barren ecozone with bog habitat like CSM, characterized by nutrient poor soils. Our present study explores the spatial influence of allochthonous seabird subsidies on terrestrial CSM by sampling biotic (phytoplankton and zooplankton) and abiotic (sediment and water) components of freshwater ecosystems at a dozen ponds that were between 226 meters to 6 km away from Bird Rock. Seabird nutrients enter freshwater ecosystems primarily via direct deposition or runoff within a lake or pond's catchment (Anderson & Polis, 1999; Clyde et al., 2021; De La Peña-Lastra et al., 2020). This is unlikely at CSM, where the seabirds are cliff nesters and do not nest within the pond catchments. We hypothesized that wind-driven seabird nutrient subsidies will impact CSM freshwater ecosystems that have no colonies within their catchments. Many studies have measured the impact of seabird nutrient subsidies in freshwater ponds in Arctic colonies (e.g. Brimble et al., 2009; Clyde et al., 2021; Keatley et al., 2009; Van Geest et al., 2007), yet the seabirds in all these studies were nesting within pond catchments or were cliff nesting above ponds, unlike CSM where the nearest pond is ~220 meters from the seabird community. Few studies have examined wind-transfer of seabird nutrients to terrestrial ecosystems inland (e.g., Bokhorst et al., 2007; Hargan et al., 2017), and those that have are within Arctic or Antarctic habitats. To our knowledge, no studies have looked at wind-transported seabird nutrients to freshwater ponds within temperate regions.

We hypothesized that zooplankton, phytoplankton and sediment samples closer to the seabird colony are enriched in $\delta^{15}N$ and $\delta^{13}C$ values compared to samples further from the bird community. To date, few studies have analyzed zooplankton isotope characteristics at seabird-influenced freshwater ponds, although there are studies documenting $\delta^{15}N$ enrichment of sediment, algae, and invertebrates at seabird colonies (Griffiths et al., 2010; Kolb et al., 2010). Nitrogen stable isotope analyses are frequently used in paleolimnological assessments of lakes to infer shifts in lake community composition throughout time as they relate to seabird populations (Duda et al., 2020; Griffiths et al., 2010; Michelutti et al., 2009). *Daphnia ephippia* in freshwater ponds with high, direct seabird influences have enriched $\delta^{15}N$ values, signaling seabird nutrient enhancements are being assimilated (Griffiths et al., 2010). Other studies show increases in zooplankton biomass and abundance is correlated with increases in seabird guano nutrient loadings (Van Geest et al., 2007; Zelickman & Golovkin, 1972), although these studies do not examine isotope enrichments. Here, we use $\delta^{15}N$ and $\delta^{13}C$ to explore whether zooplankton, sediment, and phytoplankton in freshwater ponds inland from CSM are assimilating marine-derived nutrients.

We also predict that nutrients associated with seabirds—total nitrogen, total phosphorus, magnesium, calcium, potassium, and sodium—will be elevated in ponds closer to the colony than those that are further away. We predict conductivity will similarly be elevated due to higher levels of dissolved solids from the seabird nutrients in ponds closer to the colony. We predict that primary production will increase in ponds nearer the seabirds because of these increased nutrients, which will result in elevated levels of dissolved organic carbon (DOC). Similarly, elevated pH is associated with increased primary production, thus we expect to see elevated levels of pH in the ponds nearest the colony.

3.3 Materials and Methods

3.3.1 Site Description

Cape St. Mary's Ecological Reserve (frequently CSM) is located on the south-western tip of the Avalon Peninsula, Newfoundland (46°49'N, 54°11'W) (Figure 3.1). It was established in 1993 and is considered one of the most accessible sites in all North America to see seabird colonies up close. CSM is occupied by several seabird species throughout the breeding season, including ~30,000 gannets (Environment and Climate Change Canada, n.d.), common murre (*Uria aalge*, 15,500 pairs, Wilhelm, SI. 2023 unpubl. data. Environment and Climate Change Canada), black-legged kittiwake (*Rissa tridactyla*, 4,400 pairs, Wilhelm, SI. 2023 unpubl. data. Environment and Climate Change Canada), razorbill (*Alca torda*), and the most southern thick-billed murre (*Uria lomvia*) colony (Gaston & Hipfner, 2020).

CSM is part of the "eastern hyper-oceanic barrens", an ecoregion characterized by cool summers and mild winters, and frequent foggy or windy days. This generally flat ecoregion is lacking trees except for scattered stunted balsam firs. Most of the vegetation is typical of barrens and bogs: heath mosses and lichens, low-lying berries, and an assortment of arctic-alpine plants (Protected Areas Association of Newfoundland and Labrador [PAA], 2008). As is characteristic of bogs, the soils at CSM are oligotrophic; the addition of seabird nutrients in guano may be important to this otherwise nutrient-poor ecosystem.

Dozens of ponds are scattered throughout the reserve, ranging in width from <10 to >200 meters. All of the seabirds nest on Bird Rock or the nearby mainland cliffs that are below the land—none of the birds nest in the pond catchments. Due to the geography of the colony, it is unlikely that any nutrients would be directly deposited or washed into the ponds. An exception is Pond 1, the pond nearest to the colony (Figure 3.1). Pond 1 would presumably receive the most seabird derived nutrients at CSM given that it is only 226 meters downwind from the colony, but it is also frequented by seabird bathers during the breeding season, mostly black-legged kittiwakes (author's observation). We did not directly measure wind transferred nutrients, thus cannot disentangle which nutrients in Pond 1 were directly deposited or wind deposited. None of the additional ponds in the study were observed to have seabird visitors.

Preliminary pond selections were made using Google Earth, selecting ponds in a general north-east direction from Pond 1. Ponds were ultimately chosen in the field, influenced by how safe the terrain was to traverse. We labeled ponds from closest to furthest from the colony, with the furthest pond labeled Pond 12. The winds at CSM are dominantly south and southwest; therefore, nutrients traveling via wind would pass over ponds north-east from the colony. Wind data were collected from Visual Crossing, a free online database that provides historical weather data. We collected wind data centered on Argentia, Newfoundland (approximately 55 km NNE from CSM), from January 1, 2021-December 31, 2021. Although most nutrient transfer is likely to happen from May-October when the seabirds are present at CSM and ponds are not ice-covered; the wind-rose data (Figure 3.1c.) is consistent across seasons.

3.3.2 Sample collection and processing

We sampled ponds on September 12, 14, and 18, 2021. These dates fall at the end of the seabird breeding season, when seabird nutrients would have had the longest time accumulating throughout the season. We measured pond pH and conductivity (μS cm^{-1}) on site using a Hanna Instruments portable pH/EC/TDS/Temperature reader (reported with water chemistry in Table 3.2). Distance to the colony from each pond was measured on Google Earth (Table 3.1).

Water

We collected water from each pond for chemical analysis of several analytes: total nitrogen (TN), total phosphorus (TP), dissolved organic carbon (DOC), total inorganic carbon (TIC), and cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}). We sent water samples to two different laboratories: Bureau Veritas and AGAT, both in St. John's, NL. We chose two different labs for water analysis to take advantage of the lowest reported detection limit (RDL) for our required analytes. The water was first collected from the pond surface in a 500 mL Nalgene bottle before being poured into the 5 analysis bottles–this prevented any prefilled preservatives from leaking into the pond. The bottles were placed in a cooler in the field before being transferred to a refrigerator (~4°C) until they could be brought to the analyzing lab. See Appendix C for details about water collection.

Zooplankton

Zooplankton were collected using a hand-held plankton net with a 200-µm mesh lined cup and transferred to 250 mL Nalgene bottles before being stored in a cooler in the field, until they could be kept in a -20°C freezer until processing. Some ponds proved difficult to collect zooplankton from due to the amount of suspended sediment in the water that blocked the mesh.

On the morning of zooplankton processing, we removed one bottle from the freezer to thaw. Once the bottle was thawed, we used a 125-µm sieve to remove suspended sediments and debris to make zooplankton sorting easier. We used a Leica stereoscope and a Bogorov counting chamber to view and systematically go through each bottle of zooplankton. Zooplankton were separated by order (Cladocera, Calanoida, Cyclopoida) onto glass trays using tweezers and a Pasteur pipette. Cyclopoida was ultimately not analyzed because we did not collect enough mass from any of the bottles to submit for elemental analysis and stable isotope analysis (EA/SIA). If we were not able to sort through a whole bottle in one day, we placed the bottle back into a -20°C freezer. Once we finished sorting a bottle, we placed the separated zooplankton into an oven at 55-65°C until dry, at least 12 hours. The dry zooplankton were stored in the dark in 15 mL centrifuge tubes until EA/SIA.

Phytoplankton

We collected phytoplankton onto ashed (500°C) cotton filters using a vacuum filtration system with a hand-held pump. The filters were wrapped in tin foil and were kept in a cooler in the field until they could be transferred to a -20°C freezer until processing. Filters were dried for 12 hours in an oven at 60°C, weighed, and wrapped individually in tin foil. We placed the dried filters in a desiccator cabinet until EA/SIA.

Sediments and guano

We collected ~5-10 g of sediment from the edge of each pond, placed them into a Whirl-pak® bag and stored them in a cooler until they could be transferred to a -20°C freezer. Prior to freeze-drying, the sediments were thawed and weighed. Re-frozen sediments were placed in a Labconco FreeZone 12 L Console freeze dryer for 48 hours. We reweighed the dried sediments to obtain % water measurements. We sieved the sediments with a 125-µm mesh to remove rocks and debris prior to EA/SIA. A subsample of sediments was sent to Paleoecological Environmental Assessment and Research Lab (PEARL) of Queen's University for chlorophyll *α* analysis; methods found at Michelutti et al. (2010). Gannet guano was collected and analyzed for stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) by Johanna Bosch (Bosch, 2023).

3.3.3. Elemental analysis (EA) and stable isotope analysis (SIA)

Zooplankton samples were analyzed for SIA at the Earth Resources Research and Analysis (TERRA) facility at MUN, and the Ján Veizer Stable Isotope Lab at the University of Ottawa. Initially, we had planned to have all samples analyzed at TERRA, however scheduling conflicts resulted in the samples being sent to two different labs. All samples were measured for EA (%C and %N) and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$).

We aimed to weigh out ~1 mg of zooplankton per sampling tin for SIA, however we did not end up with enough mass in every sample to reach this goal. However, we had sufficient weights to measure %C, %N, $\delta^{13}C$ and $\delta^{15}N$ for all samples. Zooplankton were weighed on an analytical scale (4 decimals) and placed into 7x7 mm tin trays that were folded into cubes. Zooplankton from ponds 1, 6, 9 and 10 were analyzed at TERRA; all others were analyzed at the

Ján Veizer Stable Isotope Lab. At TERRA, the tins were run in a Carlos Erba elemental analyser coupled to a Delta V Plus isotope ratio mass spectrometer (EA-IRMS, Thermo Scientific) via a ConFlo III interface. We also ran all sediment samples at TERRA. Approximately 5 mg of sediments were weighed out on an analytical balance and folded into tins.

Scale calibration is necessary for every run of the EA-IRMS. We used EDTA #2 (Schimmelmann Research Lab, Indiana University: δ^{13}C -40.38, δ^{15}N -0.83), caffeine #2 (Schimmelmann Research Lab, Indiana University: δ^{13}C -14.79, δ^{15}N 20.17), casein (B2155, Elemental Microanalysis: δ^{13}C -27.03, δ^{15}N 5.97), and glutamic acid (in-house standard, δ^{13}C -26.74, δ^{15}N -2.77). Acetanilide (δ^{13}C -27.14, δ^{15}N 0.63) was used to prepare a calibration curve for %C and %N.

Our phytoplankton samples were also analyzed at the Ján Veizer Stable Isotope Lab along with the remainder of the zooplankton samples. The dried phytoplankton filter samples were weighed and compared to the average dry weight of the filters without plankton, which was used to calculate how much algae were on each filter. See Table C.2 in Appendix C for the spreadsheet with filter weight data. We used a hole-punch to obtain 4 holes from each of the phytoplankton filters, which would in-theory give us >1mg of phytoplankton. These filter subsamples were placed in the 7x7 mm tin trays and weighed on an analytical balance. We added an equivalent amount of WO_2 to aid in filter combustion. Unfortunately, our phytoplankton data came back with much lower nitrogen mass than expected, and we were only able to record δ^{15}N for Ponds 1, 2, 4, 5, 9, 11 and 12. Percent C, %N and C:N was not measured for phytoplankton because the collection filters contribute to the total mass.

3.3.4 Statistical Analysis

All analyses were performed in R (v4.2.2., R Core Team, 2022). We decided to use the *model selection* approach to analyzing our data (Johnson & Omland, 2004). In this approach, we first generated competing biological hypotheses based on our study system, then fit the hypotheses to our data. Besides a linear model $y = \beta + (\alpha \times x)$, we visualized three different regression models for the δ^{15}N and nutrient datasets: exponential $y = \alpha \times e^{\beta x}$, inverse square law $y = \alpha + \beta \times x^{-2}$, and power law $y = \alpha \times x^{\beta}$, using non-linear least squares (NLS).

Prior to statistical analysis, we visualized data to determine the best fitting regression models, then checked for parametric normality. We decided to visualize diagnostic plots rather than use a definitive normality test, like Shapiro-Wilks. Tests like Shapiro-Wilks tend to be misleading, having a bias towards reporting normality in small datasets and reporting non-normality in large datasets (Wharton, 2022). Diagnostic plots allow researchers to visualize the residuals of a dataset, learning more about the data and which model is the best fit. We visualized residual versus fit plots to test for non-linearity (checking for “bowl” shaped or “fanning” residuals) and checked for normally distributed residuals via a histogram. See Appendix E for all residual diagnostic plots. We selected our final models using the Akaike information criterion (AIC) method, part of the `AICcmodavg` package in R (Mazerolle, 2020). In some cases, the best model as determined by AIC was not used if the diagnostic plots showed that the residuals were problematic for that model, meaning they were not normally distributed (as was the case for total nitrogen, calcium, and phytoplankton), and an alternative model with more normal residuals was used.

3.4 Results

3.4.1 Water chemistry

The water chemistry analysis shows elevated levels of total phosphorus, total nitrogen, calcium, magnesium, sodium, and potassium in Pond 1. Total nitrogen, sodium, and potassium remain elevated in Pond 2 before stabilizing. pH and conductivity were elevated in Pond 1. Conductivity remained elevated until Pond 5. pH recorded a downward trend as Pond distance increased, although there was considerable variation. Dissolved organic carbon (DOC) recorded an increasing trend as pond distance increased. Total inorganic carbon (TIC) levels were high in the first three ponds before dropping; however, there was an unusually high level of TIC in pond 8 (Table 3.2 and Figure 3.2).

3.4.2 Zooplankton, phytoplankton, and sediment SIA and EA, and chlorophyll α

Stable nitrogen isotopes

We recorded a decrease in δ^{15}N values in zooplankton, sediment, and phytoplankton as the pond distance from the colony increased. The decrease in Calanoida δ^{15}N best followed a power law model (AIC: power law model 29.48, null model 45.71), while the decrease Cladocera δ^{15}N values followed the inverse square function (AIC: inverse square model 42.73, null model 62.92) (Figure 3.3a.). Calanoida δ^{15}N values ranged from 4.0 to 14.6‰, and Cladocera from -0.6 to 11.33‰ for Cladocera. See Appendix F for full AIC tables. The sediment δ^{15}N data were best fit to an inverse square model (AIC: inverse square model 35.02, null model 57.85), while the phytoplankton δ^{15}N data were best fit to a power law model (AIC: power law model 31.40, null model 36.67) (Figure 3.3b.). We performed a Pearson's correlation between both Calanoida and Cladocera δ^{15}N values with both sediment and phytoplankton δ^{15}N, reporting p-values and correlation factor (r), summarized in Table 3.3.

Stable carbon isotopes

Gannet guano has a measured δ^{13}C of -20.6‰ (n = 2, Bosch, 2023). Graphical visualizations show δ^{13}C values decrease for both Calanoida and Cladocera over distance, while in the Cladocera dataset we recorded an increase in δ^{13}C values at the last two ponds. We did not find any statistically meaningful change in δ^{13}C over distance for Calanoida nor Cladocera. Calanoida δ^{13}C values ranged from -34.12 to -25.86‰, and Cladocera values ranged from -34.24 to -27.28‰.

We recorded an increase in δ^{13}C values in phytoplankton and sediment as distance from colony increased. We fit both datasets to a linear regression model. Sediment was a somewhat meaningful model ($p = 0.016$, $r^2 = 0.46$) whereas phytoplankton was not meaningful ($p = 0.67$, $r^2 = 0.02$); however, there was high correlation between the values ($p = 0.049$, correlation coefficient 0.58) (Appendix C, Figure C.1).

%N, %C, C:N and chlorophyll α

We saw a decrease in %N over distance in the Calanoida dataset ($p = 0.04$, $r^2 = 0.52$, slope = -0.34), although Ponds 11 and 12 were potentially driving the slope based on the leverage plot (Appendix E, Figure E.15). We did not record a meaningful change in %N over distance in the Cladocera dataset.

We recorded an increase in %C as pond distance increased for both Cladocera and Calanoida; however, neither dataset was statistically meaningful. Meanwhile, we recorded a decrease in %N and an increase in %C over distance in the sediment dataset, although it seemed as though the furthest ponds, Pond 11 and 12, were driving the slope. There was a large fanning of residuals for both the %N and %C models, therefore we did not feel it was appropriate to report.

C:N increased over distance in the sediment dataset, fit to a linear regression model ($p < 0.001$, $r^2 = 0.79$, slope = 5.15, $F_{1,10} = 38.38$) (Figure 3.4a.). Sediment C:N was highly positively correlated with sediment $\delta^{13}C$ ($p < 0.001$, $r = 0.84$) (Figure 3.4b.). Sediment chlorophyll α data were best fit to an inverse square law model (AIC: inverse square law -62.17, null model -46.51) (Figure 3.5).

3.5 Discussion

In this study, we explored impacts that seabirds have on the chemical and nutrient characteristics of freshwater ponds near Cape St. Mary's Ecological Reserve, Newfoundland. CSM is considered one of the most easily accessed seabird colonies in North America, making it an attractive study location. As a bog ecosystem, CSM soils have little available nutrients, thus nutrient additives from seabird waste are important to this habitat. To date, there have been no studies of seabird impacts on terrestrial nutrient loadings at CSM or at many other sites in Newfoundland and Labrador.

Our analysis suggests that seabird nutrients from the colony are entering ponds hundreds of meters away from the colony, and elevated $\delta^{15}N$ from seabird nutrient deposits is being incorporated into inland aquatic food webs.. Since the seabirds nest on cliffs separated from the mainland, or on cliffs that are below the level of the ponds, we assume the nutrients are transported to the ponds via strong southwest dominant winds. An important note is that seabirds do occasionally bathe in the pond nearest the colony (Pond 1), which likely directly deposit waste (however this is a handful of birds compared to tens of thousands on the cliffs). The C:N ratio and chlorophyll α data in sediment indicates more algal primary production in Pond 1, with more terrestrial or allochthonous carbon sources to the sediments as the ponds are distant from the community. The water from Pond 1 had high conductivity and was enriched in TN, TP, Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}, likely reflecting the direct seabird inputs and the wind transported nutrients (Anderson & Polis, 1999; Duda et al., 2021). These variables showed reduced concentrations with distance from the seabird nesting location.

Tracing nitrogen and carbon through the food web

Both Calanoida and Cladocera zooplankton recorded elevated $\delta^{15}N$ values in Pond 1 compared to the rest of the ponds. Cladocera had elevated $\delta^{15}N$ in Pond 2, but other ponds located further form the colony stabilized with lower $\delta^{15}N$ values. Not enough Calanoida mass was collected from Ponds 2-5 to analyze for stable isotopes; however, we see a similar pattern of $\delta^{15}N$ changes over distance with this limited dataset as we do in the Cladocera dataset. The range of Cladocera $\delta^{15}N$ values recorded in this study was -0.6 to 11.33‰. Prior studies of Cladocera in freshwater subarctic and temperate ponds (not influenced by seabirds) have recorded $\delta^{15}N$ values in the range of ~2 to 8‰ (France et al., 1996; Pang & Nriagu, 1977; Matthews & Mazumder, 2003; Visconti et al., 2018). Our Cladocera $\delta^{15}N$ value in Pond 1 is well out of this range (11.33‰), but the $\delta^{15}N$ values recorded in further study ponds falls within 2 to 5‰. For Calanoida in our study, the range of $\delta^{15}N$ values was 4.0 to 14.6‰. Prior studies of freshwater pond dwelling Calanoida in temperate regions record $\delta^{15}N$ values between ~5 to 13‰ (Matthews & Mazumder, 2003; Visconti et al., 2018). Our highest Calanoida $\delta^{15}N$ value in Pond 1 (14.58‰)

is ~1.5‰ higher than the maximum found in these prior studies, with the $\delta^{15}N$ values at further ponds falling between 5 to 8‰. These lower $\delta^{15}N$ values in zooplankton in our study appear consistent with these studies of ponds not influenced by seabirds, indicating that the elevated zooplankton $\delta^{15}N$ values in Pond 1 are a result of seabird-derived nutrients.

We recorded similar patterns of $\delta^{15}N$ changes over distance in the sediment and phytoplankton datasets. We also found that sediment $\delta^{15}N$ values were strongly correlated with Calanoida and Cladocera $\delta^{15}N$ values, but the relationship was not as clear with phytoplankton $\delta^{15}N$ and zooplankton $\delta^{15}N$ values. As the base of the food web, we expected phytoplankton $\delta^{15}N$ values to be highly correlated with zooplankton, with predictable increases in $\delta^{15}N$ as we moved from primary producers to primary consumers (herbivorous Cladocera) to secondary consumers (predatory Calanoida). However, this was not the case, and in some ponds the phytoplankton $\delta^{15}N$ were higher than the Cladocera $\delta^{15}N$. It is possible that the Cladocera were not feeding exclusively on phytoplankton. For example, *Daphnia* spp., a well-studied genus in the Cladocera superorder, are known to feed on suspended bacteria as well as phytoplankton (Ebert 2005), resulting in lower-than-expected $\delta^{15}N$ values.

Our $\delta^{13}C$ data were more challenging to interpret. Marine primary producers are enriched in ^{13}C when compared to terrestrial primary producers (freshwater algae and terrestrial C3 plants) (Lamb et al., 2006), so we hypothesized that $\delta^{13}C$ values from seabird nutrients would result in a decrease in $\delta^{13}C$ as ponds were further from the seabird nesting site. Gannet guano has a measured $\delta^{13}C$ of -20.6‰ (n = 2, Bosch, 2023), and black-legged kittiwake guano measured in a Scottish study recorded -22.9 to -17.8‰ (n = 213, Tait, 2017). These values are greater than any of the $\delta^{13}C$ values of zooplankton, phytoplankton, or sediment in our study. However, enriched seabird carbon isotopes are not necessarily affecting pond primary production, since terrestrial primary production typically sources carbon from atmospheric CO_2 rather than bicarbonate (Craig, 1953; Mackensen & Schmiedl, 2019). Our data show a decrease in $\delta^{13}C$ values in the first 10 ponds of the Cladocera data set, with a sharp increase for ponds 11 and 12; however there was no statistically meaningful pattern. Ponds 11 and 12 are outside of the CSM Ecological Reserve boundaries and were further from seabird community affected by seabird inputs via ground transport by water (Bokhorst et al., 2019; Brimble et al., 2009; Hentati-Sundberg et al., 2020; Vidal et al., 2003; Wojciechowska et al., 2015; Zmudczyska et al., 2012) or wind transport (Bokhorst et al., 2019; Hargan et al., 2017). We did not record correlations between Calanoida and Cladocera $\delta^{13}C$ values with sediment or phytoplankton $\delta^{13}C$. Our data do not suggest that seabird-released $\delta^{13}C$ is being assimilated up food webs, although sediment and phytoplankton $\delta^{13}C$ values are correlated.

The sediment data track record increasing $\delta^{13}C$ values over distance. Terrestrial primary producers (C3 plants) generally have higher $\delta^{13}C$ values than freshwater algae (-21 to -32‰ and -25 to -33‰, respectively) (Lamb et al., 2006). The lower sediment $\delta^{13}C$ values in the ponds nearest the seabird breeding community suggest more algal primary production in these ponds, whereas sediment in the further ponds is composed of more terrestrial plants. This indicates that increases in seabird nutrient loadings lead to increases in algal primary production in ponds.

Modeling isotope and nutrient movement

The Calanoida and phytoplankton $\delta^{15}N$ data, as well as the pH, TN, and Ca^{2+} water chemistry data as a function of distance were all best fit to power law models (Figure 3.2). The power law is described by the formula $y = \alpha \times x^{\beta}$ and is documented extensively in the natural world, describing the intensity of earthquakes, the number of species per genus of mammals, and gamma-ray solar flare intensities (Clauset et al., 2009). The Cladocera and sediment $\delta^{15}N$ data, and the conductivity, Mg^{2+}, K^+, and Na^+ water chemistry data as a function of distance were best fit to an inverse square law model, described by the formula $y = \alpha + \beta \times x^{-2}$ (Figure 3.2). The inverse square law is often used in *inverse distance weighting* (IDW), first described by Shepard (1968), which interpolates an unknown value at point *x* from known values at points *i* at different distances from *x*. Pollution modeling uses IDW to model pollution measurements from monitoring stations (de Mesnard, 2013). More research is needed at seabird colonies to determine the models of seabird nutrient movement away from colonies onto land. It is worth noting that Ca^{2+}, Mg^{2+}, K^+, and Na^+ are all also associated with sea-spray, confounding the indication that the elevated cation levels were due to seabird nutrient subsidies alone. CSM is ~100 m above sea level (Environment and Climate Change Canada, n.d.), so may be less susceptible to large sea-spray deposits. Metals, such as cadmium and zinc, are closely associated with top-trophic level marine organisms such as seabirds (Brimble et al., 2009). Future studies would be wise to measure sediment metal concentrations at CSM to further confirm seabird influence in these ponds.

Our pH data showed that seabird influence increased pond water pH, which has been recorded in other studies especially in Arctic regions where increases in primary production from seabird nutrients sequesters greater carbon dioxide in ponds, altering the inorganic carbon complex/equilibrium and driving up pH (Brimble et al., 2009). Interestingly, this is not always the case, as recorded in ponds surrounded by Leach's storm-petrels on Baccalieu Island in Atlantic Canada, where the presence of these birds decreased the pH within ponds (Duda et al., 2021). Our site differs from the Baccalieu Island sites in that there are substantially fewer birds (Baccalieu has over 3 million nesting pairs), the CSM ponds are shallower and much more abundant, and Baccalieu is covered in a much higher density of forests than CSM, thus ponds are less exposed to wind and wind-mixing (PAA, 2008; Duda et al., 2020). Our highest recorded pH was 7.5 in Pond 1 and the lowest pH was 4.1 in Pond 12 (Table 3.2). CSM is characterized by acidic plateau bogs (PAA, 2008), thus leading to more naturally acidic pond waters. Gannet guano has a pH of 5.13 ± 0.94 (Duda, M. 2023 unpubl. Data. Queen's University, Kingston, ON), much more acidic than black-legged kittiwake guano 8.77 ± 0.98 (Duda, M. 2023 unpubl. Data. Queen's University, Kingston, ON). It is likely that guano from the visiting black-legged kittiwakes is elevating the pH in Pond 1.

The pattern of movement was less clear for DOC, TIC, TP and TN. The presence of elevated levels of nitrogen and phosphorus are particularly diagnostic of seabird influence (Anderson & Polis, 1999; De la Peña Lastra, 2020), which were both highest in Pond 1. TN shows an overall decreasing trend fit to a power model until Ponds 11 and 12, where TN rises again with Pond 12 recording the second highest TN value. TP drops off after Pond 1, but spikes at Ponds 9, 11 and 12 (Figure 3.2). The DOC data were fit to an exponential model, but again Ponds 11 and 12 are driving the slope in the positive direction. As we have seen throughout this project, Ponds 11 and 12 seem to be far enough away from the seabird community that they are no longer influenced by seabird nutrient loadings. Further investigations need to occur to determine if Ponds 11 and 12 are being affected by factors not occurring inside the reserve, such as sheep grazing from nearby farms.

%N, %C, C:N and chlorophyll α

We recorded an increase in %N in the Calanoida dataset, but there were no statistically meaningful changes in %C, nor were there significant changes in %C or %N in the Cladocera or sediment datasets. In all these plots, we noticed that Ponds 11 and Pond 12 were pulling the slope either in the positive or negative direction, with opposing patterns in some cases (see Figure C.2 in Appendix C, for example). Again, Ponds 11 and 12 are outside of CSM boundaries and beyond the scope of seabird influence according to previous studies, so perhaps these ponds are reporting carbon sources extraneous to our study. Evidence from prior studies also suggests that %N and %C are not reliable indicators of seabird influence, which our data support (Anderson & Polis, 1999; Clyde et al., 2021; Kolb et al., 2010).

The C:N ratio of sediments increases with pond distance. C:N is a reliable measure that distinguishes algal sources (marine and lake) from terrestrial plants in sedimentary organic matter, with algae typically having an atomic C:N between 4 and 10 and terrestrial plants > 20 (Meyers, 1994). Our data indicate sediment in ponds nearest the seabird colony are composed of more algae than ponds further away from the colony, where the sediments are composed of more terrestrial primary producers. The data suggest that the nutrient additions from seabird waste is leading to more primary production within the algal communities in the ponds.

We also see a strong positive correlation between sediment C:N and $\delta^{13}C$ (p-value < 0.001, Figure 3.4b.). The ponds with the highest sediment C:N and $\delta^{13}C$ values are Ponds 11 and 12, indicating more terrestrial primary production than freshwater algal primary production components in the sediment in these two ponds than the ponds nearest the seabird community (Lamb et al., 2006). This does not support our hypothesis about $\delta^{13}C$, where we predicted that $\delta^{13}C$ would be higher in ponds with more seabird nutrient influence. Marine carbon is enriched in $\delta^{13}C$ relative to the terrestrial carbon source, CO_2 (Craig, 1953; Mackensen & Schmiedl, 2019). Our results indicate that enriched $\delta^{13}C$ is not assimilated into the freshwater ponds at CSM. Another explanation could be that any enriched $\delta^{13}C$ from marine carbon assimilation in pond ecosystems is being overpowered by the increased primary production of freshwater algae (due to the increase in available nutrients), which is less enriched in $\delta^{13}C$,.

Finally, chlorophyll *α* is also an indicator of primary production. Our study values ranged from 0.1099 (Pond 1) to 0.0023 mg/g (Pond 6) and stabilized around 0.0050 mg/g following a power law trend (AIC -60.67, null model -46.51). These results, along with C:N, suggest the most algal primary production in Pond 1 of the 12 ponds, directly supporting our hypothesis that increased seabird nutrient loadings results in increased primary production.

Pond 1 is enriched in $\delta^{13}C$ compared to Ponds 2 and 3, which does not follow the pattern of the rest of the ponds. A possible explanation is that enriched $\delta^{13}C$ values in gannet guano and/or kittiwake guano likely do enrich the $\delta^{13}C$ of sediments in Pond 1; however, increased autochthonous primary production in this pond from seabird nutrients likely contributes depleted $\delta^{13}C$ values to the sediment pool resulting in an intermediate $\delta^{13}C$ sediment value in Pond 1 relative to all ponds.

3.6 Conclusion and considerations

Seabird nutrients at Cape St. Mary's Ecological Reserve are being assimilated into terrestrial food webs and can be detected in ponds up to 500 m away from the "Bird Rock" seabird community. Our hypothesis aims to show that the nutrients are deposited via wind, however we did not directly measure this. Our assumptions were that since the seabirds nest on cliffs not within the catchment of the ponds, presumably the only way nutrients would assimilate into nearby ponds would be from wind transport. Of course, the presence of bathing kittiwakes in Pond 1 complicates the interpretation of our results; however, there were seabird signals in ponds further away from the colony than Pond 1, indicating direct deposit was not the only method of nutrient transport. Future studies should consider using an aerosol collector to directly measure airborne nutrients between the colony and ponds (see Bokhorst et al., 2007). Other than the C:N data, there is little evidence to support that ponds >1 km from Bird Rock are noticeably influenced by seabird nutrient subsidies, and ponds > 5 km from Bird Rock were outside the realm of seabird influence, making it difficult to compare the disparate Ponds 11 and 12 to the closer ponds. Seabird nutrients are enriched in δ^{15}N, and the zooplankton, phytoplankton, and sediment in the two ponds closest to the seabird community were similarly enriched in δ^{15}N. Increased seabird nutrients in ponds closest to the colony result in increased primary production. In conclusion, we demonstrate that seabird-derived marine nutrients are being incorporated into terrestrial aquatic food webs at a Newfoundland seabird community. This is especially important to a bog habitat like CSM, where soil nutrient availability is low. These data highlight the need to consider impacts to inland coastal ecosystems when discussing and managing the ongoing global decline of seabird populations.

Tables

Table 3.1. Summary of selected ponds including data collected during the day of sampling. All coordinates in UTM zone 21 T. Dates recorded as MM/DD/YYYY.

Pond ID	**Date of sampling**	**UTM Easting**	**UTM Northing**	**Distance to colony (km)**
Pond 1	09/12/2021	0715161	5188832	0.226
Pond 2	09/12/2021	0715258	5189031	0.414
Pond 3	09/12/2021	0715285	5189546	0.859
Pond 4	09/14/2021	0715558	5189707	1.122
Pond 5	09/14/2021	0716000	5189531	1.323
Pond 6	09/14/2021	0715804	5189793	1.343
Pond 7	09/14/2021	0716040	5190202	1.833
Pond 8	09/18/2021	0716380	5190387	2.153
Pond 9	09/18/2021	0716498	5190294	2.170
Pond 10	09/18/2021	0715963	5191123	2.518
Pond 11	09/18/2021	0717571	5193561	5.463
Pond 12	09/12/2021	0718008	5194577	6.558

Table 3.2. Measurements of chemical analytes in each pond. Measurements below the given reporting detection limit (RDL) are indicated (*) and transformed as follows: RDL*$2^{-0.5}$. TP = total phosphorus, TN = total nitrogen, DOC = dissolved organic carbon, TIC = total inorganic carbon, Ca^{2+} = calcium, Mg^{2+} = magnesium, K^{+} = potassium, Na^{+} = sodium, Cond. = conductivity.

Analyte	**TP (mg/L)**	**TN (mg/L)**	**DOC (mg/L)**	**TIC (mg/L)**	**Ca^{2+} (mg/L)**	**Mg^{2+} (mg/L)**	**K^{+} (mg/L)**	**Na^{+} (mg/L)**	**pH**	**Cond. (µS/cm)**
RDL mg/L	0.006	0.02	0.5	0.5	0.1	0.1	0.1	0.1	--	--
Pond 1	0.414	2.640	14.2	0.70	7.7	18.9	6.3	206.0	7.4	1,500
Pond 2	0.033	1.250	6.0	0.80	2.1	3.4	1.5	42.1	6.1	480
Pond 3	0.031	0.577	8.8	0.90	3.0	3.1	0.8	21.4	6.1	400
Pond 4	0.036	0.711	18.4	0.35*	3.9	8.7	1.6	57.2	7.1	460
Pond 5	0.042	0.798	12.2	0.35*	1.4	2.9	0.5	23.0	4.3	160
Pond 6	0.040	0.457	5.8	0.50	2.3	3.4	0.9	29.4	5.5	180
Pond 7	0.054	0.841	19.3	0.35*	4.7	3.0	0.9	23.2	6.0	160
Pond 8	0.050	0.451	14.3	1.60	3.7	2.4	0.6	20.1	6.7	120
Pond 9	0.028	0.574	8.2	0.35*	1.3	2.1	0.6	19.6	4.5	120
Pond 10	0.286	0.519	12.7	0.35*	2.7	2.2	0.5	21.9	5.6	130
Pond 11	0.283	0.738	24.1	0.35*	0.8	1.3	1.1	11.6	4.2	80
Pond 12	0.256	2.060	27.4	0.35*	1.2	1.9	0.5	12.7	4.1	110

Table 3.3. Summary of Pearson's correlation tests for $\delta^{15}N$ of Calanoida and Cladocera with sediment and phytoplankton. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

‡ only five ponds had data for both phytoplankton and Calanoida $\delta^{15}N$ (Ponds 1, 5, 9, 10 and 11)

	Calanoida (n = 8)		**Cladocera (n = 12)**	
	p	***r***	***p***	***r***
Sediment (n = 12)	< 0.001***	0.943	< 0.001***	0.938
Phytoplankton (n = 7)	0.105‡	0.798	0.036*	0.787

Figures

Figure 3.1. Cape St. Mary's (CSM) Ecological Reserve with study pond locations. CSM boundaries outlined in green. Avalon Peninsula in insert (a.). Photo of Bird Rock (b.) courtesy of author. Wind rose (c.) was created on www.windroseexcel.com

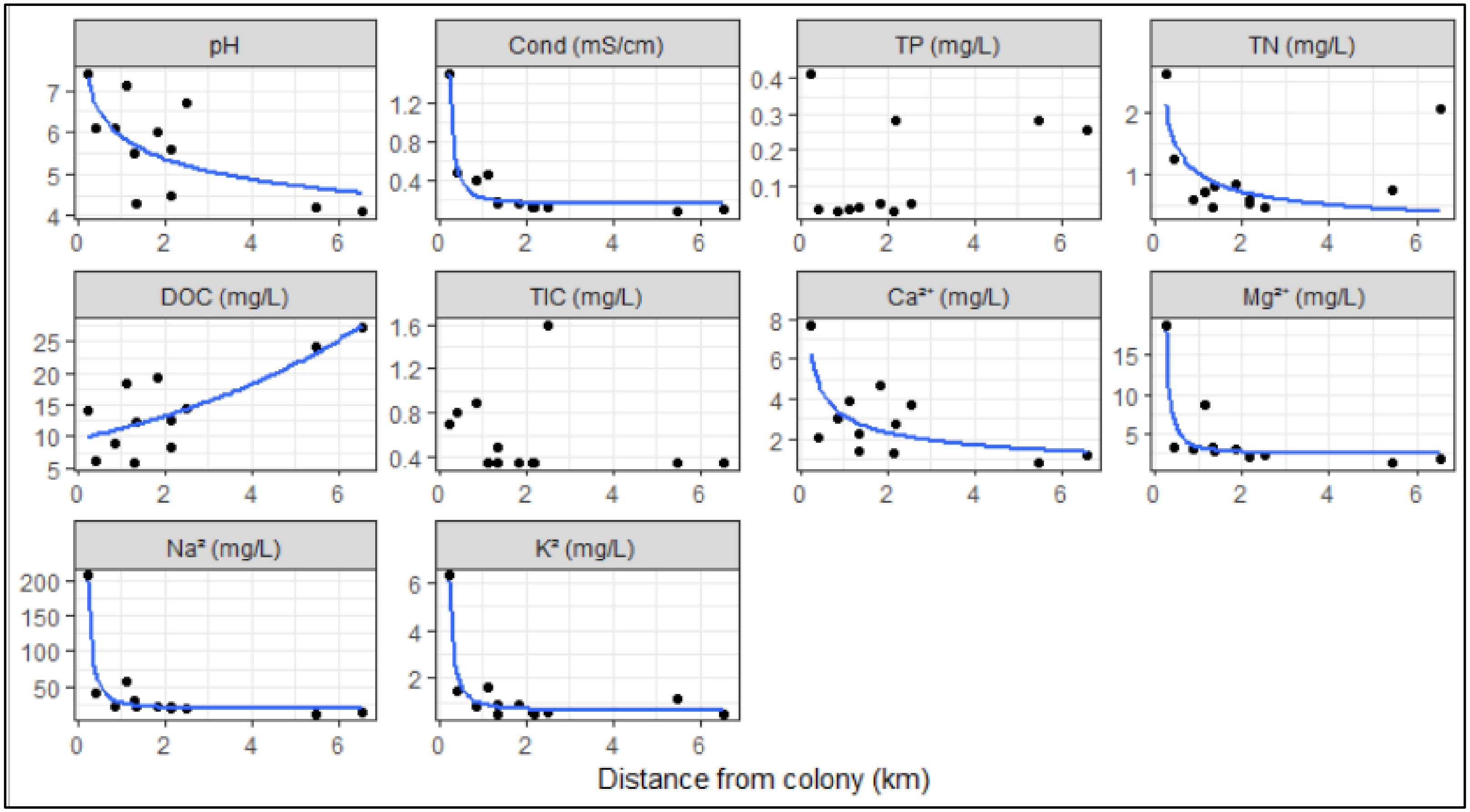

Figure 3.2. Visualizations of pH, conductivity, total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC), total inorganic carbon (TIC), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), and sodium (Na^{+}) as functions of distance from colony (km). DOC was best fit to an exponential model, pH, TN, and Ca^{2+} were best fit to a power law model, and conductivity, Mg^{2+}, Na^{+} and K^{+} were best fit to an inverse square law model. See Appendix F for full AIC tables for each analyte, and Appendix E for diagnostic plots.

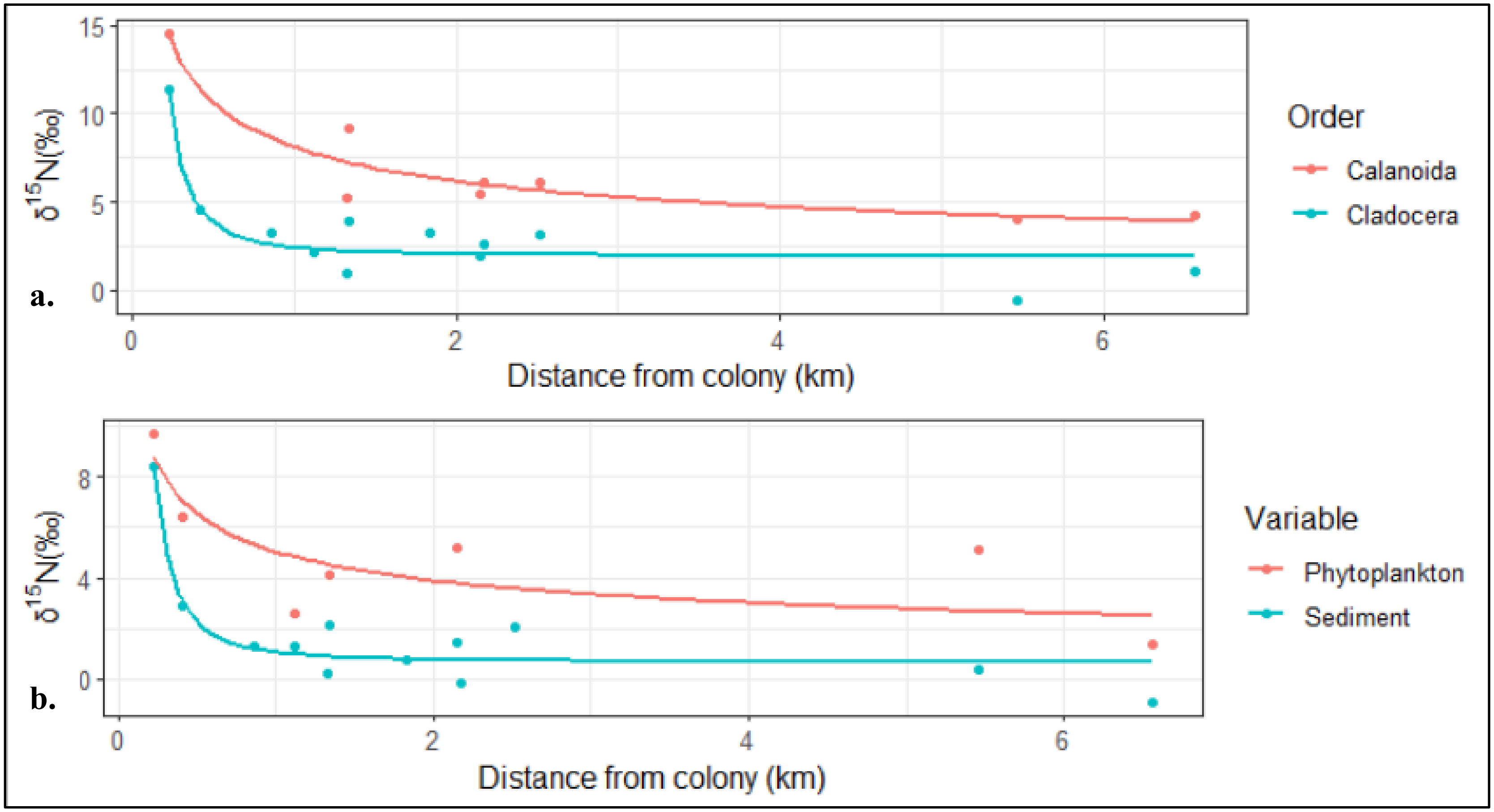

Figure 3.3. δ^{15}N values of Calanoida and Cladocera over distance from colony (a.) and sediment and phytoplankton over distance (b.). Calanoida and phytoplankton data are fit to a power law model, and Cladocera and sediment data are fit to an inverse square model.

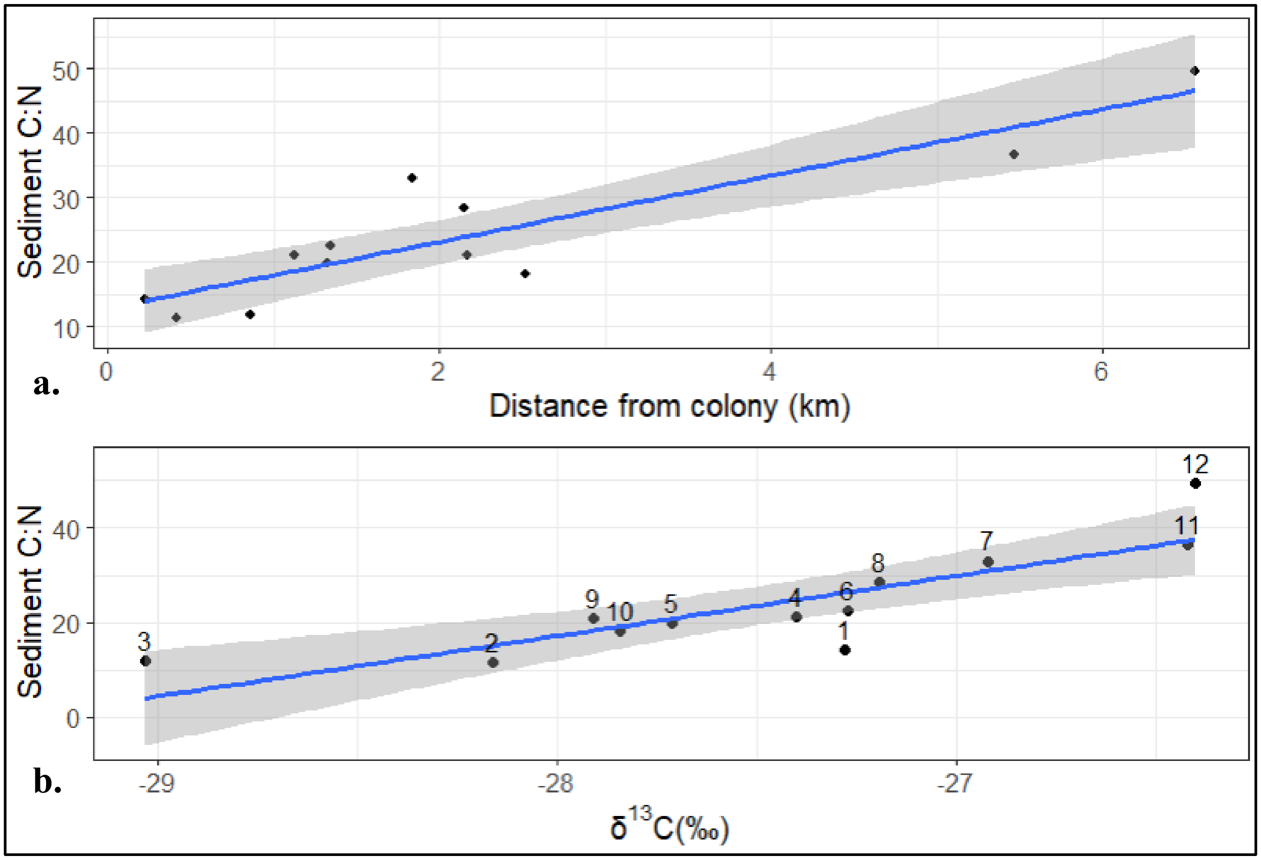

Figure 3.4. Sediment C:N over distance fit to a linear model (a) and sediment C:N correlated to δ^{13}C by pond number (b). 95% confident interval shaded in grey.

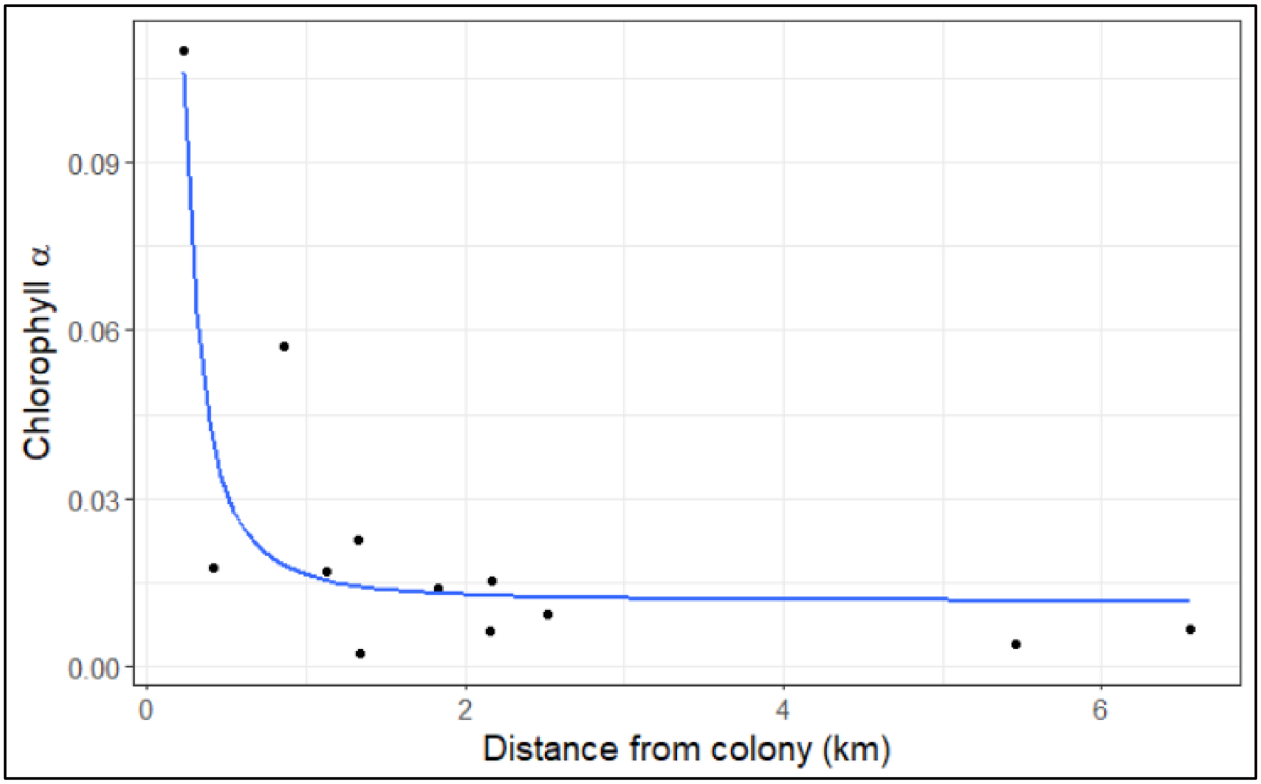

Figure 3.5. Chlorophyll *α* plotted over distance fit to an inverse square law model.

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Chapter 4. General Conclusions

Seabirds are one of the most threatened groups of birds in Canada and worldwide (NABCI, 2019). Their marine lifestyle is vulnerable to multiple destabilizing trends, including huge reductions in fish due to over-fishing (Wiley et al., 2013; Tasker et al., 2000) and the complex oceanographic changes linked to climate change (Duda et al., 2022; Hastings et al., 2020). It is crucial to investigate how seabirds are adapting to changing oceans, not only to predict population trends for future management, but to also predict how seabird population fluctuations will impact their role in global nutrient transport. I explore these themes using stable isotope analyses (SIA), a cost-effective tool used to investigate a range of ecological questions (Michener & Lajtha, 2007).

In this thesis, I used SIA to investigate temporal and spatial questions related to Newfoundland and Labrador (NL) seabird ecology: 1) are seabird trophic positions shifting in response to major food web disruptions (temporal)? and 2) how are seabird nutrient subsidies impacting freshwater ponds near colonies (spatial)? SIA of tissues allows for trophic analyses over longer periods of time, from days to weeks in blood, months in feathers, and years in bones. Metabolically inactive tissues (feathers, bones, claws etc.) "lock in" their isotope composition at the time when the tissues were grown, which opens potential for analyzing historic specimens, such as those preserved in museums (Hoenig et al., 2022). Furthermore, SIA allows for nutrient movement to be conducted via proxies, in the case of Chapter 3 where I investigated how seabird nutrients are assimilated by freshwater zooplankton.

In Chapter 2, I found evidence that NL seabird winter trophic positions (TP) are shifting in response to changes in food web dynamics over the past +120 years. The north-west Atlantic Ocean surrounding NL has been subjected to significant disturbances in the twentieth century, including the introduction of a highly efficient fishing vessel fleet in the 1950s and increasingly erratic oceanic temperature fluctuations starting in the 1970s, all of which has resulted in a regime shift (Burin et al., 2014). A regime shift is described as a significant change in the abundance and species composition of food webs within a habitat, taking place over decades (Beaugrand, 2004). This recent regime shift in the north-west Atlantic is characterized by a decrease in higher trophic species targeted by industrialized fishing, including Atlantic cod (*Gadus morhua*) and a decrease in mid-trophic forage fish species capelin (*Mallotus villosus*) due to an unusual cold water event in the early 1990s, ultimately leading to a food web dominated by smaller pelagic fishes and benthic invertebrates such as northern snow crab (*Chionoecetes opilio*) and northern shrimp (*Pandalus borealis*) (Buren et al., 2014; Frank et al., 2005; Hutchings & Myers, 1994; Pedersen et al., 2017). My seabird diet data are from the winter, and we can extrapolate fish surveys from other seasons to inform about overall winter conditions.

Relative trophic position changes of puffins and petrels over time were determined by calculating the difference in $\delta^{15}N_{AA}$ of glutamic acid and phenylalanine. Atlantic puffins' relative TP increases significantly from 1899-1929 to 1930-1959, although caution should be applied when interpreting these results because there were only three samples in the 1899-1929 time bin. Puffin relative TP decreased from 1930-1959 through 2021, albeit not significantly. Petrel relative TP did not change over time. We did not measure $\delta^{15}N_{AA}$ for the murre for the murre and razorbill samples, so we could not estimate relative TP. However, murres and razorbills stay in the northwest Atlantic during the winter (Chapdelaine et al., 2001; Hedd et al., 2011; Huettmann et al., 2005; McFarlane Tranquilla et al., 2013), similar to puffins (Fayet et al., 2017). The puffin $\delta^{15}N_{Phe}$ values do not change over time (although there was considerable variation), thus we cautiously interpret bulk $\delta^{15}N$ of murres and razorbills as a proxy for TP. Both murre and razorbill data record decreasing $\delta^{15}N$ over time, indicating TP is decreasing for both species.

We interpret lower TP in puffins, murres and razorbills as an indication that the modern birds are feeding on lower-trophic level fish and/or more invertebrates than they were historically. Murres and razorbills are deep-diving piscivores, although less is known about their winter diet (Elliot et al., 1990; Huettmann et al., 2005; Montevecchi et al., 2019; Pratt et al., 2017). Puffins are considered generalist feeders, although no data exist on winter diets for North American puffin populations (Hedd et al., 2010). Leach's storm-petrels (*Hydrobates leucorhous*) appear to have stable winter diets throughout our study time series, showing that either their preferred prey species populations have remained stable, or they are able to substitute with prey in a similar trophic position.

In Chapter 3 we investigated to what extent seabird marine nutrient subsidies are being incorporated into freshwater pond food webs near the bird community at Cape St. Mary's Ecological Reserve (CSM). I recorded elevated values of seabird associated nutrients (N, P, K^+, Ca^{2+}, Mg^{2+}, and Na^+) in the pond closest to the seabirds, decreasing to a lower, stable level in the further ponds. Additionally, there is evidence that seabird nutrients are assimilating into the pond food webs, as evidenced by the elevated $\delta^{15}N$ values in phytoplankton, sediments, and zooplankton at the ponds closest to the seabirds.

Major changes in NL seabird diets could be consequential to the biology and chemistry at their breeding grounds. In this study system, I imply that any changes in feeding choices at-sea will show up in freshwater ponds at CSM as lowered $\delta^{15}N$ values in sediment, phytoplankton, and zooplankton. It is unclear whether nutrient composition would change because of differing diets; however, if diet choices are leading to seabird populations with poorer health or lower numbers, it is reasonable to believe that fewer seabird nutrients will be incorporated into the ponds and surrounding soils and flora near the seabird community.

4.1 Model selection approach

I used the *model selection* approach to analyze pond nutrient and isotope data in Chapter 3. Model selection creates several interpretable models for the system being studied and analyzes the likelihood of these competing models (Johnson & Omland, 2004). Traditional null hypothesis testing assumes a null model where the researcher creates a null hypothesis that, in many cases, is surely false before any evidence is collected (Anderson et al., 2000). I based our selection on several models found in the natural world: the power law ($y = \alpha \times x^{\beta}$), used to describe the intensity of earthquakes, the number of species per genus of mammals, and gamma-ray solar flare intensities (Clauset et al., 2009), and an inverse square law ($y = \alpha + \beta \times x^{-2}$), used in pollution modeling (Shepard 1968; de Mesnard, 2013). The results show nutrient and isotope movement out from the source (seabird community), reaching a lower, stable value before 500 m. Many of our nutrients and isotope systems fit into the power law and inverse square law models. Researchers should consider using these modeling frameworks in other seabird colony ecosystems.

4.2 Future research

Both bulk and amino-acid specific SIA are dynamic techniques in ecology, and there are many more questions to be answered in my study system. One possibility to further this study would be to include prey in a dietary proportion study using Bayesian mixing-models (Stock et al., 2018). These analyses need to include many data that I did not have for my study: species-specific trophic discrimination factors (TDF) for any possible prey species, isotope values of prey species caught in the winter, and historical isotope values of prey species. There is a dearth of knowledge of what seabirds eat during the winter, when many species migrate to offshore wintering grounds (Gómez et al., 2018). It would be prudent to first gather winter diet information for these seabirds via stomach sampling, then use those data to inform what prey species to include in Bayesian mixed-modeling; otherwise, one could create a model that applies to extremely unlikely prey sources (as the author of this thesis initially did!).

Future studies of seabird nutrients at CSM could go in many different directions. First, this study was a quick snapshot of nutrients and isotopes over a period of a few days. Taking samples at multiple times of the seabird breeding and non-breeding season could answer questions about the permanence of seabird nutrients and isotopic enhancements in the surrounding freshwater habitats. Another limit to my study was the location of the ponds. Sampling vegetation or soil along a transect moving away from the seabird community would give researchers greater freedom to measure nutrients and isotopes along a fixed gradient. This study also lacked species composition data. One interesting question that could be asked is the presence or absence of carnivorous plants near the seabird community. With overall nutrient poor and acidic soils, CSM is home to several carnivorous plant species, including sundews *Drosera* spp*.,* and purple pitcher plant *Sarracenia purpurea,* the provincial emblem of NL. With increasing soil seabird derived nutrient subsidies, especially nitrogen, we could hypothesize that these plants would be less abundant than they would be further away from enhanced nutrient source.

4.3 Research implications

My research provides evidence of a changing diet of three diving piscivorous auk species over 120 years, supporting reports of decreased fish biomass in the north-west Atlantic (Frank et al., 2005; Myers et al., 1997; Rose, 2003; Trites et al., 2006). These species appear to be shifting to a lower trophic level invertebrate dominated diet, coinciding with worsening chick conditions (Baillie & Jones, 2004; Davoren & Montevecchi 2003), although their populations appear stable. Our results imply some diet plasticity, but continued population monitoring is crucial to assess the consequences. This study also records large depositions of seabird nutrients at ponds near the seabird community at CSM, which are assimilated up freshwater food webs. As major transporters of marine nutrients to terrestrial ecosystems (Anderson & Polis, 1999; 2016; Otero et al., 2018), changes in seabird diet will have far reaching biological and chemical changes at and around their colonies. This study provides data for both researchers and managers working in seabird conservation and considers broader terrestrial implications of declines in seabird populations.

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Appendix A: Chapter 2, supplemental tables and figures

Table A.1: Comparison of $\delta^{13}C_{AA}$ values of chicken feathers pre-treated with bleach and methanol.

	Methanol Treatment					**Bleach Treatment**				
	1	**2**	**3**	**Mean**	**Std. Dev.**	**1**	**2**	**3**	**Mean**	**Std. Dev.**
Ala	-12.6	-11.7	-10.5	-11.6	**1.0**	-9.6	-11.9	-14.2	-11.9	**2.3**
Val	-27.9	-27.0	-26.8	-27.2	**0.5**	-25.8	-27.4	-28.3	-27.1	**1.3**
Gly	-15.2	-14.3	-14.0	-14.5	**0.6**	-13.6	-15.7	-17.0	-15.4	**1.8**
Leu	-29.7	-29.9	-29.4	-29.7	**0.2**	-28.2	-30.0	-30.2	-29.5	**1.1**
Ileu	-24.9	-25.5	-24.9	-25.1	**0.4**	-25.2	-25.4	-26.1	-25.6	**0.5**
NorLeu	-29.4	-28.7	-29.1	-29.0	**0.4**	-28.4	-29.3	-29.7	-29.1	**0.7**
Pro	-22.5	-21.0	-21.7	-21.7	**0.7**	-20.6	-21.8	-22.9	-21.8	**1.1**
Thr	-14.6	-14.6	-15.5	-14.9	**0.5**	-14.2	-12.5	-15.6	-14.1	**1.5**
Asp	-17.6	-15.7	-16.1	-16.5	**1.0**	-15.0	-17.4	-18.8	-17.0	**1.9**
Ser	-9.8	-9.1	-9.3	-9.4	**0.4**	-6.0	-9.9	-10.2	-8.7	**2.3**
Glu	-13.6	-12.3	-12.6	-12.8	**0.7**	-10.9	-12.8	-13.7	-12.5	**1.4**
Phe	-27.5	-26.9	-27.0	-27.2	**0.3**	-25.8	-27.0	-27.2	-26.7	**0.8**
Tyr	-25.3	-24.0	-23.7	-24.3	**0.9**	-23.5	-24.9	-24.6	-24.3	**0.8**
Lys	-20.1	-21.2	-19.8	-20.3	**0.7**	-20.2	-20.5	-23.0	-21.2	**1.5**

Table A.2: AA mixture used for $\delta^{15}N_{AA}$ analysis. Individual AAs were weighed out into a 25 mL volumetric flask and filled to 25 mL with 0.01M HCl to create AA standards.

Amino Acid	**Ala**	**Val**	**Gly**	**Leu**	**Ile**	**Pro**	**Thr**	**Asp**	**Ser**	**Glu**	**Phe**	**Tyr**	**Lys**
Mass (mg)/ 25 mL 0.01 M HCl	8	15	18	13	8	25	12	15	19	27	9	5	5

A 1-factor Kruskal-Wallis test revealed differences between species for $\delta^{15}N$ ($p < 0.001$) and $\delta^{13}C$ ($p < 0.001$), while the post-hoc Dunn test further explained differences in groups: Figures A.1 and A.2, Tables A.3 and A.4.

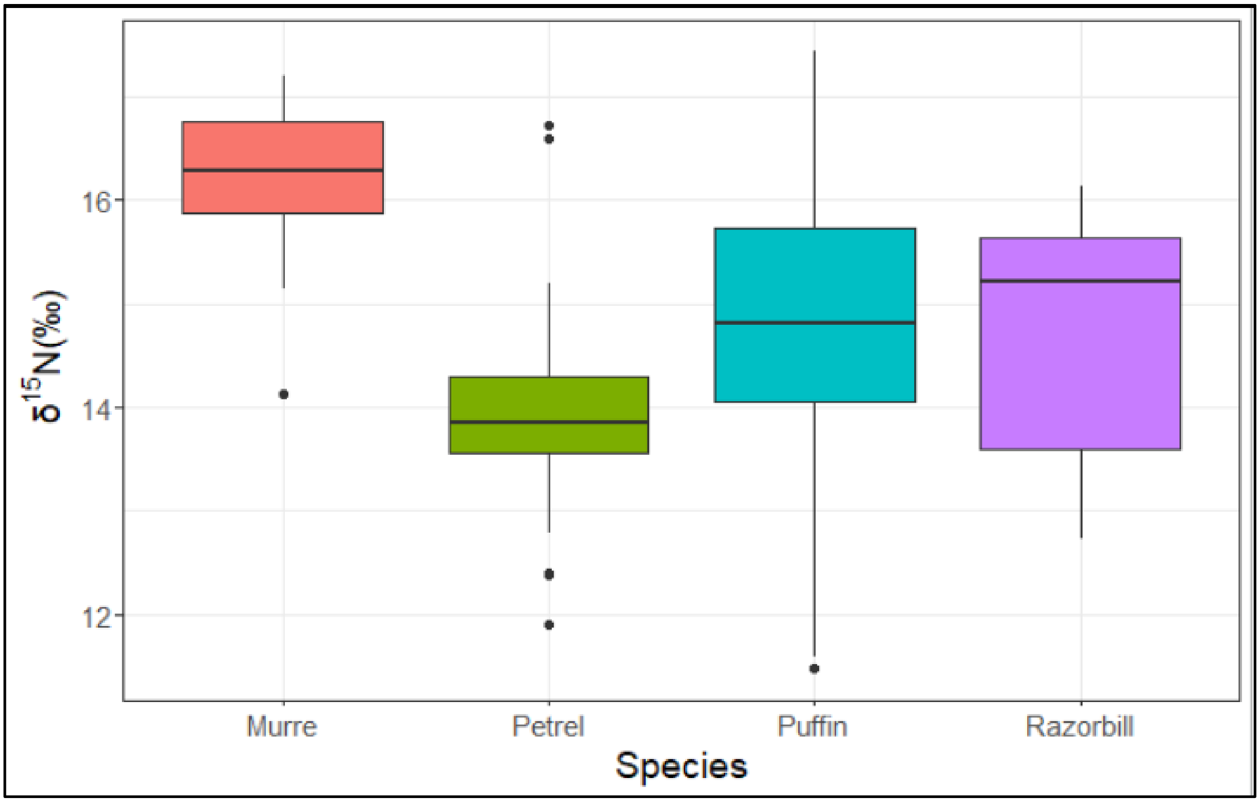


Figure A.1. Mean bulk $\delta^{15}N$ by species.

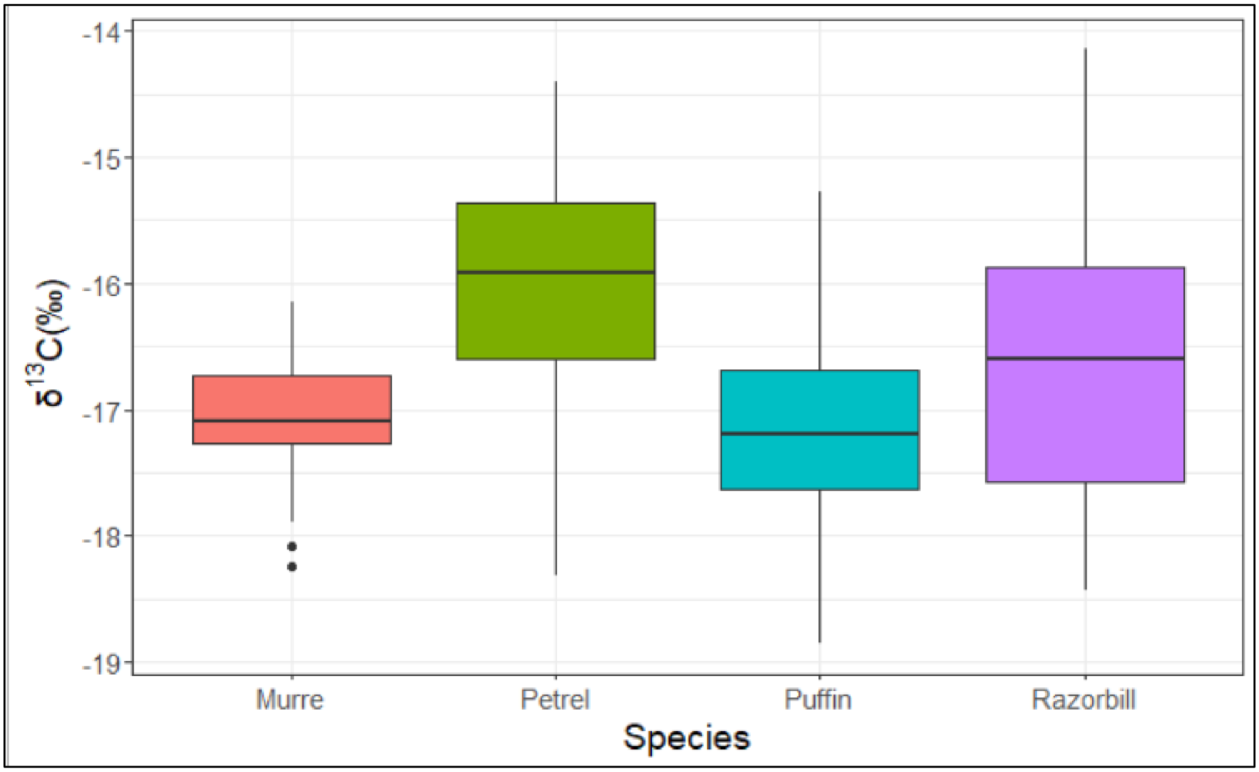


Figure A.2. Mean bulk $\delta^{13}C$ by species.

Table A.3. Mean bulk $\delta^{13}C$ and $\delta^{15}N$ for all species over time.

	$\delta^{13}C$ (‰)	**$\delta^{15}N$ (‰)**
Petrel	-16.1	13.9
Puffin	-17.2	14.7
Murre	-17.1	16.2
Razorbill	-16.7	14.7

Table A.4: Summary of Dunn test results of bulk $\delta^{15}N$ and $\delta^{13}C$ differences between species.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	$\delta^{15}N$			$\delta^{13}C$		
	Petrel	**Puffin**	**Murre**	**Petrel**	**Puffin**	**Murre**
Petrel						
Puffin	0.033*			< 0.001***		
Murre	< 0.001***	< 0.001***		< 0.001***	1	
Razorbill	0.284	1	0.006**	0.299	0.998	1

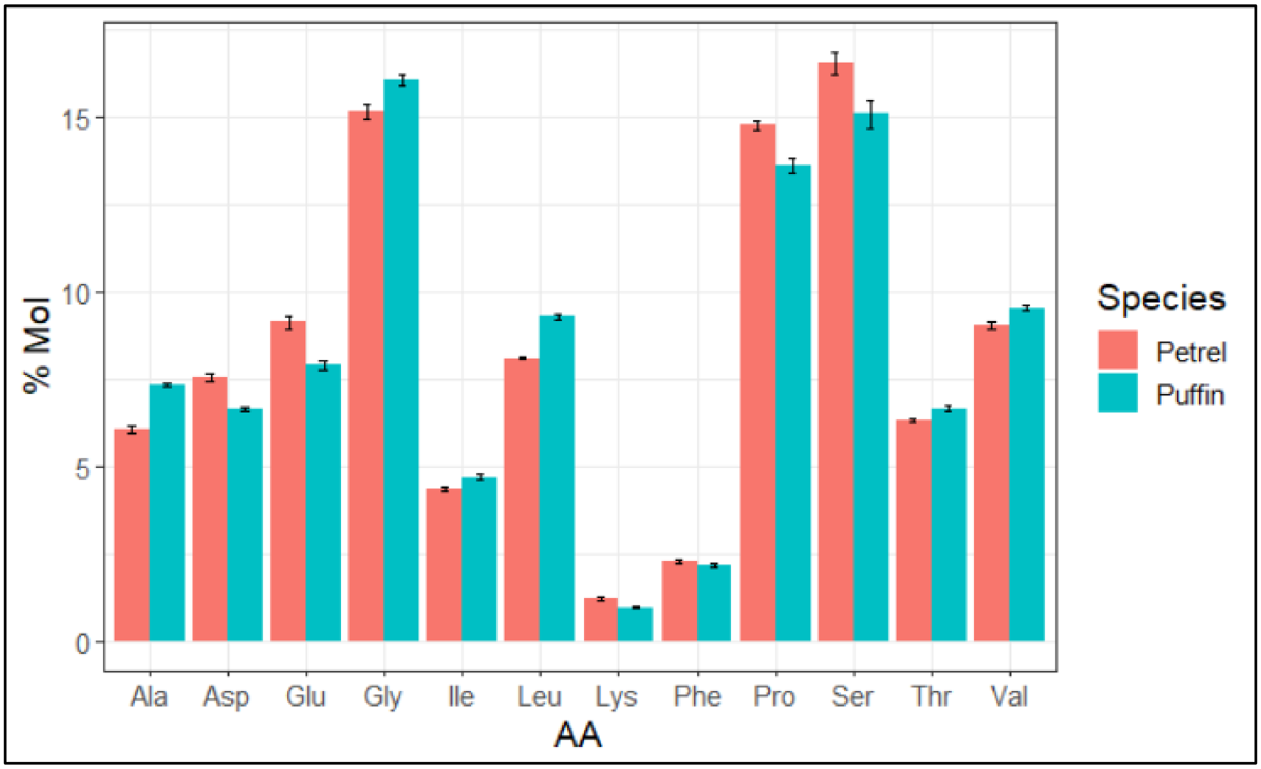


Figure A.3. Concentration of amino acids in puffin and petrel feathers analyzed for $\delta^{15}N_{AA}$, measured in %Mol.

Table A.5. Select stable isotope ($\delta^{15}N$ and $\delta^{13}C$) values from select prey species of auk seabirds in NL

Prey	**n**	**$\delta^{15}N$**	**$\delta^{13}C$**	**Source**
Copepod	5	7.61 ± 0.22	-23.13 ± 0.10	Jenkins & Davoren, 2021
Euphausiid	5	7.67 ± 0.16	-21.97 ± 0.17	Jenkins & Davoren, 2021
Euphausiid	3	9.3 ± 0.66	-20.6 ± 0.23	Sherwood & Rose, 2005
Capelin *Mallotus villosus*	15	12.12 ± 0.54	-20.63 ± 0.29	Jenkins & Davoren, 2021
Capelin *Mallotus villosus*	21	12.2 ± 0.09	-21.0 ± 0.10	Sherwood & Rose, 2005
Snailfish *Liparis* spp.	10	10.33 ± 0.57	-20.78 ± 0.42	Jenkins & Davoren, 2021
Dusky snailfish *Liparis gibbus*	5	12.6 ± 0.43	-20.3 ± 0.17	Sherwood & Rose, 2005
Sandlance *Ammodytes* spp.	9	10.11 ± 0.26	-21.19 ± 0.18	Jenkins & Davoren, 2021
Small fish*	23	9.26 ± 0.09	-22.58 ± 0.03	Jenkins & Davoren, 2021
Atlantic cod *Gadus morhua*	155	14.3 ± 0.05	-19.0 ± 0.04	Sherwood & Rose, 2005
Polychaetes	2	16.6 ± 1.49	-18.0 ± 0.38	Sherwood & Rose, 2005
Northern shrimp *Padalus borealis*	20	11.4 ± 0.30	-18.2 ± 0.12	Sherwood & Rose, 2005

* “Small fish” defined as fish < 100 mm in length.

Appendix B: Chapter 2, diagnostic plots

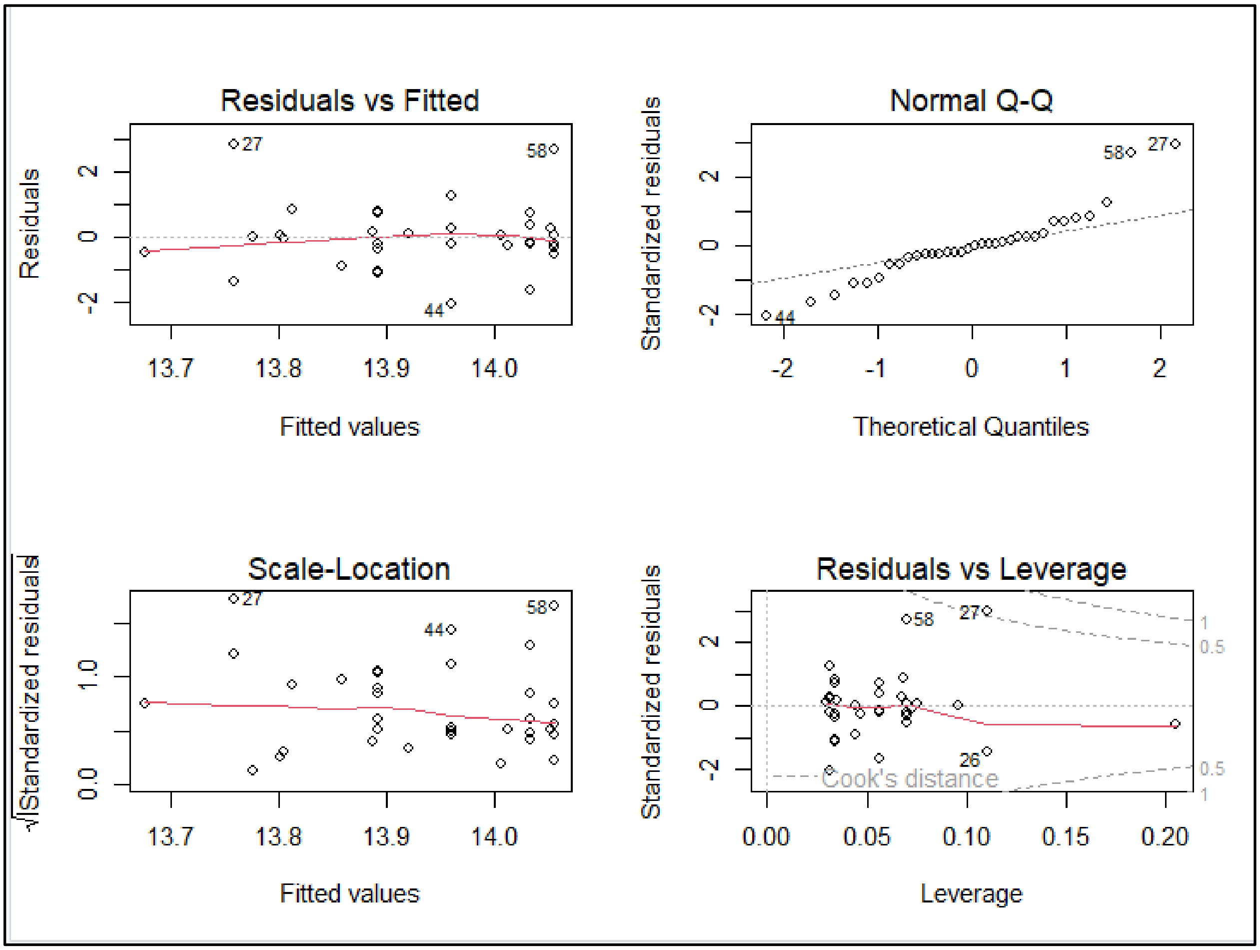


Figure B.1. Petrel δ^{15}N, linear model

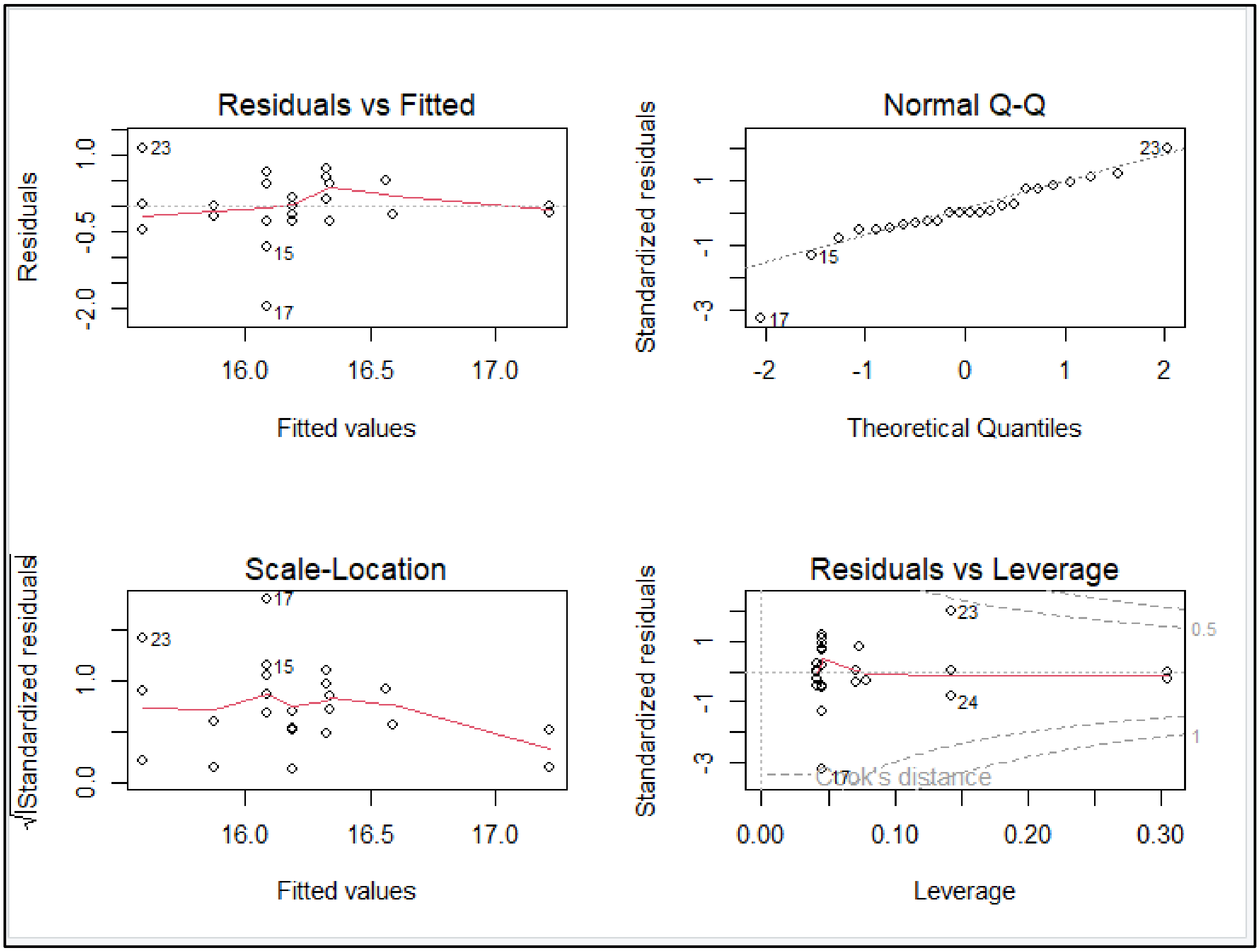


Figure B.2. Murre δ^{15}N, linear model

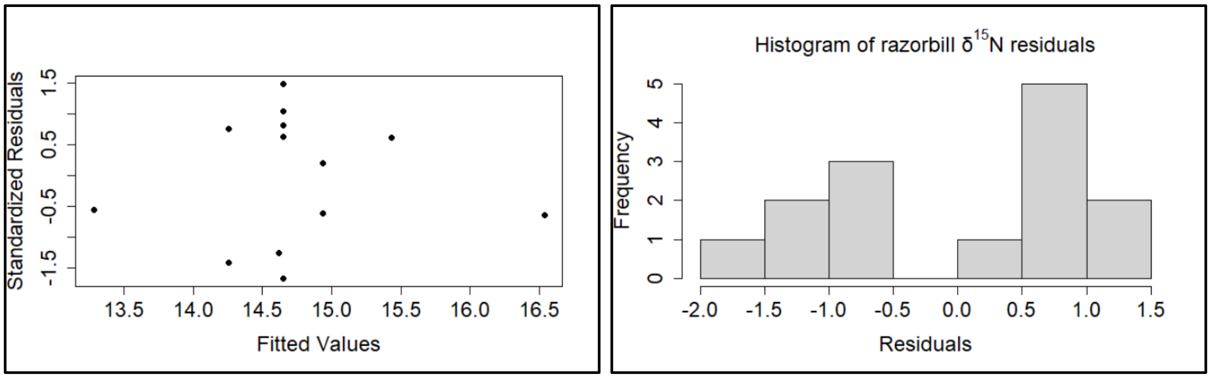


Figure B.3. Razorbill δ^{15}N, GAM

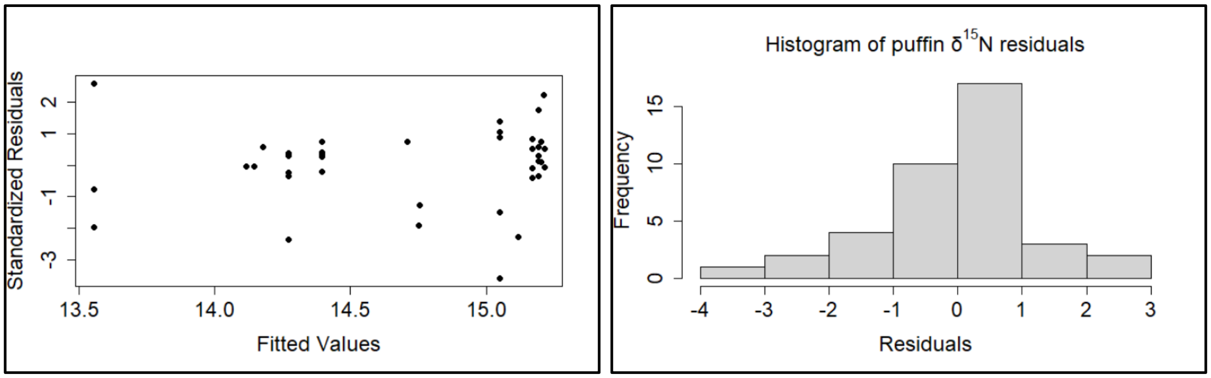


Figure B.4. Puffin δ^{15}N, GAM

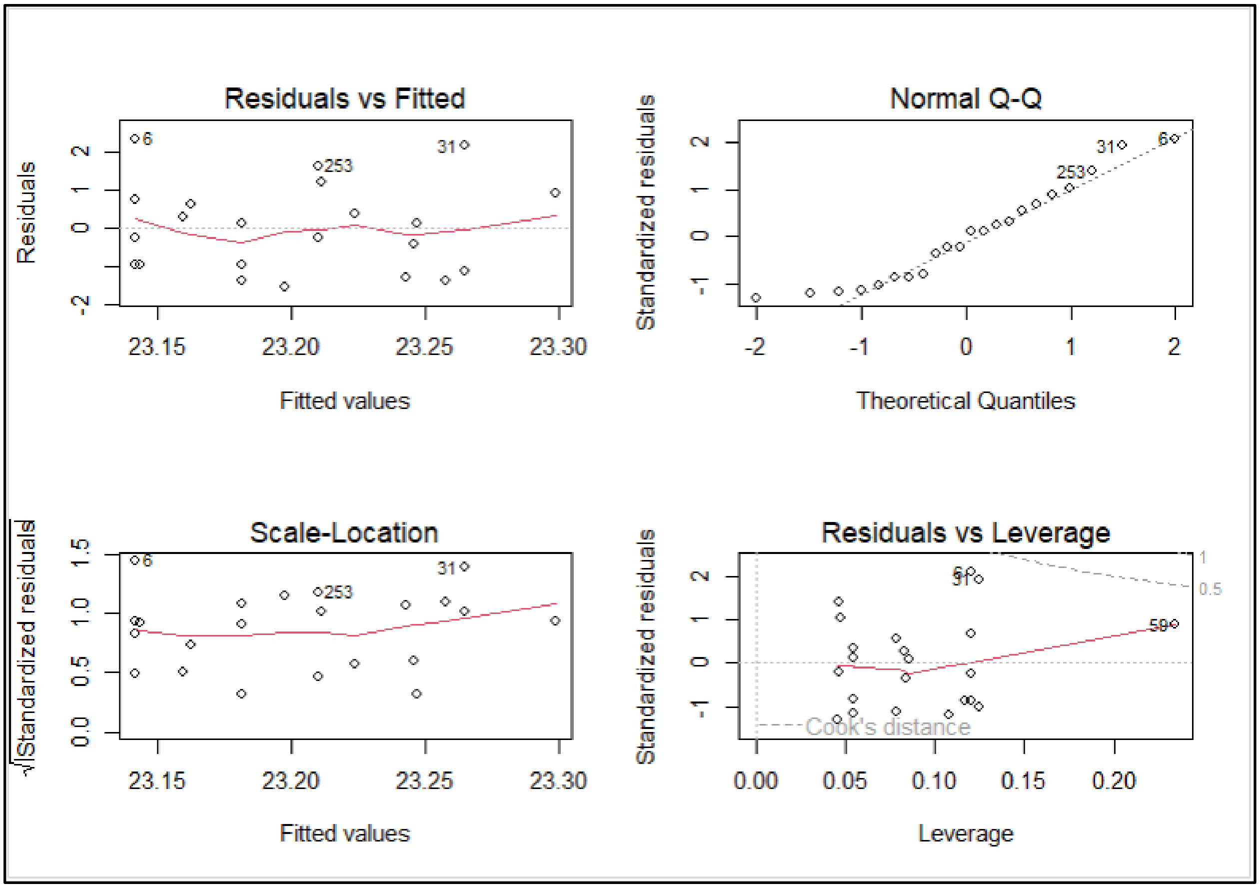


Figure B.5. Petrel glutamic acid δ^{15}N, linear model

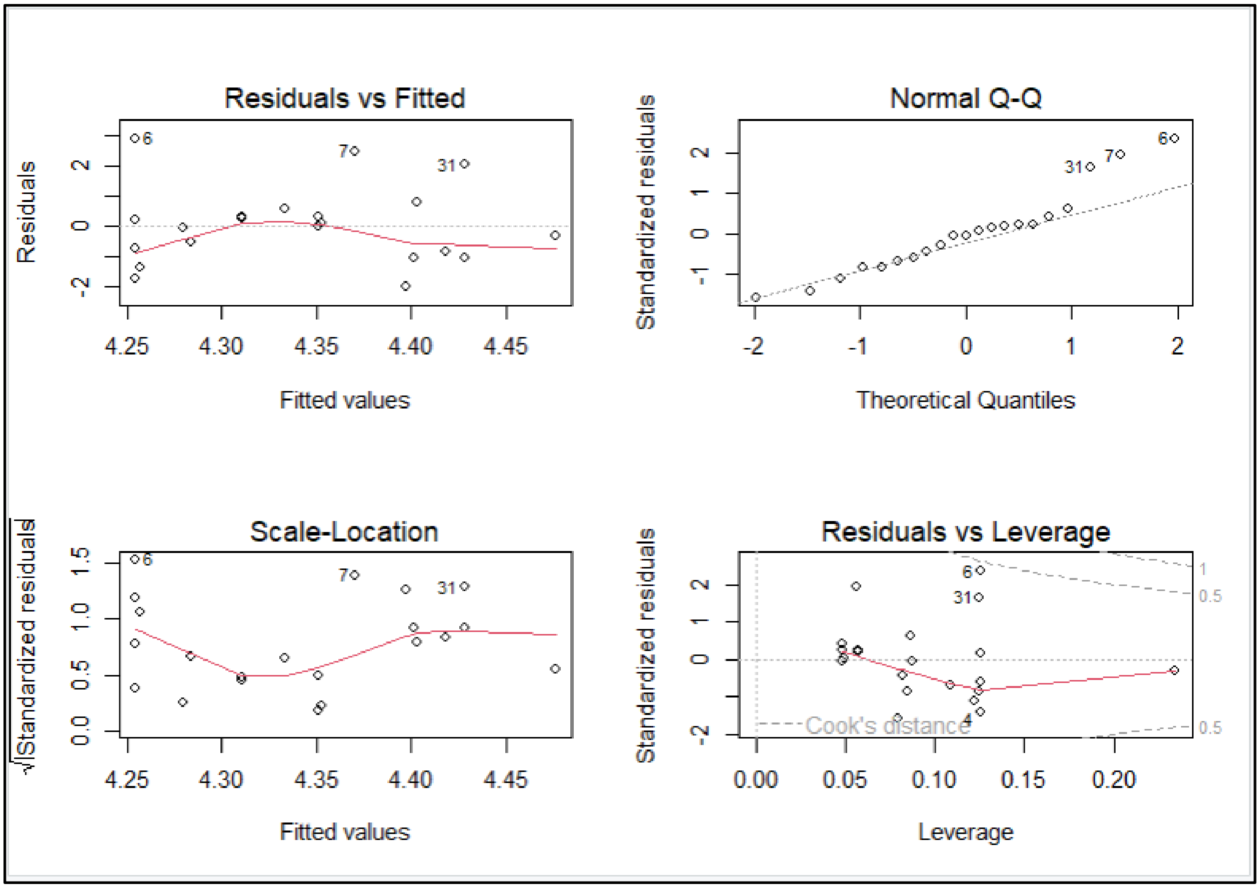


Figure B.6. Petrel phenylalanine δ^{15}N, linear model

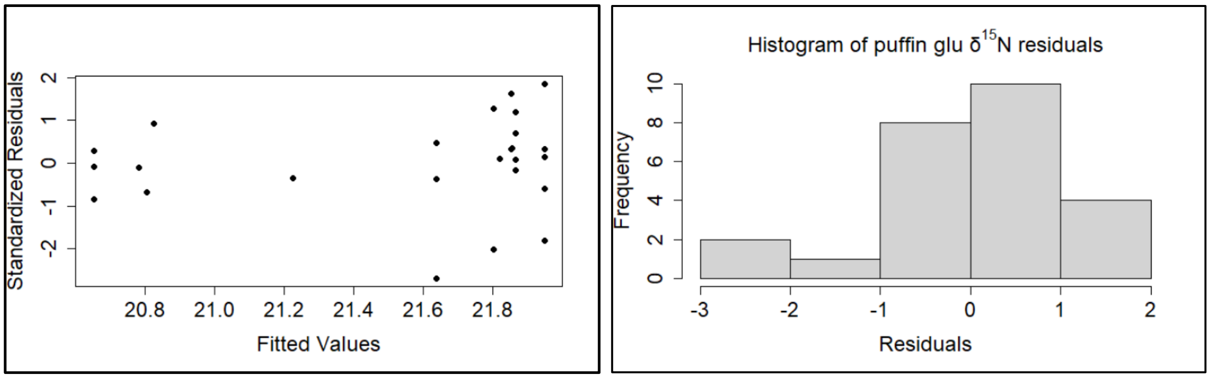


Figure B.7. Puffin glutamic acid δ^{15}N, GAM

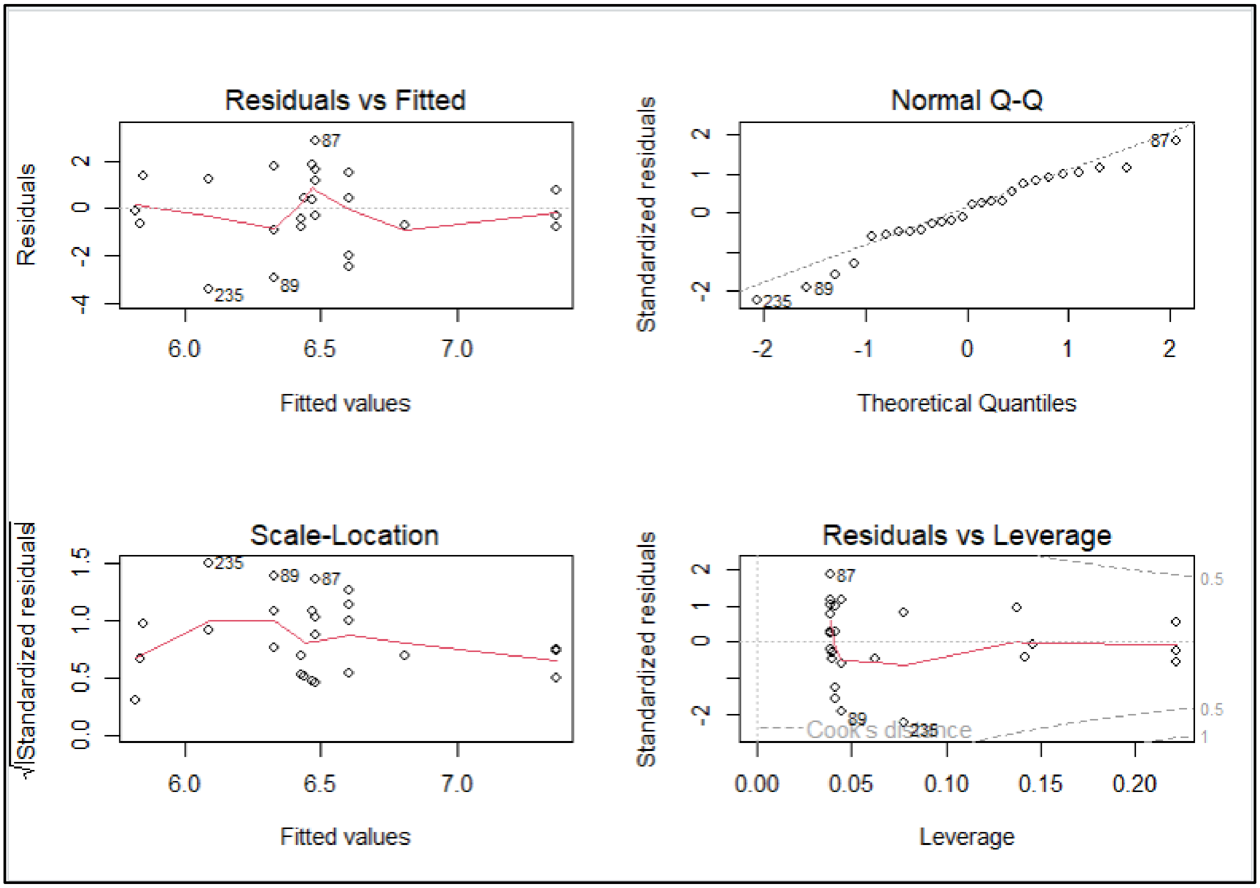


Figure B.8. Puffin phenylalanine δ^{15}N, linear model

Appendix C: Chapter 3, supplemental tables and figures

Table C.1. Water collection bottles

Analyte	**Size of bottle (mL)**	**Preservative**	**Laboratory**
Total nitrogen (TN)	100	H_2SO_4	Bureau Veritas
Total phosphorus (TP)	200	H_2SO_4	AGAT
Dissolved organic carbon (DOC)	100	NA	AGAT
Total inorganic carbon (TIC)	100	NA	AGAT
Cations (Ca^{2+}, Mg^{2+}, K^+, Na^+)	200	HNO_2	AGAT

Table C.2. Phytoplankton filter worksheet

ID	**Vol H_20 filtered (mL)**	**Avg. dry wt (g)**	**Dry wt w/ algae (g)**	**Phyto on filter (g)**	**Phyto on filter (mg)**	**Phyto per mg/cm^2**	**mg/one hole punch**	**# of punches**
CSM 1	470	0.0946	0.2871	0.1925	19.25	1.266	0.358	4
CSM 2	1000	0.0946	0.2822	0.1876	18.76	1.234	0.349	4
CSM 3	1000	0.0946	0.2817	0.1871	18.71	1.231	0.348	4
CSM 4	1000	0.0946	0.2687	0.1741	17.41	1.145	0.324	4
CSM 5	1000	0.0946	0.2716	0.177	17.7	1.164	0.329	4
CSM 6	1000	0.0946	0.2828	0.1882	18.82	1.238	0.350	4
CSM 7	500	0.0946	0.2732	0.1786	17.86	1.175	0.332	4
CSM 8	1000	0.0946	0.2807	0.1861	18.61	1.224	0.346	4
CSM 9	1000	0.0946	0.2809	0.1863	18.63	1.225	0.346	4
CSM 10	1000	0.0946	0.2798	0.1852	18.52	1.218	0.344	4
CSM 11	500	0.0946	0.2742	0.1796	17.96	1.181	0.334	4
CSM 12	300	0.0946	0.2868	0.1922	19.22	1.264	0.357	4

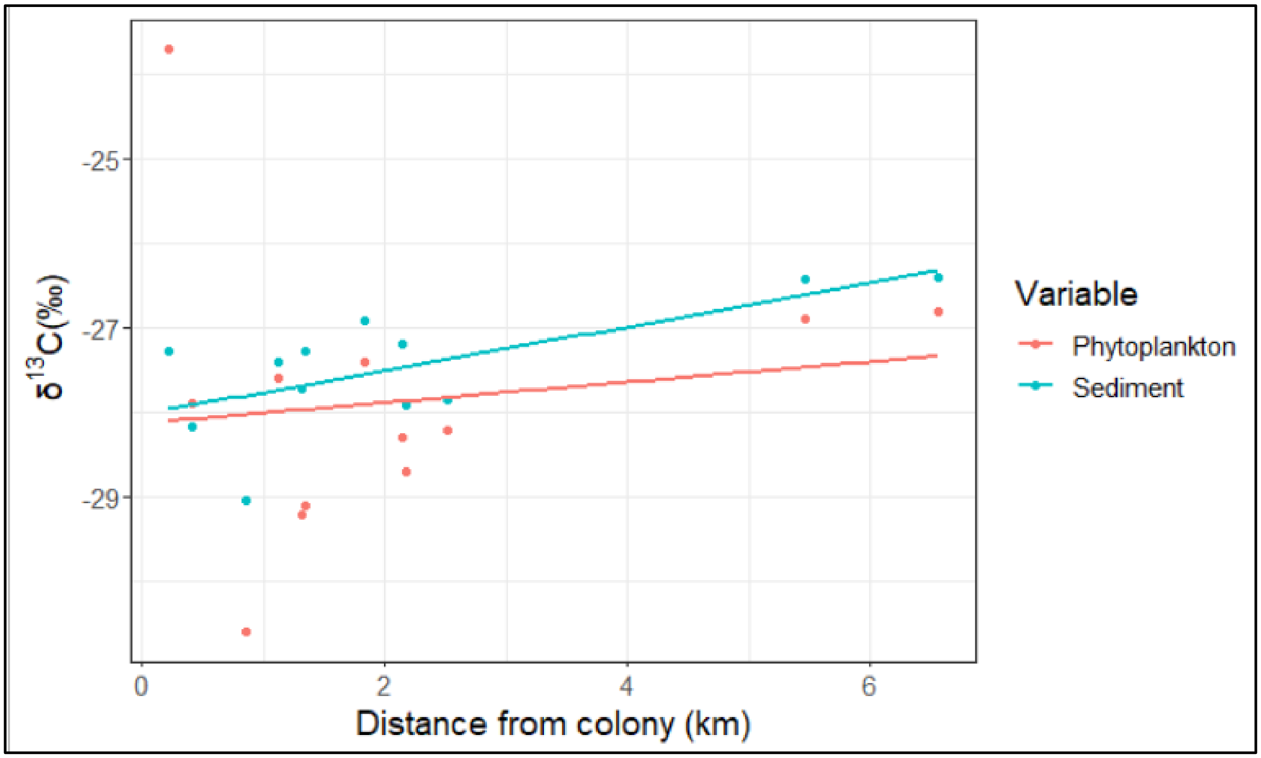


Figure C.1. $\delta^{13}C$ of sediment data over distance, fit to a linear regression model. Confidence intervals left out due to large residuals.

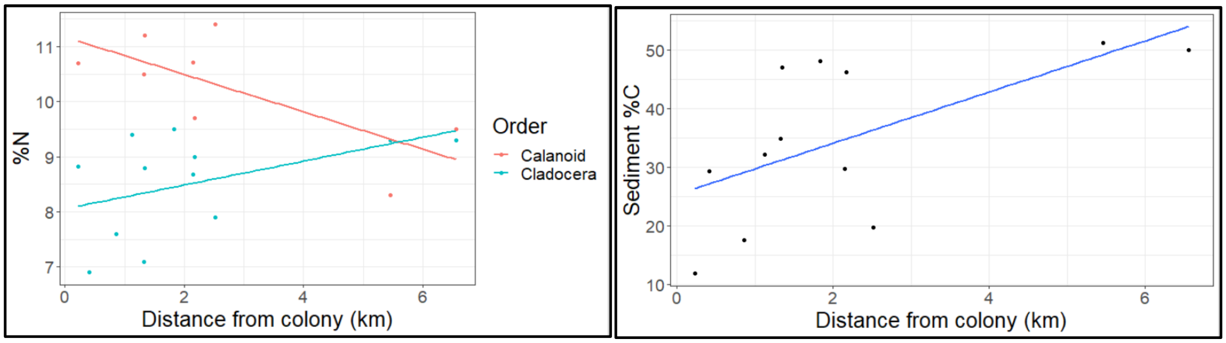


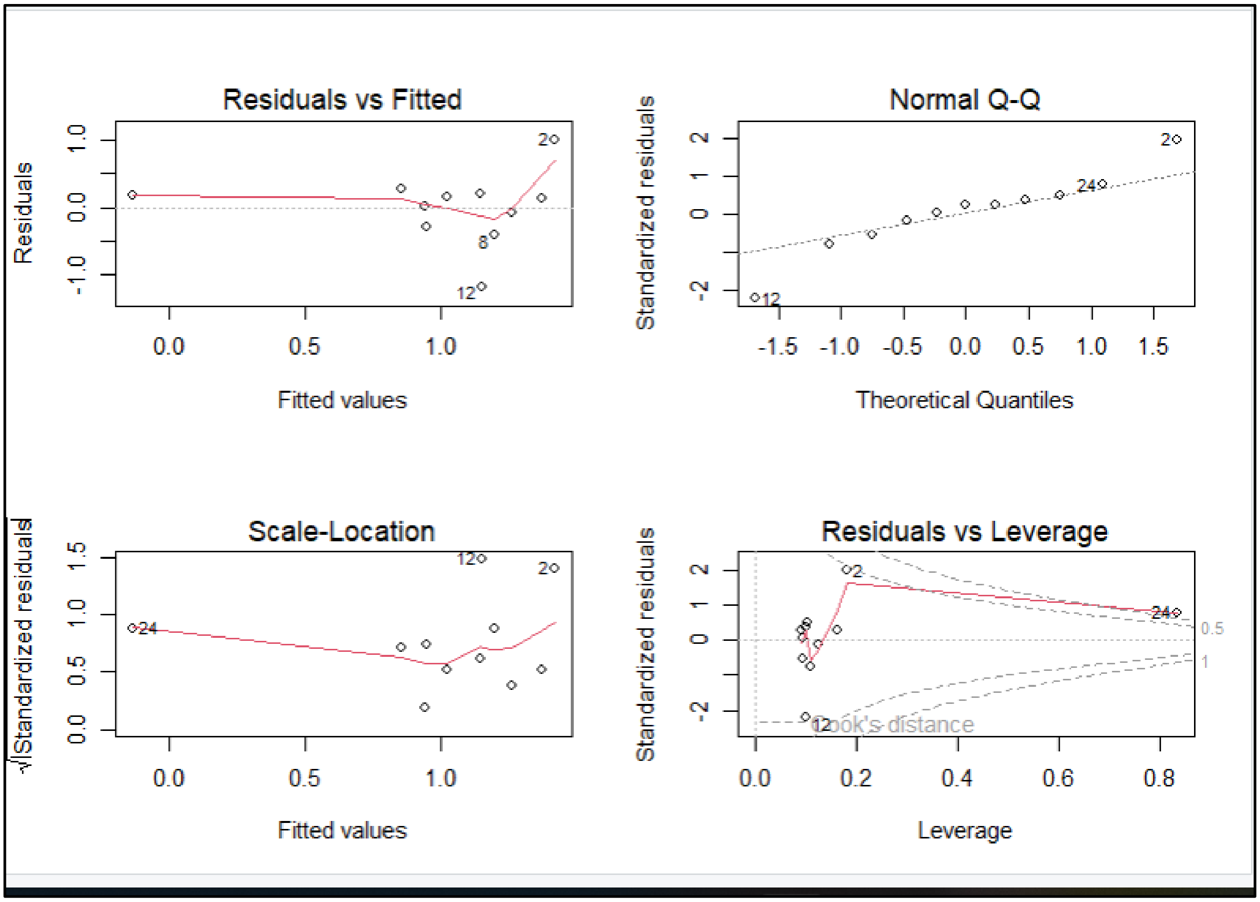
Figure C.2. %N of Calanoida and Cladocera, and %C of sediment. Notice that the furthest Ponds 11 and 12 seem to be driving the slope.

Appendix D: Chapter 3, model selection

Our strategy for data analysis was the *model selection* approach, which allowed us to test several hypotheses to find one that fit the best statistically, while also being the most interpretable in our system. The model selection approach differs from the traditional null hypothesis testing, where the researcher creates a null hypothesis that, in many cases, is surely false before any evidence is collected (see Anderson et al., 2000, for general examples of a priori false null hypotheses). In this approach, a rejection of a null hypothesis is good enough for an acceptance of an alternative hypothesis, even though the rejection says nothing about how well the alternative model supports the actual data (nor does the p-value) (Johnson & Omland, 2004; Wasserstein et al., 2019). Here, we will run through our model selection using the Cladocera δ^{15}N data.

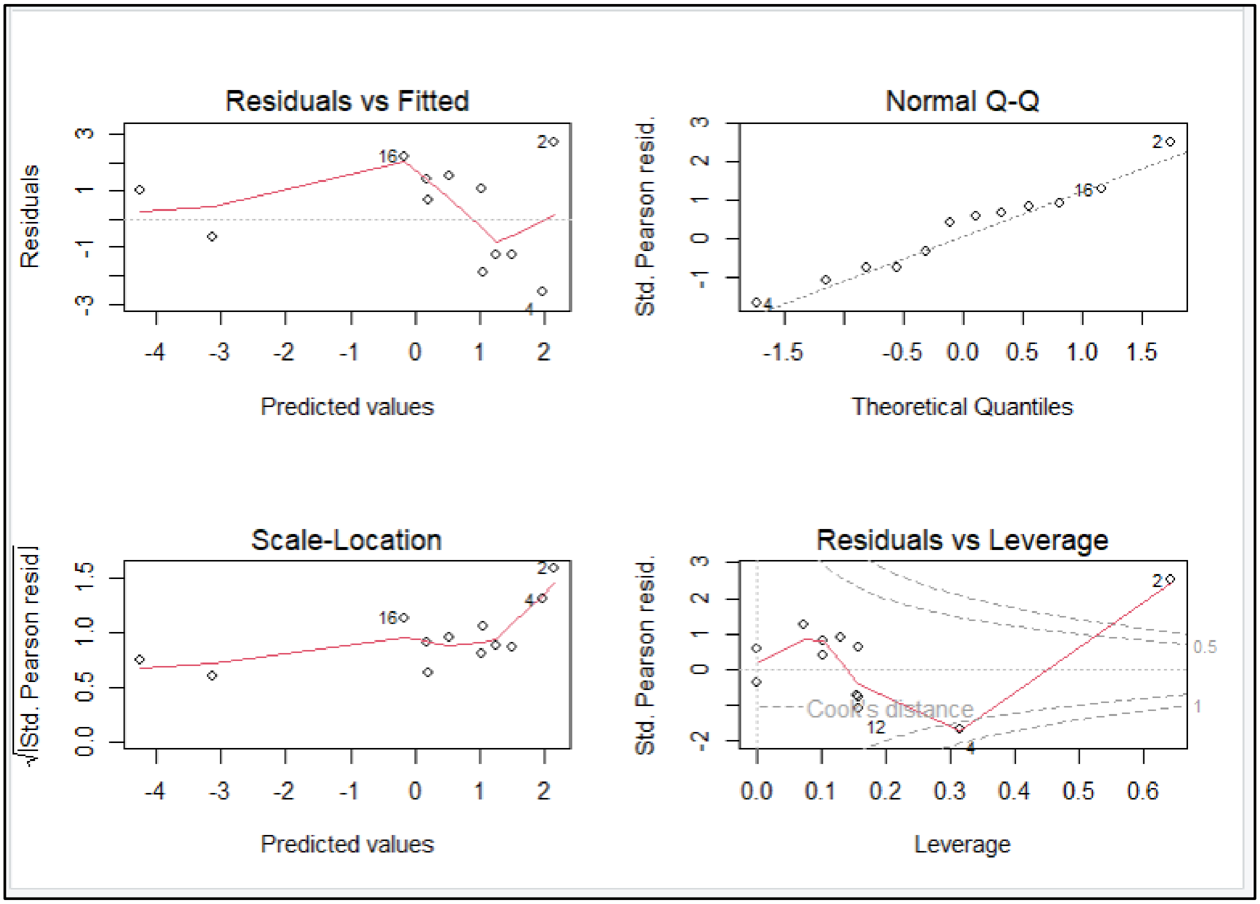
Our first visualization of the Cladocera δ^{15}N told us that δ^{15}N was decreasing over distance in a non-linear fashion. We first created an exponential regression model $y = \alpha \times e^{\beta x}$. There are multiple ways to do this in R: using the linear model (LM) `lm()` command, using the generalized linear model (GLM) `glm()` command, or using the nonlinear least square (NLS) `nls()` command. Using `lm()` log-transforms the data to create a linear relationship, whereas `glm()` and `nls()` do not transform the data. We created three models using `lm()`, `glm()`, and `nls()` and printed out the diagnostic plots and summaries:

```
> lm1<-lm(formula = log(d15N) ~ Distance,data=Cladocera_SIA)
```



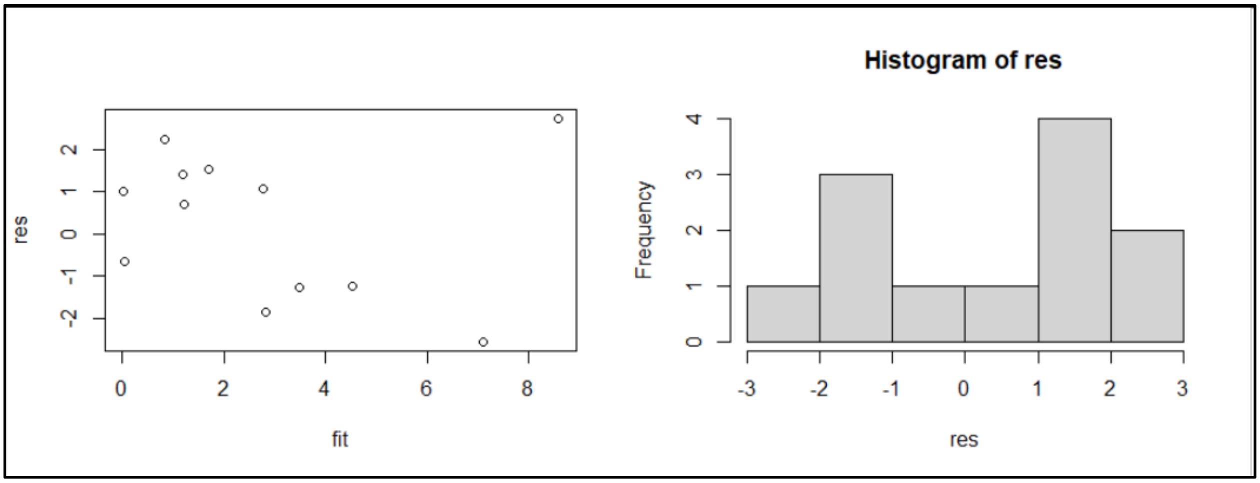
The residuals showed a bowl shape, meaning that the model may not fit the data at the extreme ends. We also see a pond with high leverage on the Residuals vs Leverage plot (point "24", which is pond 12) – this means that pond 12 may have a large influence on the model. A high leverage point is any point with an unusual independent value (x), which may cause that point to have undue influence on the fit of the entire model (Wharton, 2022). If possible, models should contain no large leverage points. Pond 11 had a negative δ^{15}N value, which cannot be log transformed thus is not included in this model (n=11).

```
> glm1<-glm(formula=d15N~Distance,family="gaussian"(link=log),
                    data=Cladocera_SIA,start=c(10,-1))
```



The GLM model also shows a bowl shape, however in this model Pond 2 is a potential leverage point versus Pond 12 in the LM model. This may seem odd- wouldn't the same ponds be leverage points if the two models are the same, in theory? Since the LM model is log-transforming the response variable, they carry different weights in the GLM vs LM models.

```
> nls1<-nls(d15N~ a*exp(b*Distance),start=list(a=10,b=1),
                   data=Cladocera_SIA)
```



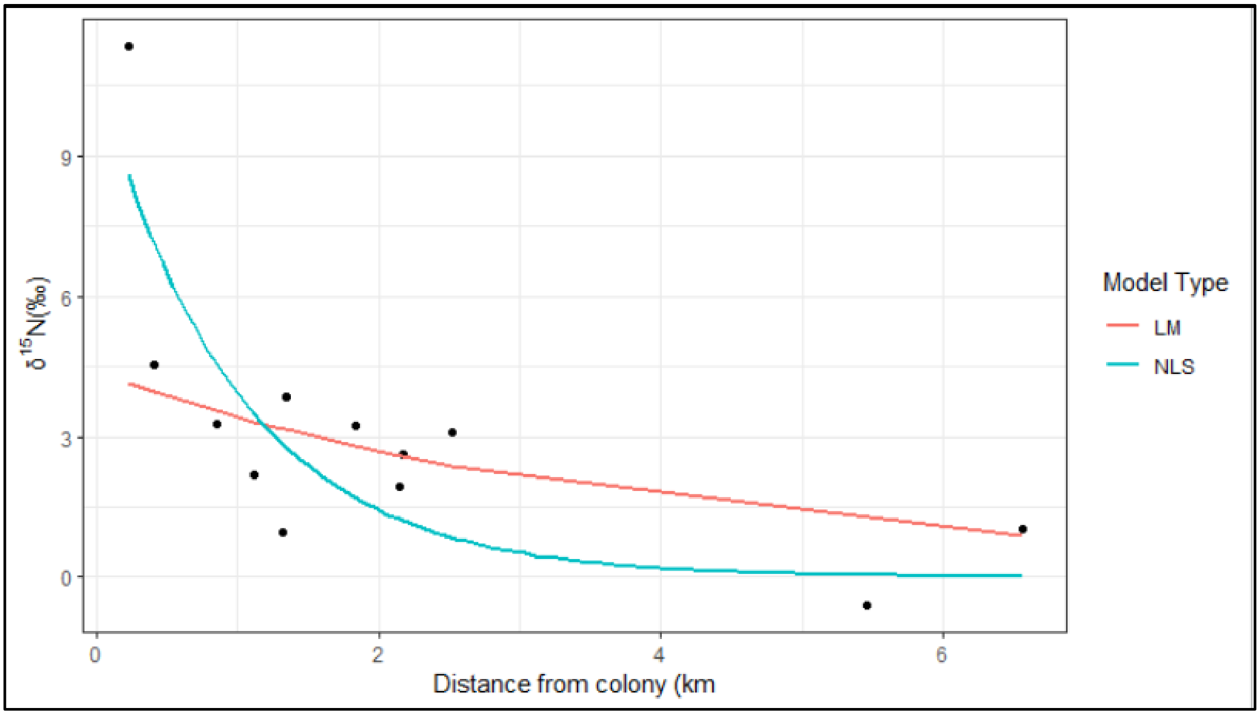
We see a bowl shape in the residuals vs. fit plot, as well as dual peaks in the residuals histogram, indicating issues with the fit of the model at the extreme ends of the data.

When comparing the models, we noticed that the models had different parameters:

	α	**β**
LM	4.3806	-0.2464
GLM	10.7872	-1.0095
NLS	10.7874	-1.0095

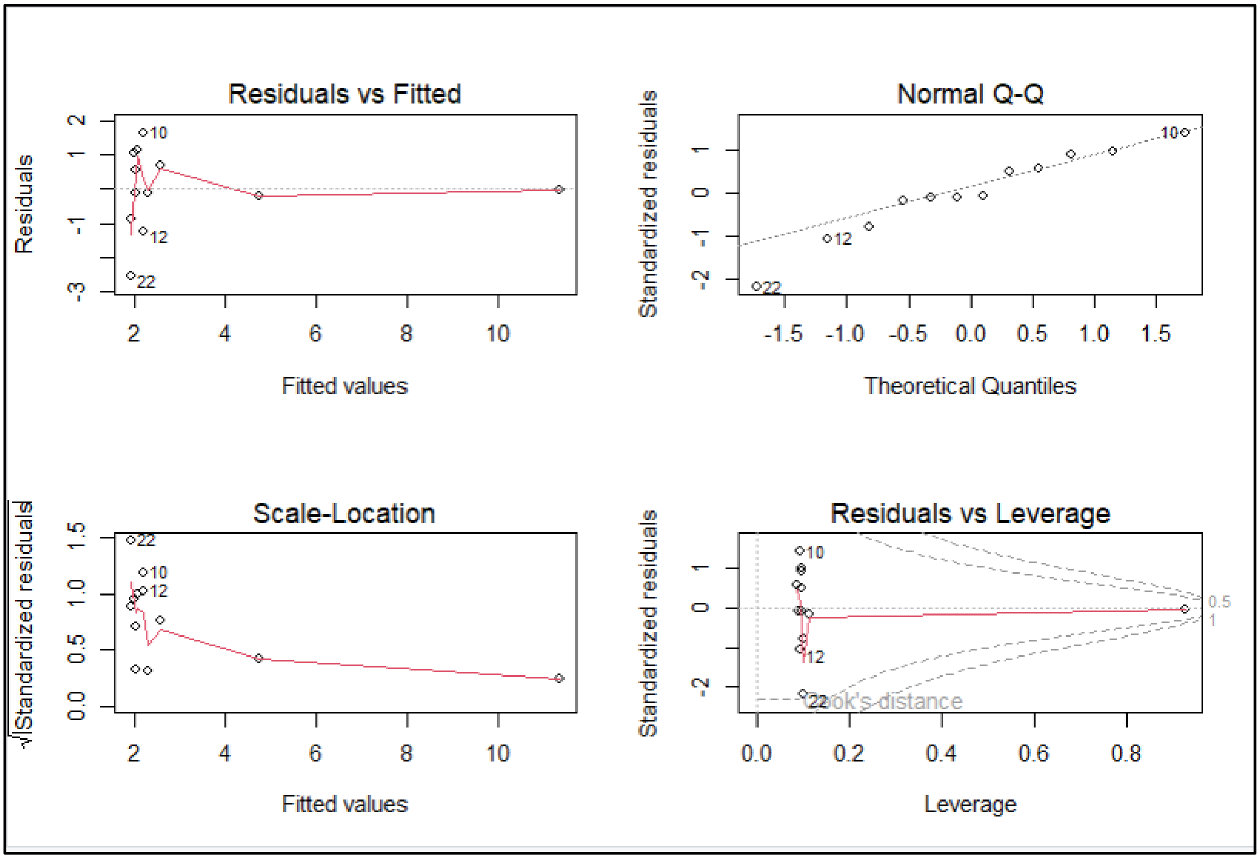
The GLM and NLS models have nearly identical parameters.

Finally, we plotted both the GLM and LM models with the data:



We next looked at an “inverse square law” model $y = \alpha + \beta \times x^{-2}$. First, we created an LM model:

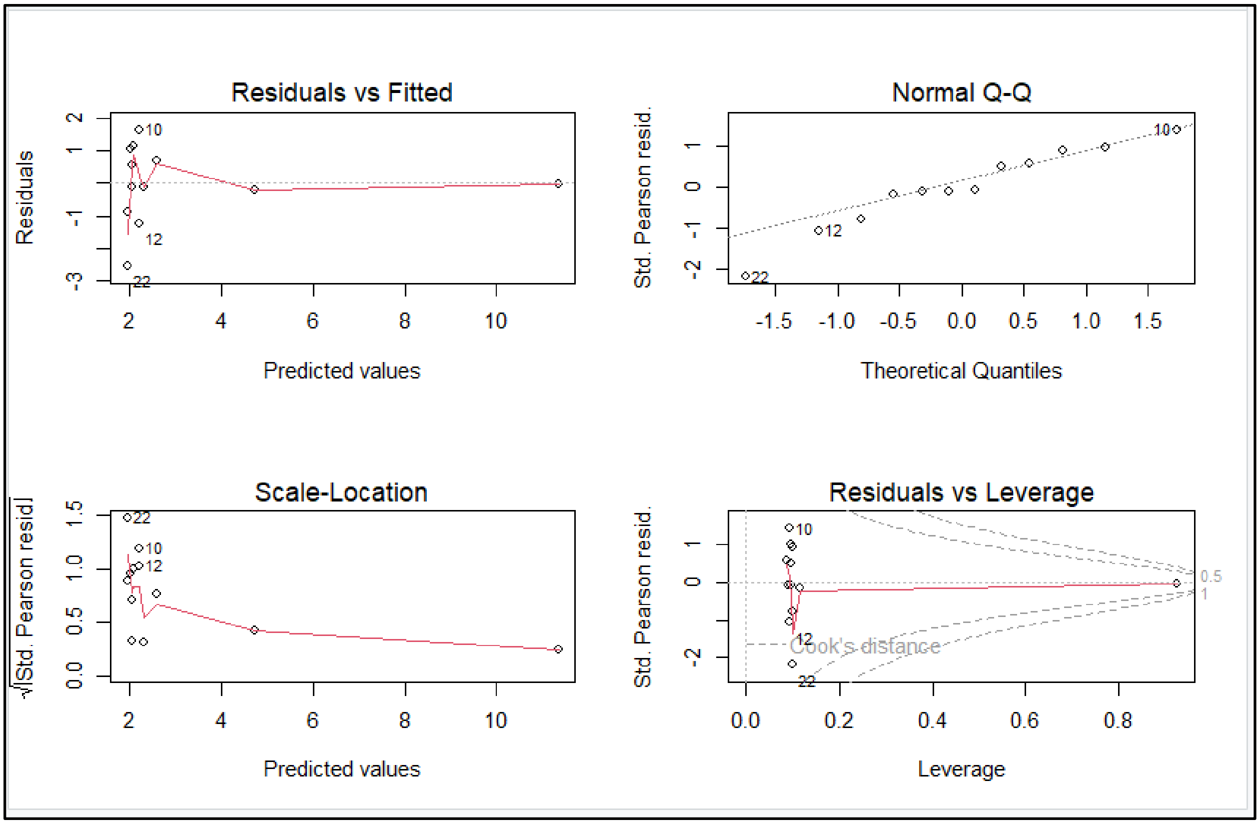
```
> lm2<-lm(d15N~I(1/Distance^2),data=Cladocera_SIA)
```



We see no obvious bowl shape with the residuals, although they are fanning to the left strongly. We also do not see any strong leverage points with this model.

Next, we made a GLM model:

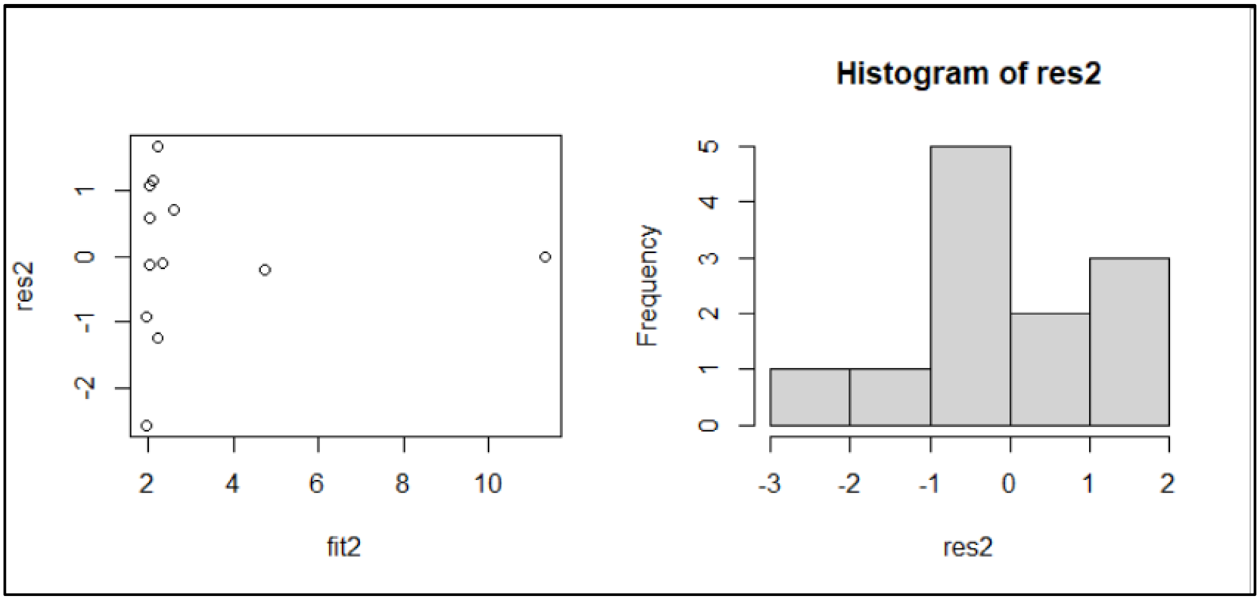
```
> glm2<-glm(d15N~I(1/Distance^2),
      family=gaussian,data=Cladocera_SIA)
```



These diagnostic plots look identical to the LM model. This makes sense, since there is no data transformation/ links.

Finally, we created a NLS model:

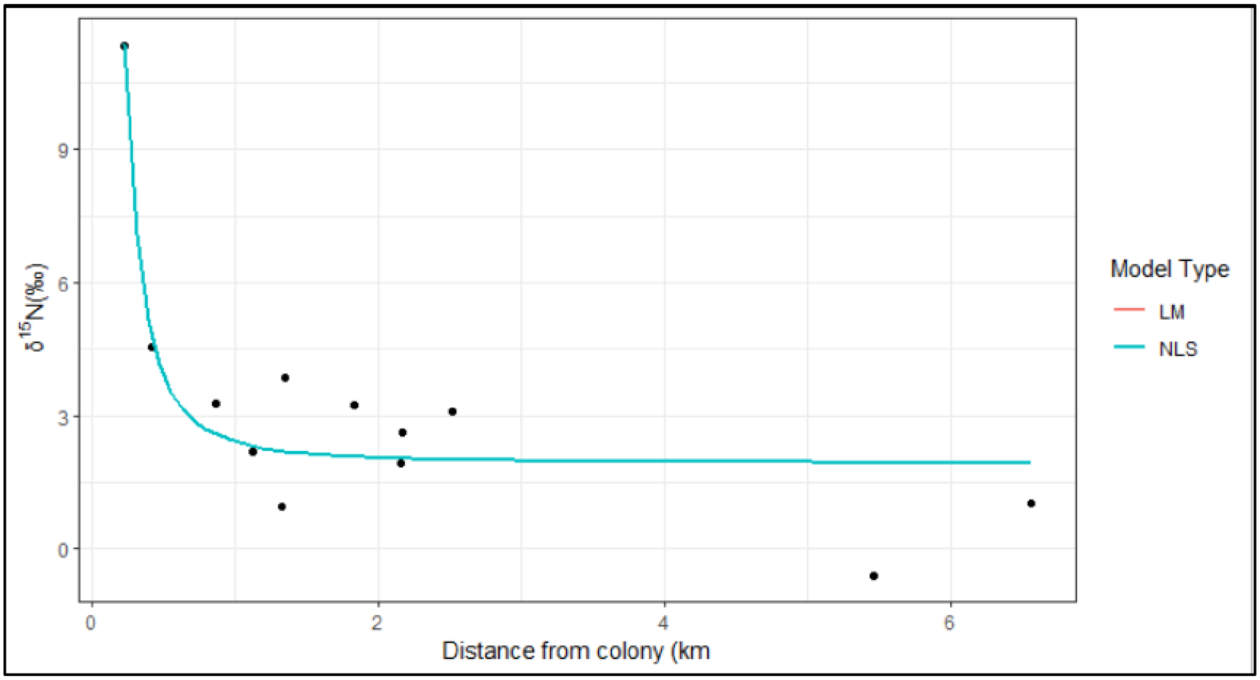
```
> nls2<-nls(d15N~a+b*I(1/(Distance^2)),start=list(a=1,b=1),
      data=Cladocera_SIA)
```



Here, we see a similar residual shape as the LM and GLM models, with no obvious bowl shape and a strong fanning to the left. The residual histogram shows a generally normal curve.

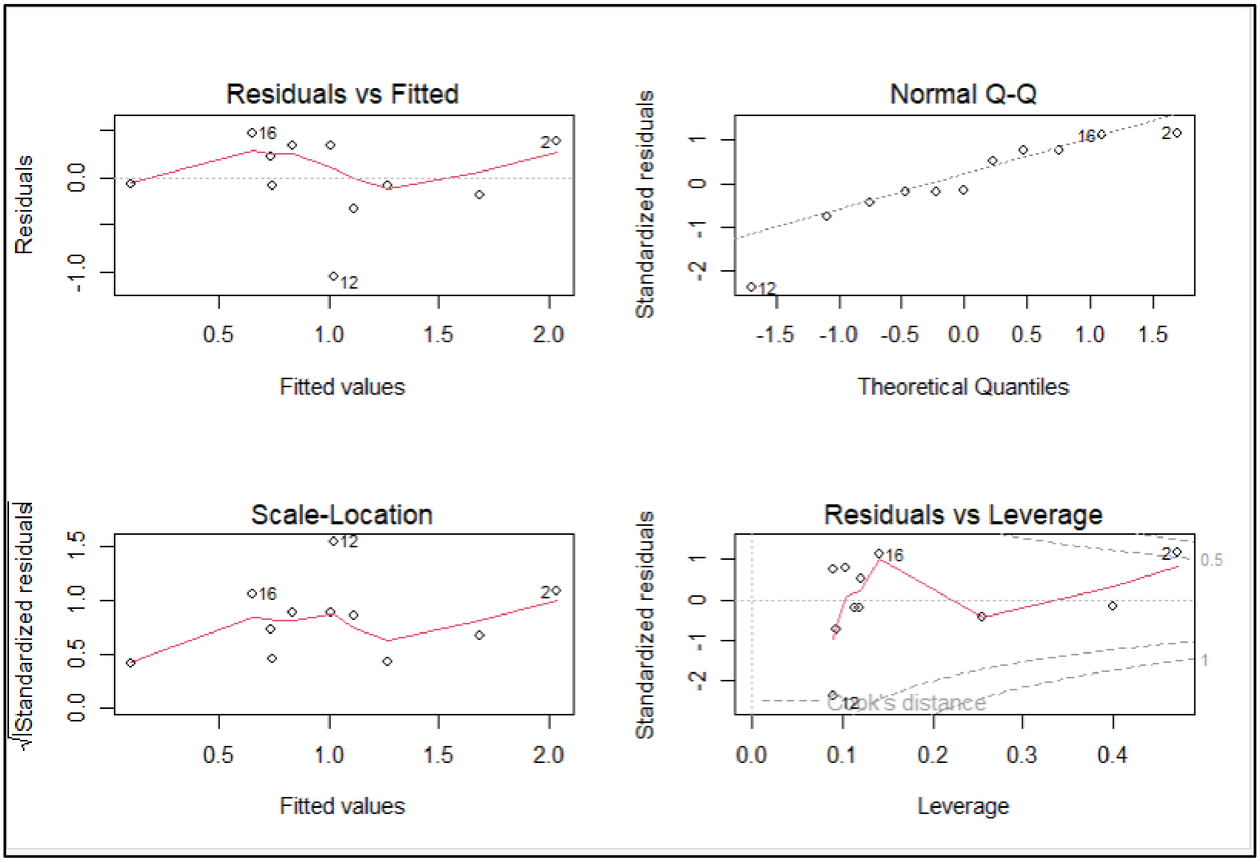
This method resulted in the same parameters for all models, thus only one curve shows up on the plot:

	α	**β**
LM	1.9362	0.4808
GLM	1.9362	0.4808
NLS	1.9362	0.4808



Finally, we wanted to check the “power law” $y = \alpha \times x^{\beta}$. We first created a LM:

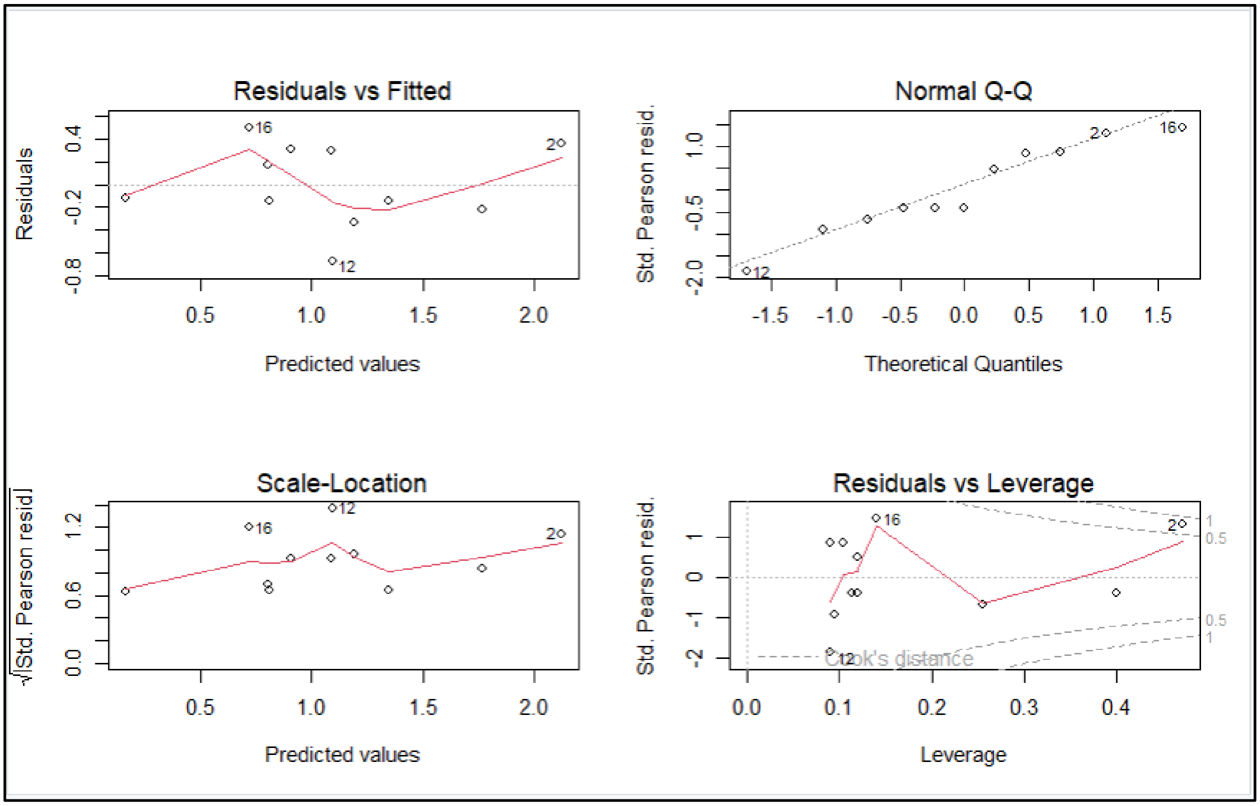
```
> lm3<-lm(log(d15N)~log(Distance),data=Cladocera_SIA)
```



We see a slight bowl shape in the residuals vs fit plot, and no strong leverage points. Again, pond 11 was excluded from this model because a negative value cannot be log-transformed.

Next, we created a GLM:

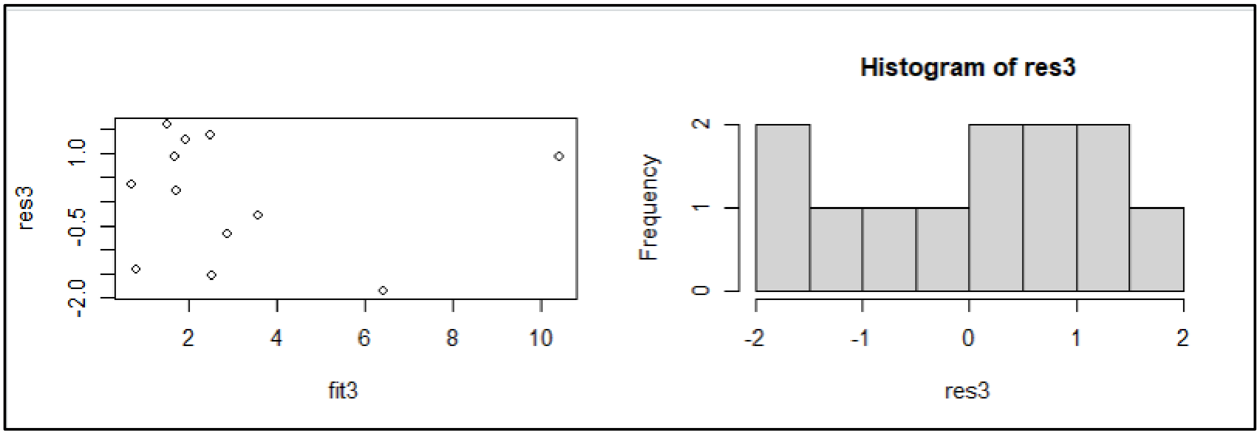
```
> glm3<-glm(d15N~log(Distance),family=Gamma(link='log'),
           data=Cladocera_SIA[Cladocera_SIA$d15N >0, ])
```



Again, we see a bowl shape in the residuals, and no strong leverage points. Pond 11 was not included in this model because negative values are not allowed for the "Gamma" family.

And finally, NLS:

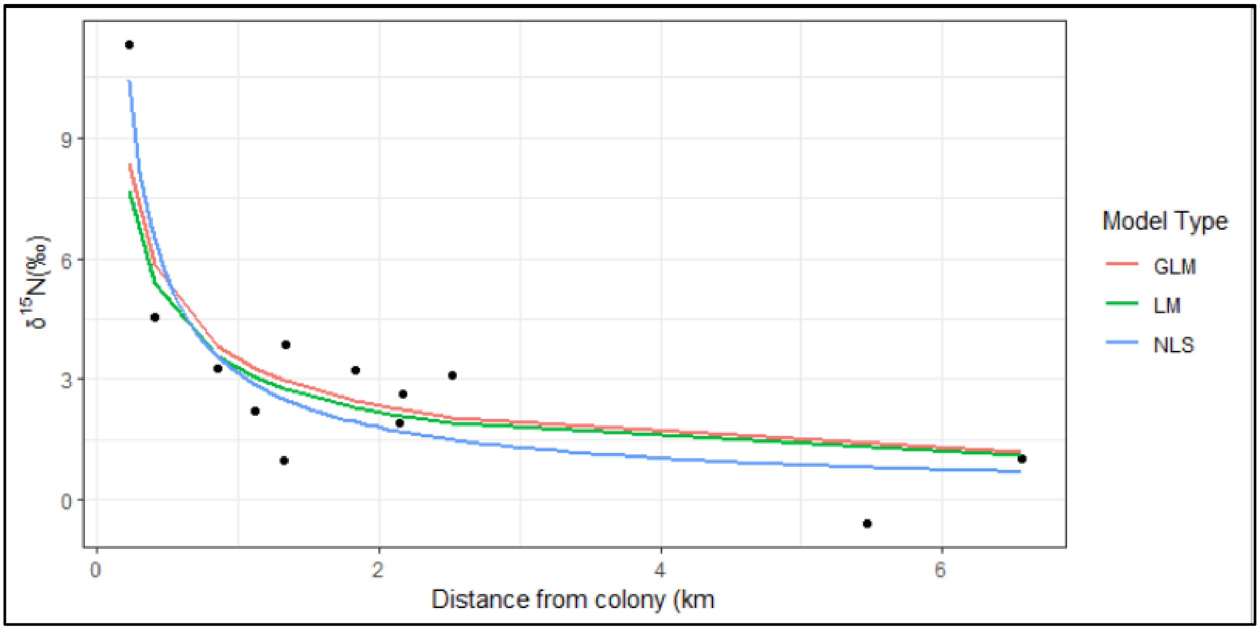
```
> nls3<-nls(d15N~a*Distance^b,
                  start=list(a=3,b=1),data=Cladocera_SIA)
```



We see a bowl shape in the residuals, and a left-skewed histogram of residuals.

These three methods resulted in different parameter values:

	α	**β**
LM	3.2567	-0.5753
GLM	3.5145	-0.5811
NLS	3.1558	-0.8030



So, which model should we go with? Whenever possible, it is best to first rank the models based on knowledge of the study system, rather than which one gives the best statistical significance – this avoids finding meaningless models with spurious correlations to your system! In this case, we are studying the movement of nutrients via wind transport and groundwater. After we deduced that the inverse square model and the power law model were both possibilities in our system, we checked the goodness of fit of the three models using the Akaike information criterion (AIC) method, in the `AICcmodavg` library in R. AIC penalizes models for having more parameters, however all three of our models have the same number of parameters so the point is moot (Johnson & Omland, 2004). We decided to compare the three NLS models because NLS does not omit negative values, nor does it log-transform the data; NLS is the most appropriate for non-linear data. We decided to use the inverse law model for our Cladocera δ^{15}N data based on the output from AIC:

```
                  df      AIC
Clad_exponential   3 52.28701
Clad_inverse       3 42.72966
Clad_power         3 43.74569
Clad_null          2 62.92381
```

Works Cited

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Appendix E: Chapter 3, diagnostic plots

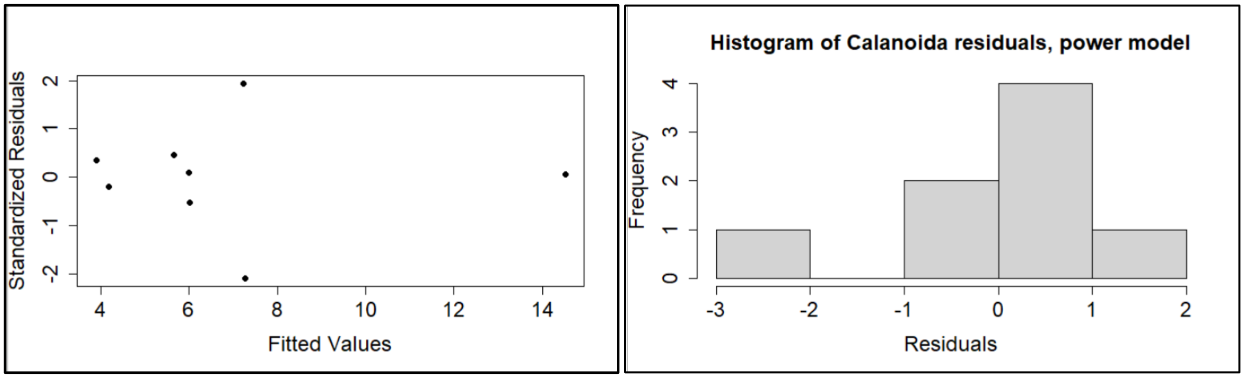

Figure E.1. Calanoida δ^{15}N, power model

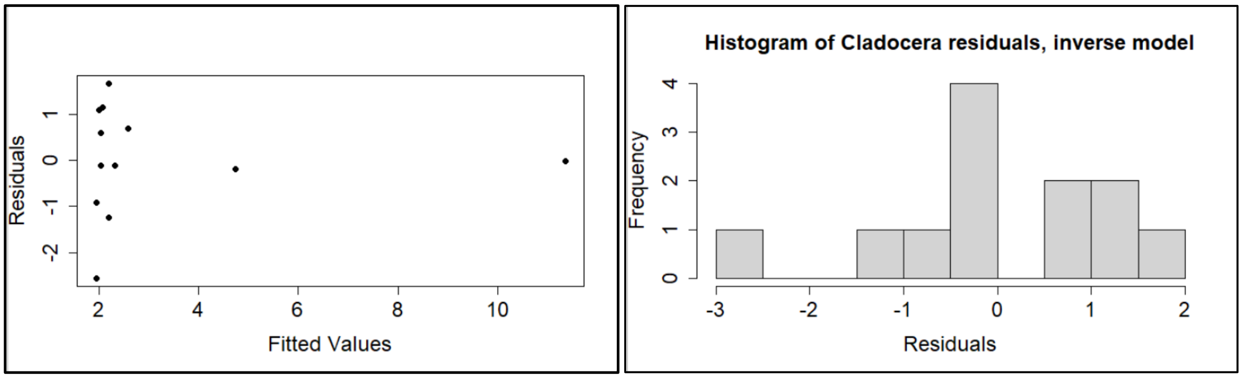

Figure E.2. Cladocera δ^{15}N, inverse model

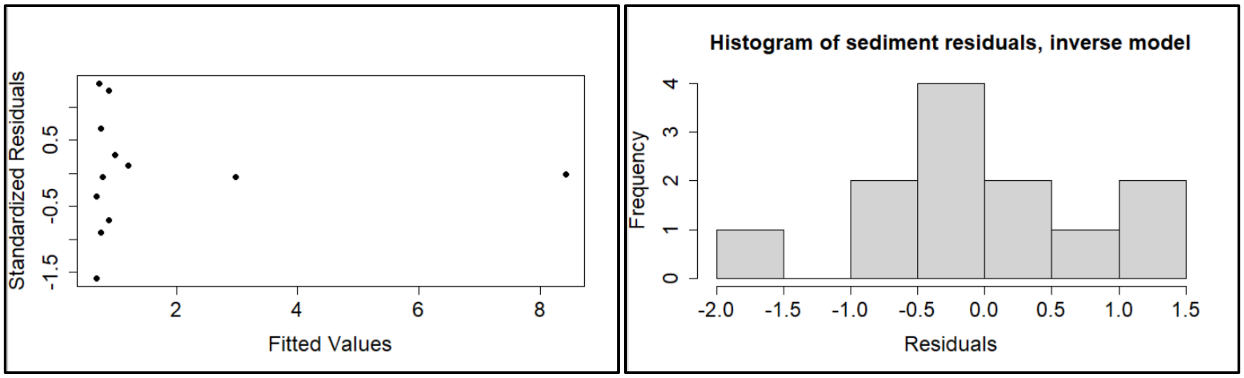

Figure E.3. Sediment δ^{15}N, inverse model

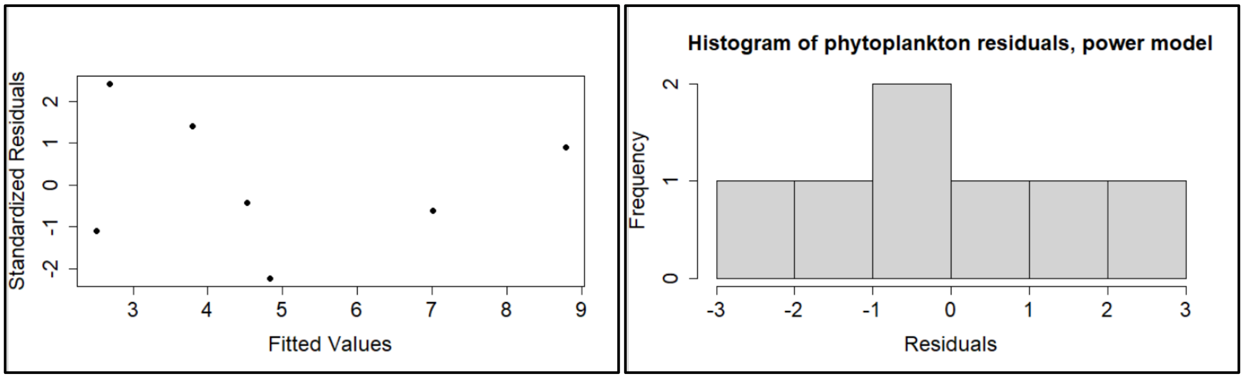

Figure E.4. Phytoplankton δ^{15}N, power model

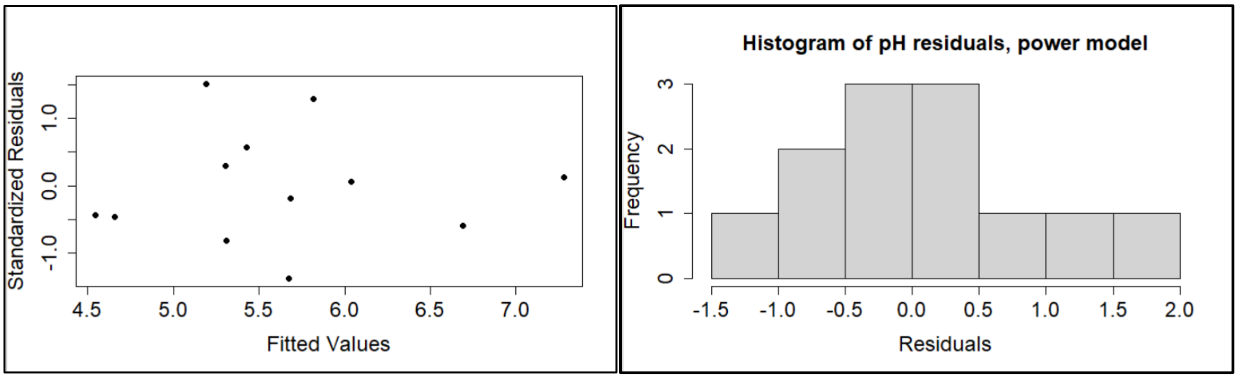

Figure E.5. pH, power model

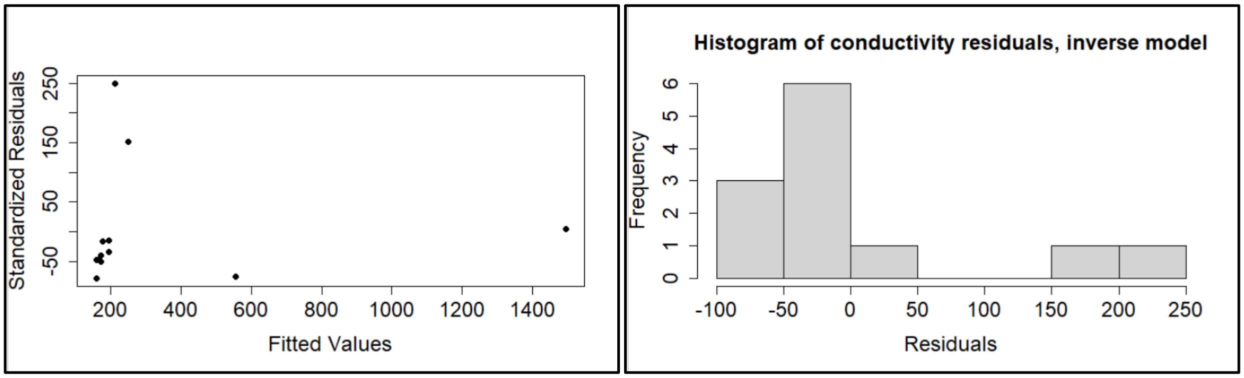

Figure E.6. Conductivity, inverse model

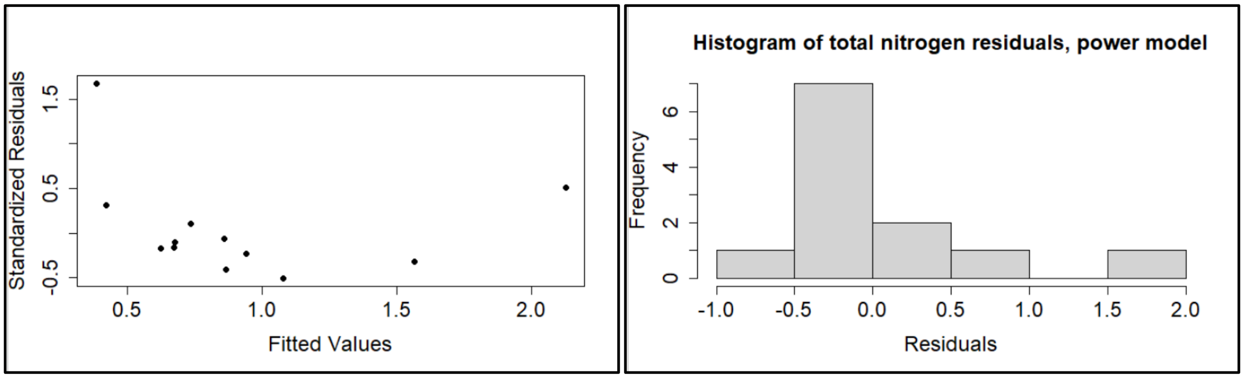

Figure E.7. Total nitrogen, power model

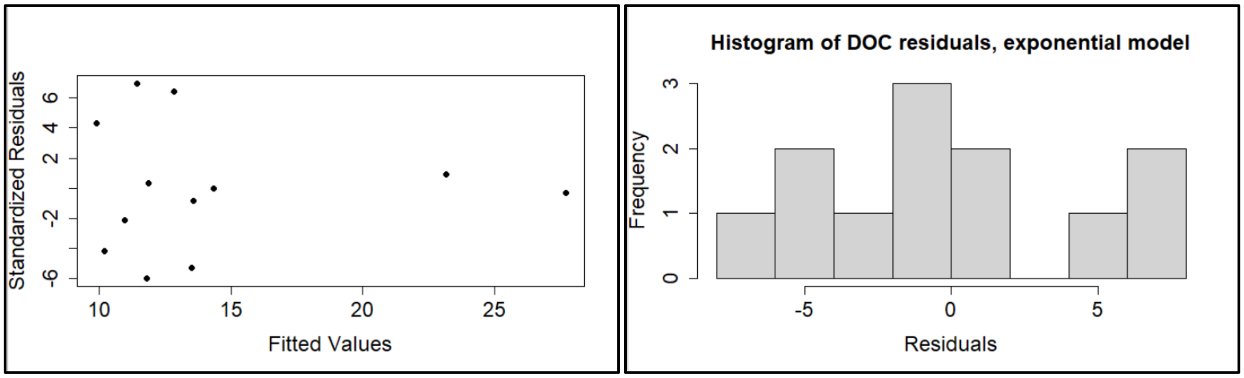

Figure E.8. DOC, exponential model

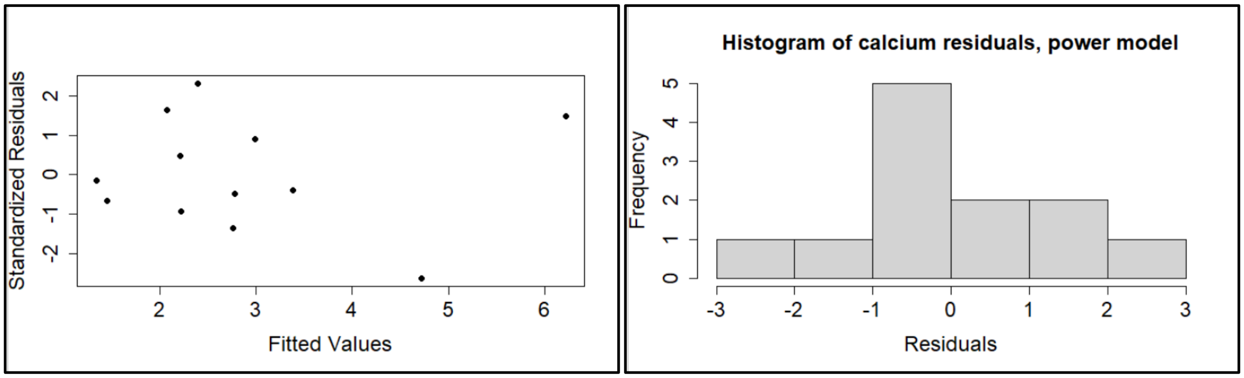

Figure E.9. Ca^{2+}, power model

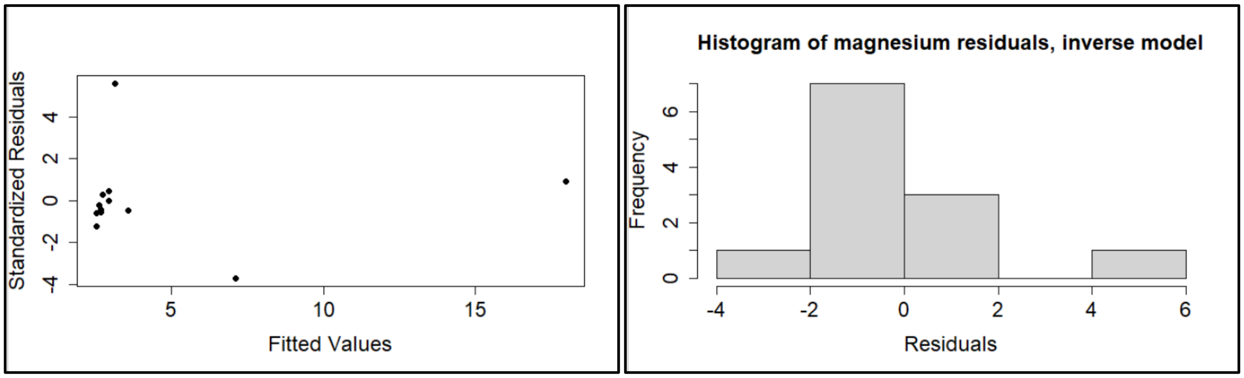

Figure E.10. Mg^{2+}, inverse model

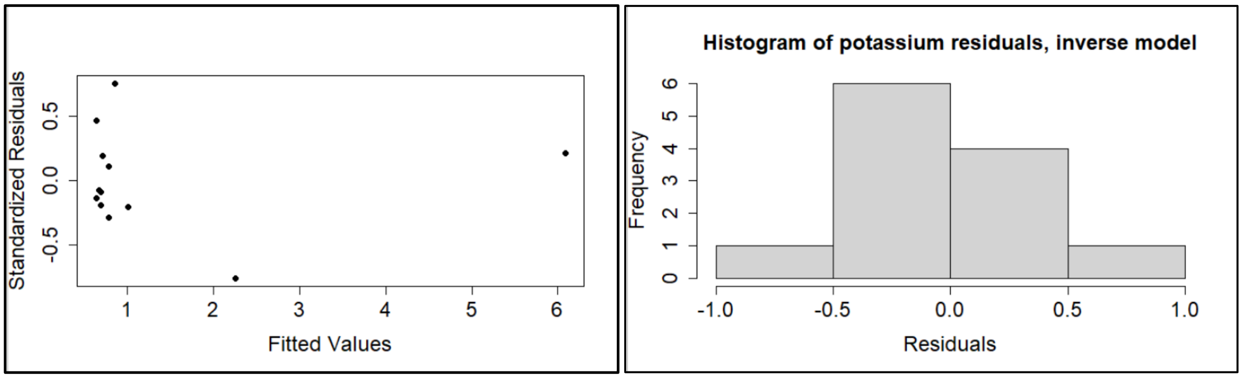

Figure E.11. K^+, inverse model

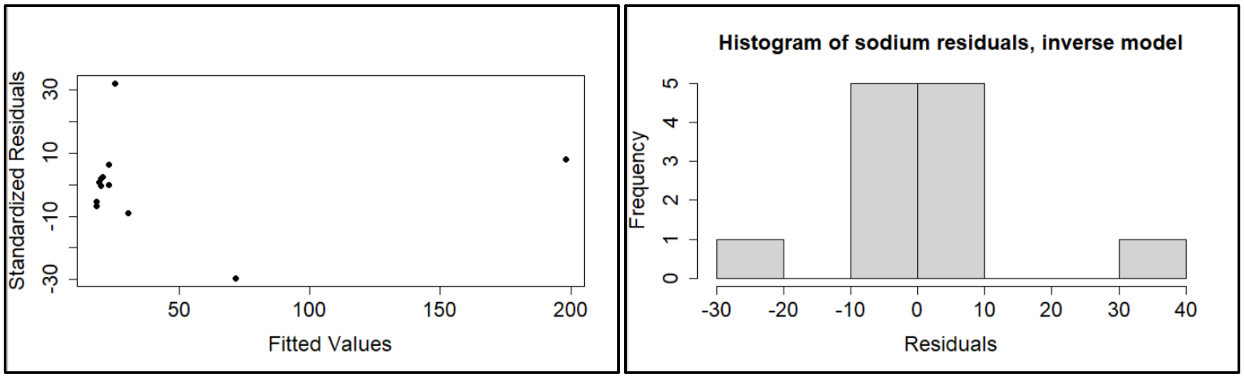

Figure E.12. Na^+, inverse model

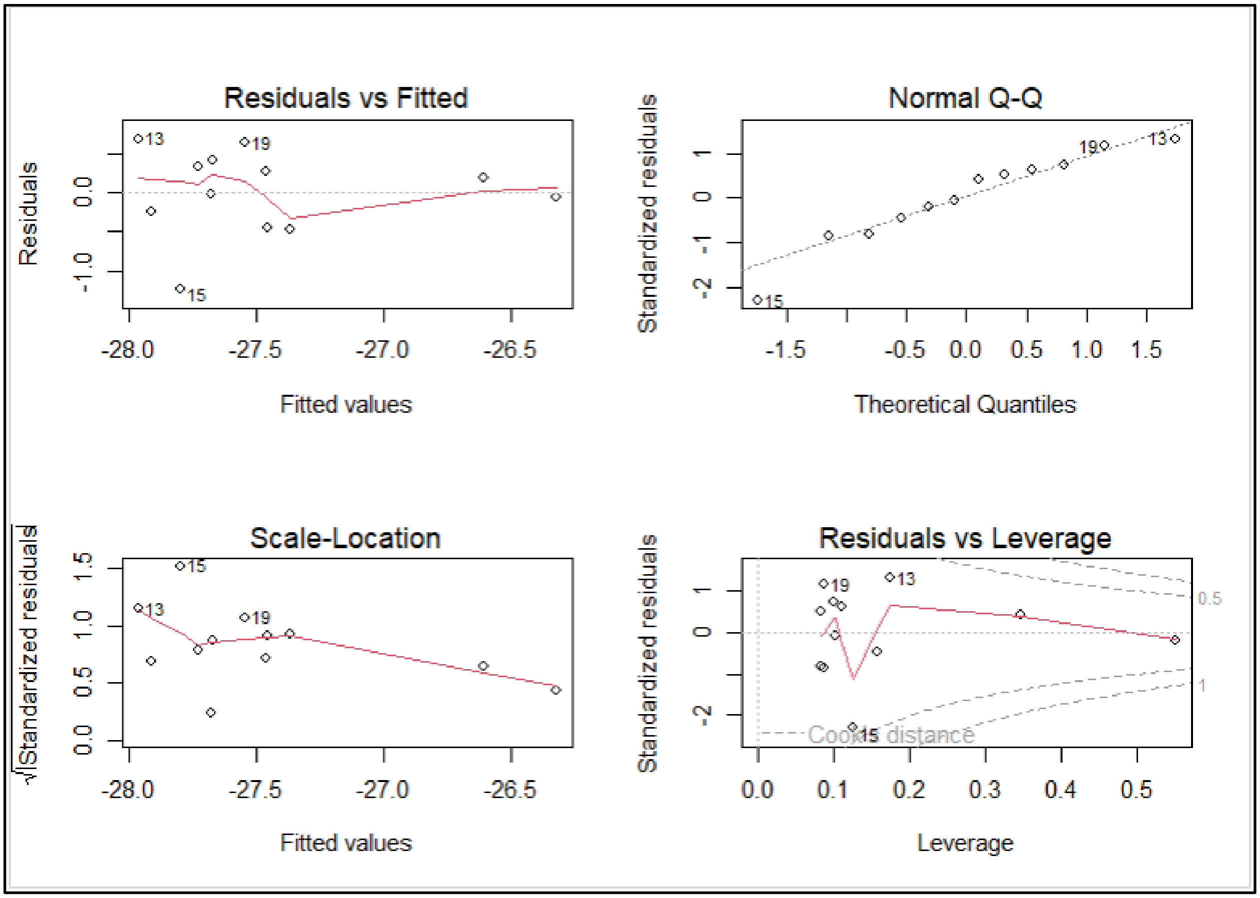

Figure E.13. Sediment $\delta^{13}C$, linear regression

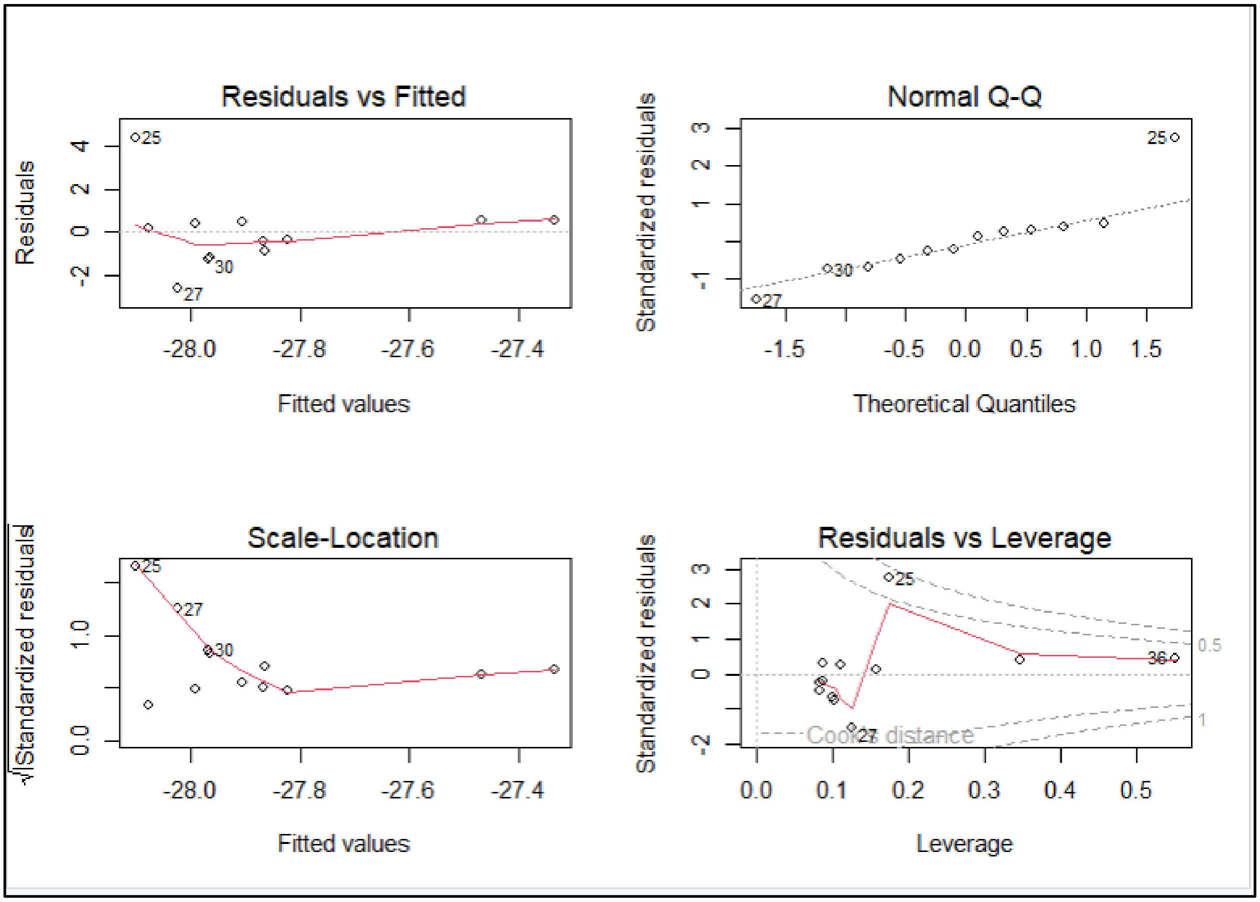

Figure E.14. Phytoplankton δ^{13}C, linear regression

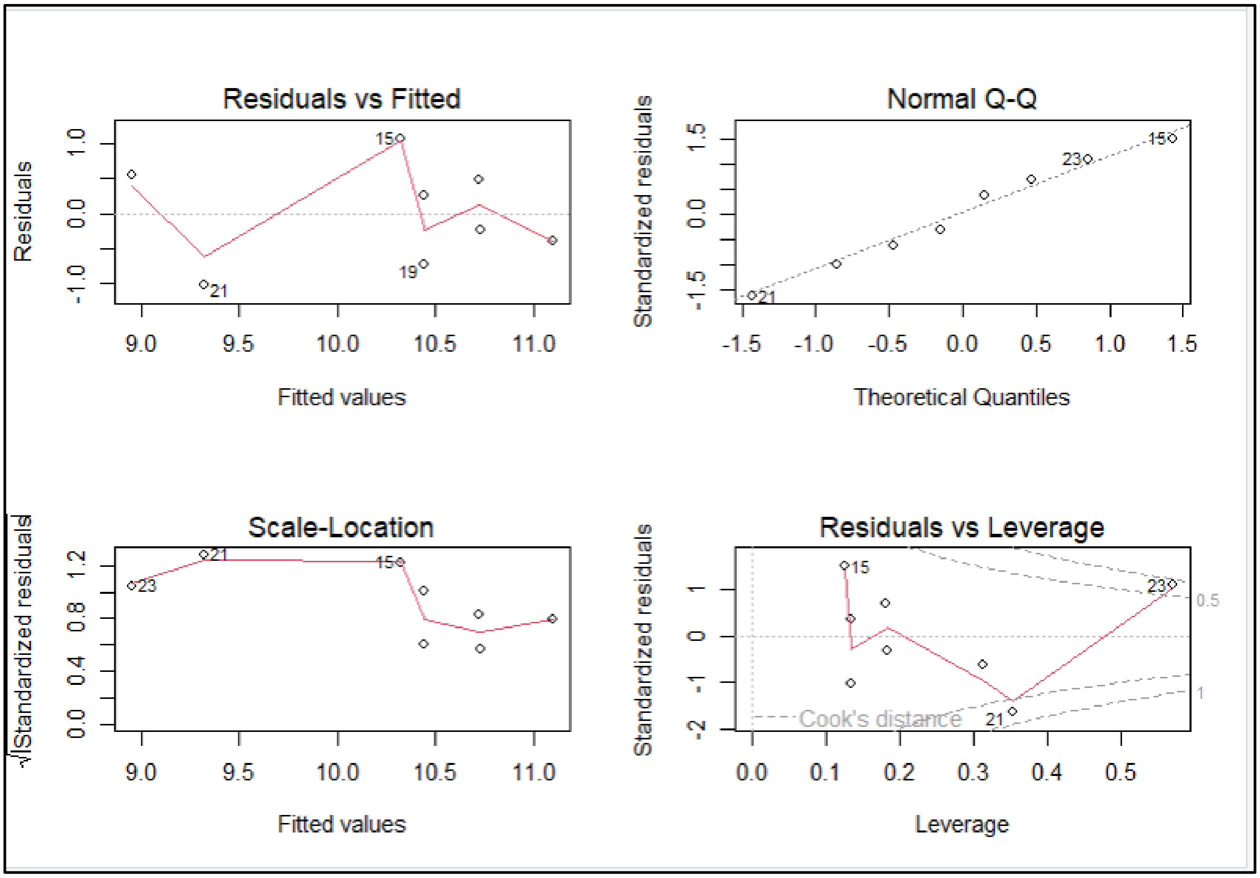

Figure E.15. Calanoida %N, linear regression

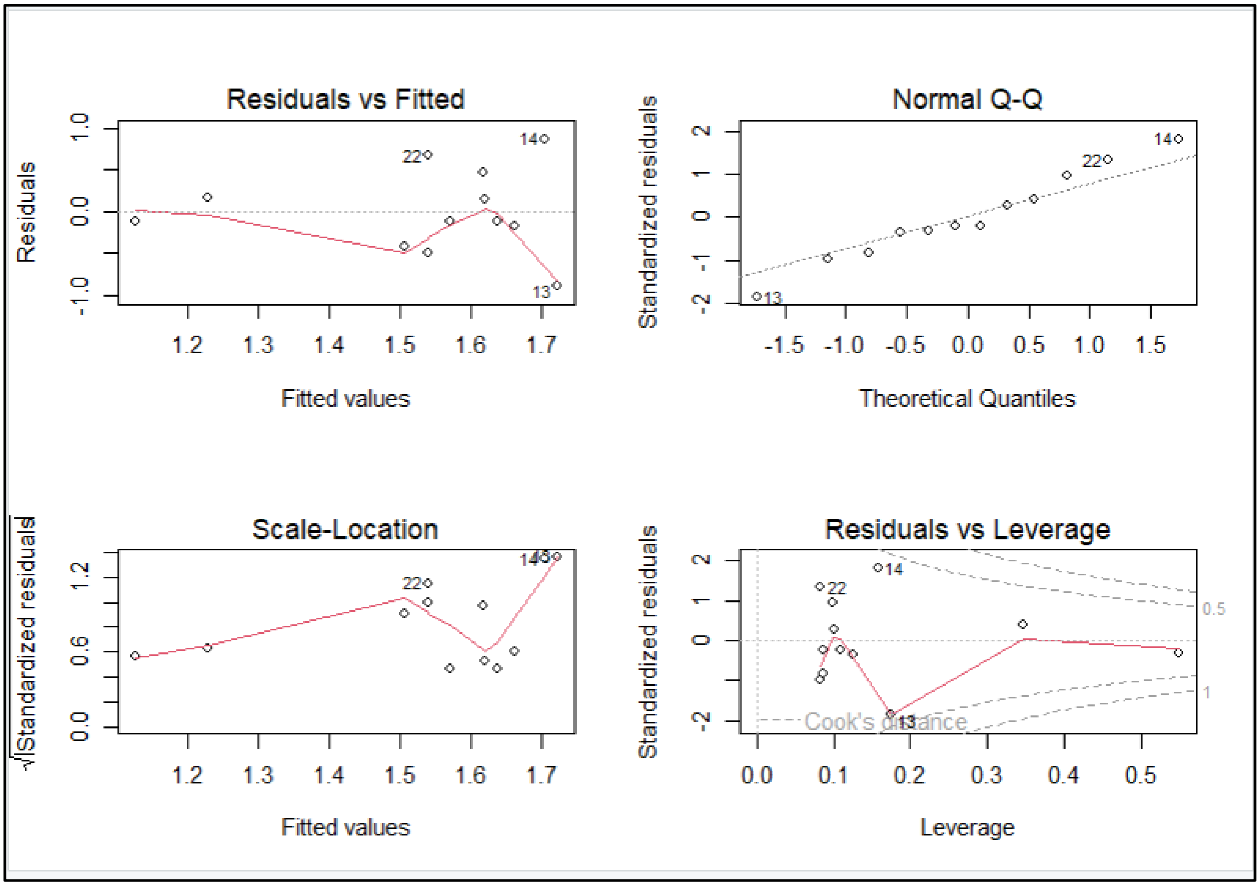

Figure E.16. Sediment %N, linear regression

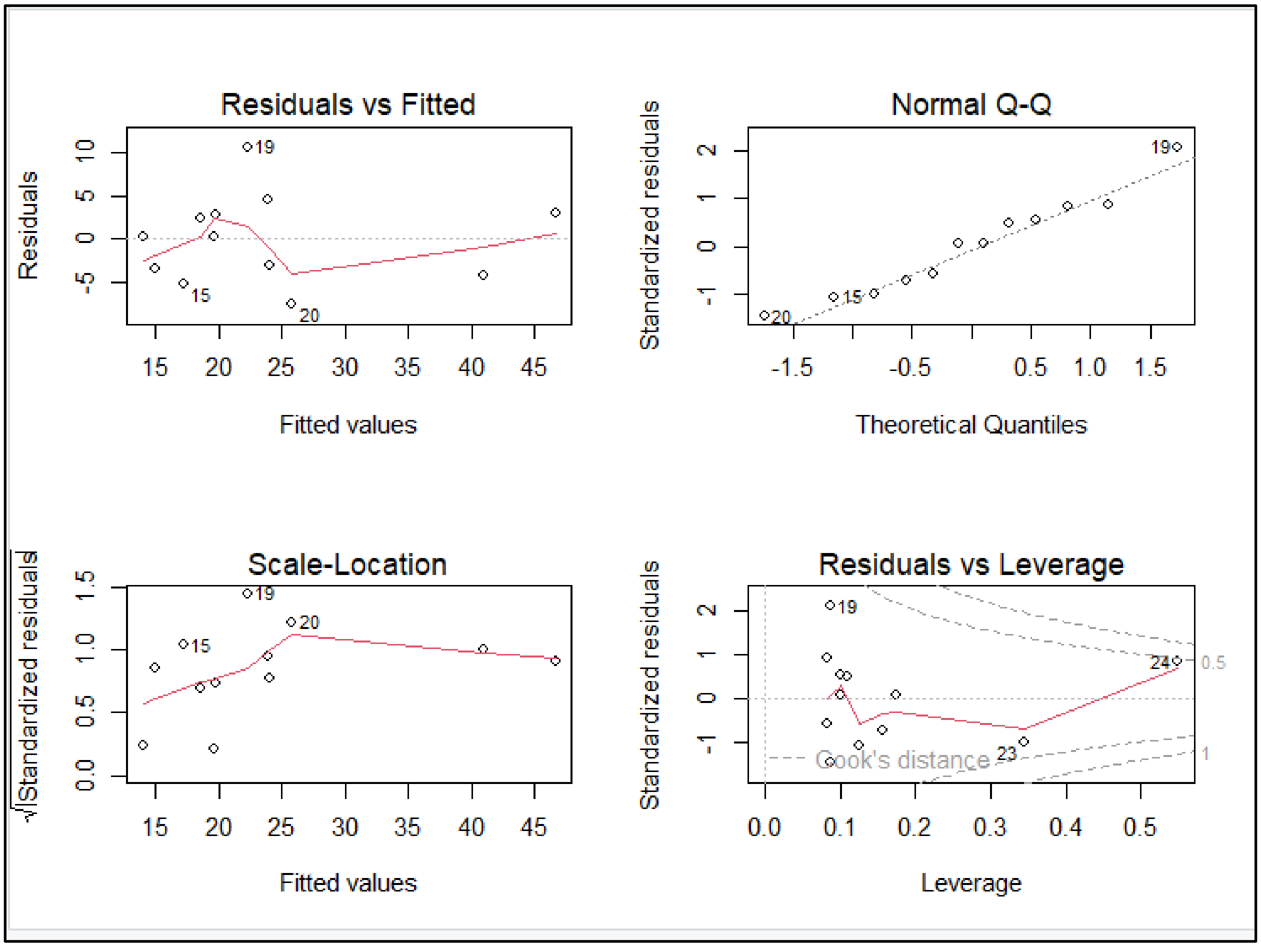

Figure E.17. C:N sediment, linear regression

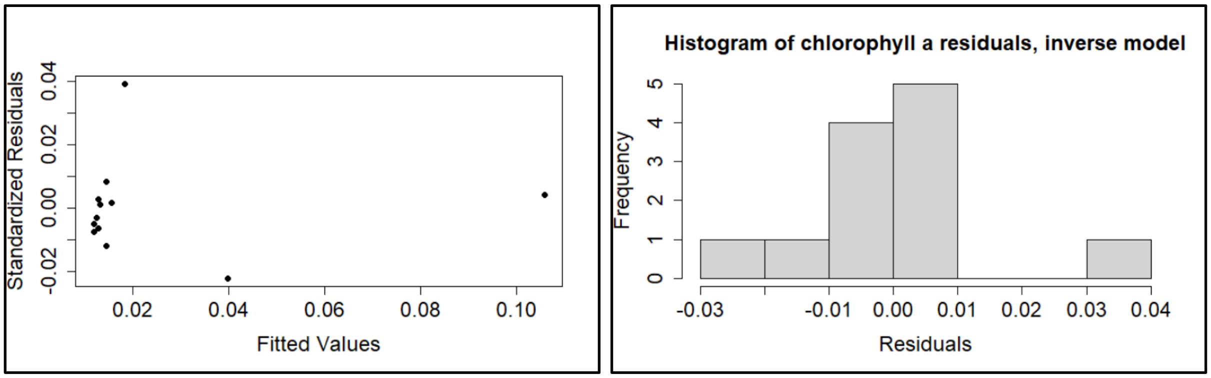

Figure E.18. Chlorophyll *α*, inverse model

Appendix F: Chapter 3, AIC model comparison

Table F.1. Calanoid δ^{15}N

```
                   df      AIC
Cal_exponential     3 38.96886
Cal_inverse         3 34.47441
Cal_power           3 29.47691
Cal_null            2 45.71023
```

Table F.2. Cladocera δ^{15}N

```
                   df      AIC
Clad_exponential    3 52.28701
Clad_inverse        3 42.72966
Clad_power          3 43.74569
Clad_null           2 62.92381
```

Table F.3. Sediment δ^{15}N

```
                   df      AIC
Sed_exponential     3 42.18941
Sed_inverse         3 35.01731
Sed_power           3 35.54242
Sed_null            2 57.84667
```

Table F.4. Phytoplankton

```
                   df      AIC
Phyto_exponential   3 35.43438
Phyto_inverse       3 29.60728
Phyto_power        3 31.40158
Phyto_null         2 36.66825
```

Table F.5. Total Nitrogen

```
                   df      AIC
TN_exponential      3 31.77084
TN_inverse          3 19.36585
TN_power            3 26.32982
TN_null             2 28.17928
```

Table F.6. DOC

```
          df      AIC
DOC_exponential    3 73.66125
DOC_inverse        3 85.16100
DOC_power          3 77.03160
DOC_null           2 83.39726
```

Table F.7. Ca^{2+}

```
               df      AIC
Ca_exponential  3 49.94476
Ca_inverse      3 45.03230
Ca_power        3 47.10733
Ca_null         2 52.82190
```

Table F.8. Mg^{2+}

```
               df      AIC
Mg_exponential  3 67.77763
Mg_inverse      3 56.85249
Mg_power        3 61.52388
Mg_null         2 75.26505
```

Table F.9. K^{+}

```
              df      AIC
K_exponential  3 34.21174
K_inverse      3 16.09227
K_power        3 27.29351
K_null         2 48.49190
```

Table F.10. Na^+

```
               df      AIC
Na_exponential  3 116.7552
Na_inverse      3 102.3685
Na_power        3 110.5861
Na_null         2 132.5559
```

Table F.11. pH

```
               df      AIC
pH_exponential  3 34.96016
pH_inverse      3 38.17437
pH_power        3 34.68387
pH_null         2 40.29781
```

Table F.12. Conductivity

```
                 df      AIC
cond_exponential  3 162.6314
cond_inverse      3 149.2399
cond_power        3 151.6084
cond_null         2 180.6152
```

Table F.13. Chlorophyll α

```
                    df       AIC
chla_exponential     3 -56.32563
chla_inverse         3 -62.17083
chla_power           3 -60.67327
chla_null            2 -46.51334
```