Subarctic Crustose Coralline Algae as Recorders of Past Climatic and Environmental Change

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of Earth Sciences
University of Toronto

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Abstract

If unabated, the continued anthropogenic release of carbon dioxide is expected to lead to warming and acidification of ocean waters, with widespread and detrimental impacts on marine ecosystems. Proxy records stored in biomineralized shells and skeletons of long-lived paleoclimate archives are essential for understanding long-term climate variability - previously unresolvable based on spatiotemporally limited observations. In this dissertation, geochemical and physical proxies from Clathromorphum spp. crustose coralline algae (CCA) are used for interpreting past climatic and environmental changes in the subarctic North Pacific and North Atlantic Oceans.

Micro-computed tomography techniques are used to examine the algal skeleton, and reveal changes in skeletal density in relation to recently observed acidification off the Aleutian Islands, Alaska. Metabolic trade-offs associated with continued growth and calcification in acidifying seawaters may have resulted in the production of weaker (less-dense) skeletons since 1980. However, correlations indicate that in addition to acidification, sunlight availability and temperature stress are also important factors influencing the ability of CCA to calcify.
Furthermore, barium-to-calcium (Ba/Ca) ratios are utilized as proxies for phytoplankton productivity in northern Labrador, Canada, such that: Higher (lower) algal Ba/Ca values are interpreted as decreased (increased) productivity coinciding with the expansion (melting) of sea-ice. This multi-centennial record of algal Ba/Ca indicates a long-term increase in North Atlantic productivity that is unprecedented in the last 365 years. Conversely, in mountainous coastal regions surrounding the Gulf of Alaska where high sediment loads are present in seasonal runoff, algal Ba/Ca is used as an indicator for freshwater runoff. Ba/Ca is inversely correlated to instrumental salinity, and indicates a unique period of freshening (2001 – 2006) that is related to increasing glacial melt and precipitation on mainland Alaska. The results presented here illustrate the physiological responses of coralline algae to acidification, and provides much-needed data for future projections of climate and environmental change.
Acknowledgements

Ten years ago, as a first-year student at the University of Toronto, I would have never imagined that I would one day graduate with a PhD in the Earth Sciences. Throughout my bachelor’s degree, I dabbled in a wide range of subjects ranging from plant and animal ecology, to invertebrate zoology, to climatology, and eventually to earth and environmental sciences. It wasn’t until I took a course entitled “Past and Present Climate Change” by (my now supervisor) Professor Jochen Halfar, that I realized the “missing link” that united my seemingly unrelated areas of study, and sparked my fascination - and so began my long and arduous road to pursue a graduate degree. Several people have helped me along the way to whom I am indebted:

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A special thanks goes out to Steffen Hetzinger and Branwen Williams for their helpful advice, comments, suggestions, and especially for their help with statistical analyses, and editing of manuscripts during their postdoctoral appointments in the Halfar lab.

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“You raise me up, so I can stand on mountains;
You raise me up, to walk on stormy seas;
I am strong when I am on your shoulders;
You raise me up to more than I can be.”

– Josh Groban

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Phoebe Tsz-Wai Chan
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Introduction, Objectives, and Structure of Thesis

1 Introduction

1.1 Our Changing Climate System

Since the beginning of the industrial revolution, anthropogenic release carbon dioxide (CO$_2$) through fossil fuel combustion, cement manufacture, and land use changes have increased atmospheric CO$_2$ concentrations by over 40% from preindustrial levels ~ 280 parts per million (ppm) [Caldeira and Wickett, 2003] to 390.5 ppm [Hartmann et al, 2013]; resulting in an average global sea-surface temperature (SST) rise of 0.7 °C in the top 100 m [IPCC, 2013]. Current levels of atmospheric CO$_2$ are the highest they have been in the last 800,000 years [Luthi et al., 2008], and are projected to continue increasing to levels approaching 1000 ppm by the end of the century - warming global SST by an additional 0.6 – 2.0 °C [Collins et al., 2013].

Over the last few decades, the rise in Arctic surface air temperatures as a result of increasing atmospheric greenhouse gas (GHG) concentrations has been almost twice as large as the global average [Screen and Simmonds, 2010; Serreze et al., 2009] – a process known as Arctic amplification [Holland and Bitz, 2003; Manabe and Stouffer, 1980]. As the climate continues to warm, the summer ice-melt season lengthens and intensifies, creating open water areas that can absorb more shortwave solar radiation, which in turn, further inhibits or delays the formation of sea-ice in the following fall and winter [Perovich and Richter-Menge, 2009; Serreze et al., 2009]. Arctic amplification is hypothesized to be driven by positive feedbacks associated with diminishing Arctic ice and snow cover, leading to a reduction in surface albedo, and allowing for enhanced heat transfer from the ocean to the atmosphere [Holland and Bitz, 2003; Screen and Simmonds, 2010; Serreze et al., 2006; Serreze et al., 2009; Stroeve et al., 2012]. Both observational and modelling studies have indicated significant changes in atmospheric circulation, precipitation, temperature, and weather patterns as a result of sea-ice loss [Francis and Vavrus, 2012]; However, the amplified response to increasing temperature resulting from ice-melt is expected to be more pronounced in the ocean rather than land areas [Serreze et al., 2009].
1.1.1 Melting Arctic Sea-Ice and Phytoplankton Blooms

Arctic sea-ice extent has been declining at an accelerated rate of ~ 9 % per decade since 1978, accompanied by reductions in the proportion of perennial ice cover, and an increase in the duration of the ice-melt season [Comiso, 2012; Perovich and Richter-Menge, 2009]. The progressive shift from thick (2 – 4 m) multi-year sea-ice to substantially thinner (0.5 – 1.8 m) first year ice allows for more solar radiation to be transmitted into the surface ocean, supporting higher levels of primary productivity [Arrigo et al., 2012; Arrigo and van Dijken, 2015]. In fact, according to the most recent satellite estimates of chlorophyll a, rates of phytoplankton net primary production (NPP) in the Arctic Ocean have increased by 30% since 1998 [Arrigo and van Dijken, 2015]. Phytoplankton growth in mid-to-high latitude oceans is often limited by low light levels due to deep vertical mixing [Doney, 2006; Sarmiento et al., 2004]. The continued freshening associated with warming and ice melt can supply additional buoyancy to the water column, reducing the mixed layer depth and leading to higher levels of productivity [Arrigo et al., 2012; Behrenfeld et al., 2006; Doney, 2006]. In addition, expanding open water areas (increasing suitable ice-free habitats for phytoplankton growth) and longer open water seasons (prolonging the time between spring melt and fall freeze-up) result in a lengthened and more productive phytoplankton growing season [Arrigo et al., 2008]. Enhanced biological productivity is predicted to significantly alter patterns of nutrient cycling in the ocean, carbon export to the deep sea, impact fishery yields, and the marine ecosystem - resulting in wholesale shifts in local species abundance and diversity, particularly in mid-to-high-latitude regions where there are a low number of trophic links [Arrigo et al., 2008; Drinkwater and Pepin, 2013; Frajka-Williams and Rhines, 2010; Guay et al., 2009].

1.1.2 Ocean Acidification

Over the same time period, the oceans have absorbed approximately one-third of anthropogenically-derived carbon over the industrial era through continuous air-sea gas exchange [Sabine et al., 2004] - a process commonly known as ocean acidification, also known as “The other CO2 problem” [Doney et al., 2009]. While oceanic uptake of atmospheric CO2 has curtailed GHG-induced warming realized to date, it has also resulted in widespread changes on seawater chemistry. Surface seawater CO2 equilibrates with the atmospheric CO2 concentrations
on the timescale of approximately one year, and undergoes speciation through a series of chemical reactions:

\[
\begin{align*}
\text{CO}_2 \text{(atm)} & \leftrightarrow \text{CO}_2 \text{(aq)} + \text{H}_2\text{O} \\
& \leftrightarrow \text{H}_2\text{CO}_3 \\
& \leftrightarrow \text{H}^+ + \text{HCO}_3^- \\
& \leftrightarrow 2\text{H}^+ + \text{CO}_3^{2-}
\end{align*}
\]  

(1)

Dissolution of CO₂ in seawater forms carbonic acid (H₂CO₃), which can then dissociate by losing hydrogen ions (H⁺) to form bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) ions. The hydrolysis of CO₂ in seawater increases the hydrogen ion concentration (thereby lowering pH), and can also react with carbonate to form more bicarbonate - ultimately lowering the seawater carbonate saturation state (Ω) [Doney et al., 2009]. Ocean pH has declined by 0.1 units since preindustrial times [Caldeira and Wickett, 2003; Orr et al., 2005], and is expected to decrease an additional 0.3 – 0.5 pH units by 2100 as the ocean continues to absorb anthropogenic CO₂ (Figure 1) [Caldeira and Wickett, 2005].

**Figure 1 | Observed and projected carbonate concentration for world oceans.**

A) Global Ocean Data Analysis Project (GLODAP) - based CO₃²⁻ at sea surface for year 1995. B) Community Climate System Model 3.1 (CCSM3) - modeled decadal mean CO₃²⁻ at sea surface projected for year 2095. White areas indicate regions with no data. Images adapted from [Feely et al., 2009].
Ocean acidification can have profound effects on marine organisms that produce shells, tests, and skeletons out of calcium carbonate (CaCO₃). As oceans become more acidic, the availability of CO₃ for organisms to secrete calcium carbonate structures decreases, compromising the ability for marine organisms to calcify, which may lead to the production of weaker skeletons that are more vulnerable to biological and or physical erosion [Andersson et al., 2003; Feely et al., 2009; Kroeker et al., 2013; Ragazzola et al., 2012]. The rapidly growing body of experimental research examining the impacts of ocean acidification on a variety of marine calcifiers reveals a broad spectrum of responses depending on factors such as: life history stage, degree of control over calcification, presence of biogenic coverings, and the amount and type of calcium carbonate in the shell or skeleton [Kroeker et al., 2013; Ries et al., 2009]. Despite differences among taxonomic groups, a recent comprehensive meta-analysis synthesizing data pooled together from 228 studies indicates that ocean acidification (roughly representing year 2100 scenarios) had a significantly negative effect on factors such as growth, development, abundance, survival and calcification [Kroeker et al., 2013]. Among the key taxonomic groups investigated, coralline algae, corals, and molluscs exhibited the largest magnitude of biological responses, followed by echinoderms and coccolithophores [Kroeker et al., 2013; Kroeker et al., 2010]. Furthermore, it has been hypothesized that high-latitude marine calcifiers will be most vulnerable to undersaturated conditions owing to naturally depressed Ω resulting from increased CO₂ solubility in colder water temperatures [Andersson et al., 2008; Büdenbender et al., 2011; Feely et al., 2004; Orr et al., 2005; Ragazzola et al., 2012]. As such, adverse biological consequences on key taxonomic groups - particularly those residing in high-latitude regions, is likely to result in wholesale shifts in distributional ranges of species, a loss in species biodiversity and abundance, and altered tropic interactions [Connell et al., 2013; Fabry, 2008; Kleypas and Yates, 2009].

Therefore, continued greenhouse warming and acidification of the world’s oceans are expected to lead to widespread and detrimental consequences on marine ecosystems - and are projected to intensify in the future [IPCC, 2013]. Climate model simulations, used for making projections of future climate scenarios, rely upon the analyses of temporally and spatially diverse instrumental and proxy climate information. However, historical records and instrumental observations from the marine realm are spatiotemporally sparse prior to the mid-20th century - particularly in Arctic and Subarctic regions, where difficulties of navigating in remote ice-laden waters and harsh polar
climates have oftentimes resulted in short and incomplete records. The scarcity of climate data, especially from extratropical oceans, can greatly limit our understanding of important low-frequency climate forcing mechanisms. High-resolution reconstructions of past ocean climate and environments have therefore relied upon archival information stored in long-lived marine biota in order to place currently observed changes in the context of natural long-term climate variability [Wanamaker Jr et al., 2011].

1.2 Sclerochronology: The Study of Past Climates

Since the initial discovery of annual density banding patterns in skeletons of reef-building hermatypic corals, the analyses of chemical and physical growth parameters have enabled paleoclimate scientists to derive information about the seawater environment in which the corals grew [Buddemeier et al., 1974; Knutson et al., 1972]. Sclerochronology is the study of marine organisms that form incremental growth patterns in biomineralized shells and skeletons as recorders of past environmental and oceanographic conditions [Buddemeier et al., 1974; Hudson et al., 1976; Jones, 1983]. The formation of banding patterns in a multitude of long-lived biogenic archives (ex. corals, bivalve molluscs, coralline algae, fish otoliths, etc.) can occur inter-annually to subannually, and can extend back in time on multidecadal and even multicentennial timescales. Layer counting methods allow for long calendar-dated records of proxy marine climate information to be obtained at high temporal resolution, analogous to records of terrestrial environmental change through the study of tree-rings in dendrochronology [Hughes, 2002]. Thus, using these precisely dated archives, proxy information about past oceanographic conditions may be obtained through geochemical analyses and measurements of physical growth properties (ex. growth increment widths, skeletal density, and calcification rates).

1.3 High-Latitude, High-Resolution Marine Proxies

To date, the vast majority of high-resolution proxy-based paleoclimate reconstructions have been concentrated in warm tropical and subtropical seas due to the limited spatial distribution of many
reef-forming coral species. However, in order to address the rapidly changing climate and environment in high-latitudes, a renewed focus has been placed on the search for additional high-resolution sclerochronological archives in mid-to-high latitude oceans. Here, I will briefly review annually to subannually resolved, multidecadal-to-centennial sclerochronological archives for understanding past changes in the climate and environment in mid-to-high latitude regions of the northern hemisphere (Figure 2 and Table 1):

1.3.1 Cold-Water Corals

Although corals have traditionally been associated with shallow tropical and subtropical seas, recent advancements in deep-sea exploration have revealed a variety of cold-water azooxanthellate corals (without symbiotic dinoflagellates) such as: stony corals, soft corals, black corals, and hydrocorals [Freiwald et al., 2004]. Cold-water corals generally reside in regions where ocean temperatures remain between 4 – 12 °C; at depths of ~ 50 – 1000 m in high latitudes, and at depths of up to 4000 m in low latitude regions [Roberts et al., 2006; Robinson et al., 2014]. While cold-water corals have mostly been observed in mid-to-high latitude Atlantic, they are likely to exhibit global distribution - limited only by our ability to observe them at depth [Freiwald et al., 2004].
Figure 2 | Distribution of high-resolution, mid-to-high latitude marine paleoclimate and paleoenvironment proxy records.

Symbols represent distinct groupings of marine archives, broadly categorized into: coralline algae (star), bivalve molluscs (circle), and deep-sea corals (square). Colours differentiate distinct organisms within the same grouping (ex. geoduck vs. ocean quahog). Numbers correspond to information listed in Table 1, which briefly summarizes the archive type, genus/species, paleoclimate and/or paleoenvironment analyses performed, sample location, and reference.
Table 1 | Summary of high-resolution, mid-to-high latitude marine proxies. Brief summary of archive type, genus/species, paleoclimate and/or paleoenvironmental reconstruction performed, location of sample collection, and references from high-resolution marine sclerochronological archives. Information is broadly categorized into three groups: deep-sea corals, bivalve molluscs, and coralline algae. Reference number in the first column corresponds to numbering found in Figure 2.

<table>
<thead>
<tr>
<th>Ref. #</th>
<th>Archive</th>
<th>Species</th>
<th>Reconstruction</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gorgonian Coral</td>
<td><em>Primnoa resedaeformis</em>, <em>Primnoa willeyi</em></td>
<td>$\delta^{13}$C and $\delta^{15}$N for surface productivity</td>
<td><em>P. resedaeformis</em> from Gulf of Maine in NE Channel, Nova Scotia, Canada; <em>P. willeyi</em> from British Columbia, Canada; Aleutian Islands and Prince William Sound, Alaska, USA.</td>
<td>[Sherwood et al., 2005a]</td>
</tr>
<tr>
<td>2</td>
<td>Gorgonian Coral</td>
<td><em>Primnoa spp.</em></td>
<td>C Suess effect and $^{15}$N plankton composition</td>
<td>Gulf of Alaska, USA</td>
<td>[Williams et al., 2007]</td>
</tr>
<tr>
<td>3</td>
<td>Bamboo Coral</td>
<td><em>Isididae (Keratoisis, Isidella, or Acanella spp.)</em></td>
<td>Surface water $^{14}$C and total dissolved inorganic carbon (DIC) of intermediate waters</td>
<td>Warwick Seamount, Alaska, USA</td>
<td>[Roark et al., 2005]</td>
</tr>
<tr>
<td>4</td>
<td>Bamboo Coral</td>
<td><em>Isididae or Coralliidae family</em></td>
<td>Intermediate depth ocean temperatures</td>
<td>Warwick Seamount, Alaska, USA</td>
<td>[Kimball et al., 2014]</td>
</tr>
<tr>
<td>Ref. #</td>
<td>Archive</td>
<td>Species</td>
<td>Reconstruction</td>
<td>Location</td>
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<td>5</td>
<td>Gorgonian Coral</td>
<td><em>Primnoa resedaeformis</em></td>
<td>Mg/Ca as proxy for seawater temperature at depth</td>
<td>In-between Georges Bank and Browns Bank within pathway of NE Channel, Nova Scotia, Canada</td>
<td>[Sherwood et al., 2005b]</td>
</tr>
<tr>
<td>6</td>
<td>Gorgonian Coral</td>
<td><em>Primnoa resedaeformis</em></td>
<td>δ¹⁵N as tracer for nutrient and slope water origin</td>
<td>Gulf of Maine, in pathway of NE Channel, Nova Scotia, Canada</td>
<td>[Sherwood et al., 2011]</td>
</tr>
<tr>
<td>7</td>
<td>Gorgonian Coral</td>
<td><em>Primnoa resedaeformis</em></td>
<td>Surface water ¹⁴C and productivity</td>
<td>Northeast Channel, SW Nova Scotia, Canada</td>
<td>[Sherwood et al., 2005c]</td>
</tr>
<tr>
<td>8</td>
<td>Gorgonian Coral</td>
<td><em>Primnoa resedaeformis, Keratoisis ornata</em></td>
<td>Surface and intermediate depth ¹⁴C – relative contributions of Labrador Slope Water vs. Warm Slope Water</td>
<td>SW Grand Banks, Baffin Bay, Davis Strait, Hudson Strait, Sable Gully, NE Channel, Canada</td>
<td>[Sherwood et al., 2008]</td>
</tr>
</tbody>
</table>

### Bivalve Molluscs (Circle Symbols)

<table>
<thead>
<tr>
<th>Ref. #</th>
<th>Archive</th>
<th>Species</th>
<th>Reconstruction</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pacific Geoduck</td>
<td><em>Panopea abrupta</em></td>
<td>SST</td>
<td>British Columbia, Canada</td>
<td>[Black et al., 2009]</td>
</tr>
<tr>
<td>2</td>
<td>Pacific Geoduck</td>
<td><em>Panopea abrupta</em></td>
<td>SST and surface air temperature</td>
<td>Ladysmith Harbour, British Columbia, Canada</td>
<td>[Noakes and Campbell, 1992]</td>
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<td>Strait of Juan de Fuca, Washington State, USA</td>
<td>[Strom et al., 2004; Strom et al., 2005]</td>
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<td>Summer SST and food supply (associated with primary productivity)</td>
<td>[Schöne et al., 2005a]</td>
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<td>West of island of Grimsey, Northern Iceland</td>
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<td>SST</td>
<td>[Butler et al., 2013]</td>
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<td>$^{14}$C reservoir ages</td>
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<td>SST, Primary productivity, and human activity (land-use change and marine aquiculture)</td>
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<td>Primary productivity (Copepod abundance)</td>
<td>[Witbaard et al., 2003]</td>
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<td>NE North Sea and SW Baltic Sea</td>
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<td>SST and primary productivity</td>
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<td>North Atlantic Oscillation (NAO) reconstruction</td>
<td>[Helama and Hood, 2011]</td>
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<td>SST [Hetzinger et al., 2009] (\delta^{13}C) Suess effect, and upwelling associated with the Aleutian Low pressure system [Williams et al., 2011]</td>
<td>Attu Island and Amchitka Island, Aleutian Islands, Alaska, USA</td>
<td>[Hetzinger et al., 2009; Williams et al., 2011]</td>
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<td><em>Clathromorphum nereostratum</em></td>
<td>SST</td>
<td>Attu Island, Amchitka Island, and Akun Island, Aleutian Islands, Alaska, USA</td>
<td>[Williams et al., 2014]</td>
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<td>SST, Pacific Decadal Oscillation (PDO) and El Niño Southern Oscillation (ENSO) teleconnections</td>
<td>Attu Island, Aleutian Islands, Alaska, USA</td>
<td>[Halfar et al., 2007]</td>
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<td>Aleutian Low (AL), upper-ocean mixing, light-levels</td>
<td>Attu, Alaid, Rat, Amchitka, Ogliuga, Akun Islands, Alaska, USA</td>
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<td>SST, teleconnection pattern between North Pacific AL Pressure System and NAO</td>
<td>Amchitka Island, Aleutian Islands, Alaska, USA</td>
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<td>SST, PDO, salinity / freshwater variability</td>
<td>Akun Island, Aleutian Islands, Alaska, USA</td>
<td>[Chan et al., 2011]</td>
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<td><em>Lithophyllum impressum, Lithothamnion phymatodeum, Pseudolithophyllum muricatum, Pseudolithophyllum whidbeyense</em></td>
<td>Skeletal response to decreasing pH</td>
<td>Tatoosh Island, Washington, USA</td>
<td>[McCoy and Ragazzola, 2014]</td>
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<td>Crustose</td>
<td><em>Clathromorphum compactum</em></td>
<td>Sea-ice variability</td>
<td>Arctic Bay, Nunavut; and Kingitok Island, northern Labrador, Canada</td>
<td>[Halfar et al., 2013]</td>
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<td>9</td>
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<td><em>Clathromorphum compactum</em></td>
<td>SST, NAO and Atlantic Multidecadal Oscillation (AMO) periodicity</td>
<td>Bay Bulls, Cape St. Martin, and Quirpon Island, Newfoundland, Canada</td>
<td>[Halfar et al., 2011a]</td>
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<td>10</td>
<td>Rhodolith</td>
<td><em>Lithothamnium crassiusculum, Lithothamnium glaciale</em></td>
<td>SST</td>
<td>Strait of Belle Isle, Newfoundland, Canada</td>
<td>[Halfar et al., 2000]</td>
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<td>11</td>
<td>Rhodolith</td>
<td><em>Lithothamnium glaciale</em></td>
<td>SST, and glacial runoff / salinity</td>
<td>Søndre Strømfjord, Greenland</td>
<td>[Kamenos et al., 2012]</td>
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<td><em>Clathromorphum compactum</em></td>
<td>SST</td>
<td>Bay Bulls, Cape St. Martin, and Quirpon Island, Newfoundland, Canada</td>
<td>[Gamboa et al., 2010]</td>
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<td>13</td>
<td>Crustose</td>
<td><em>Clathromorphum compactum</em></td>
<td>Freshwater variability / salinity and ocean water</td>
<td>Quirpon Island and Bay Bulls, St. Johns, Newfoundland</td>
<td>[Hetzinger et al., 2013]</td>
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In the cold, dark, and nutrient-rich waters of the deep, cold-water corals grow very slowly, but can form large reefs that can live for hundreds to even thousands of years [Druffel et al., 1995; Roberts et al., 2006]. In fact, visual and radiometric evidence from annual layer-forming deep-sea gorgonian coral *Primnoa resedaeformis* (subclass *Octocorallia* – also known as Sea fan or red tree coral) have revealed individual ages of 100 - 300 years using $^{14}$C dating and $^{210}$Pb dating [Andrews et al., 2002; Risk et al., 2002; Sherwood et al., 2005c]. *P. resedaeformis* typically reside in water depths of 200 – 700 m, and form a unique two-part skeleton comprised of alternating bands of calcite and proteinaceous gorgonin, that provides direct measures of dissolved inorganic carbon (DIC) at depth, and particulate organic matter (POM – via sinking coral food source) at the surface, respectively [Risk et al., 2002; Sherwood et al., 2008; Sherwood et al., 2005a]. Analysis of the red tree coral growth structure enabled radiocarbon reservoir ages of North Atlantic slope waters along the entire northwest North Atlantic to be made [Sherwood et al., 2008]. Similarly, radiocarbon dating of *P. resedaeformis* off of southwest Nova Scotia, Canada, has also been used to reconstruct the radiocarbon bomb spike curve [Sherwood et al., 2005c]; and was found to be identical to $\delta^{14}$C previously measured in nearby ocean quahog Arctica Islandica [Weidman and Jones, 1993], and inner ear bones (otoliths) of haddock *Melanoglossmus aeglefinus* [Campana, 1997]. The coherence of the radiocarbon signal
among multiple sclerochronological archives further confirms that all specimens recorded the same regional signal. Additionally, Mg/Ca trace element ratios extracted from the calcite skeleton of *P. resedaeformis* have been shown to be in general agreement with local instrumental temperatures recorded at depth [Sherwood et al., 2005b]. However, investigations conducted on other cold-water coral species found that internal variability of temperatures reconstructed from Mg/Ca were more than double the range of instrumental temperatures measured – likely reflecting food availability rather than in-situ SSTs [Aranha et al., 2014; Cohen et al., 2006; Gagnon et al., 2007]. More recently, methods such as compound-specific analysis of amino acids (δ⁰¹⁵N-AA) have been used to examine δ¹⁵N variability in gorgonian corals associated with an North Atlantic Oscillation (NAO) driven increase in subtropical Gulf Stream slope waters since the 1970s [Sherwood et al., 2011]. Other members of the genus *Primnoa spp.* collected from the Gulf of Alaska have also demonstrated potential as paleoclimate archives, with stable isotopes of carbon (δ¹³C) and nitrogen (δ¹⁵N) from the coral skeleton being associated with depletions in ¹³C related to the anthropogenic injection of carbon into the atmosphere known as “The Suess Effect”; and depletions in ¹⁵N corresponding to changes in the coral food source [Williams et al., 2007]. Other gorgonian octocorals such as the Bamboo coral (order Alycyonacea, family Isididae) also form a two-part skeleton in a candelabralike shape, with several centimeter-long cylindrical calcite internodes interspaced with proteinaceous gorgonin nodes 4-8 mm wide [Roark et al., 2005]. Δ¹⁴C dated skeletons of bamboo coral correspond to oscillations in Sr/Ca ratios, indicating annual cycles; while the apparent fine-scale banding pattern have been hypothesized to represent monthly lunar cycles influencing zooplankton biomass - hence coral growth rates [Roark et al., 2005]. Similar to the red tree coral, bamboo corals have also recorded elevated ¹⁴C associated with the bomb-spike signature from atmospheric nuclear testing in late 1950s and 1960s, and have shown potential for recording biogeochemical changes at the surface and at depth [Roark et al., 2005].

While cold-water corals are capable of providing dual paleoceanographic records of surface and deepwater processes in high-latitude regions where proxy archives are scarce, sclerochronological analysis of annual growth increments identification may be challenging. Annual couplets of light brown (calcite) and dark brown (gorgonin) are more easily identified towards the center of the *P. resedaeformis* coral branch; However outer layers of the coral skeleton are mostly made up of finely banded translucent calcite (with very little gorgonin)
complicating the identification of annual growth [Risk et al., 2002; Sherwood et al., 2005b]. Thus, layer counting methods must always be supplemented with radiometric dating techniques (ex. $^{12}$C, $^{210}$Pb, U/Th), leading to error estimates in growth increment ages [Sherwood et al., 2005a; Williams et al., 2007]. Furthermore, irregular growth direction, uneven growth rates (inner vs. outer layers) and deposition of individual growth layers may result in wide or narrow, and even discontinuous sections around the circumference of a single increment [Risk et al., 2002]. Similarly, growth rates in bamboo corals are not uniform over the lifetime of individual specimens [Roark et al., 2005]. This can be especially problematic in the analysis of samples exhibiting ambiguous growth banding - where constant growth is commonly assumed and specimen ages are linearly extrapolated from growth rates taken from regions where banding is clear [Risk et al., 2002; Roark et al., 2005; Williams et al., 2007]. Therefore complex skeletal morphologies, combined with extremely slow growth rates, often complicates the precise dating and identification of growth banding patterns in cold-water corals.

1.3.2 Bivalves

Shells of nearshore bivalve molluscs currently supply the bulk of annually-to-subannually resolved marine paleoclimate data to date. A number of bivalve species exhibit pronounced longevity on multi-decadal and even multi-centennial timescales, some of which include: Pacific geoduck clams [Black et al., 2009; Strom et al., 2004], ocean quahogs [Butler et al., 2013; Schöne, 2013; Wanamaker et al., 2008], and deepwater oysters [Wisshak et al., 2009]. In addition, cross-dating techniques adapted from the field of dendrochronology can be applied to bivalve sclerochronology in order to generate absolutely dated master chronologies spanning several centuries [Black et al., 2009; Butler et al., 2009; Butler et al., 2013; Witbaard et al., 1997]; and can even incorporate specimens from archaeological shell middens and fossil shells in floating chronologies [Andrus, 2011; Helama and Hood, 2011]. Annual growth increment chronologies from Pacific geoduck *Panopea abrupta*, have been used as proxies for SST changes in the northeast Pacific [Black et al., 2009; Noakes and Campbell, 1992; Strom et al., 2004; Strom et al., 2005]. In the northeast Atlantic, ocean quahog *Arctica islandica* represents the most comprehensively studied bivalve mollusk species in the field of palaeoclimatology due to: 1) extreme longevity – with individual solitary organisms with maximum reported lifespans of 375
to 507 years [Butler et al., 2013; Schöne et al., 2005a; Wanamaker et al., 2008]; 2) the formation of annual and even daily growth lines – allowing for precise calendar dating of shell material [Jones, 1980; Schöne et al., 2005b]; 3) Wide geographical distribution across the Atlantic Ocean – in the west from North Carolina, USA to Newfoundland Canada, and to the east extending along the European coastline from France and Iceland to Norway [Dahlgren et al., 2000; Schöne, 2013]; and 4) variable growth increment widths and geochemical composition – that has been shown to record changes in environmental conditions [Schöne, 2013; Wanamaker Jr et al., 2011]. Furthermore, examination of the oxygen isotopic composition of *A. islandica* has confirmed that growth bands are indeed formed at annual periodicities, and that its shell is deposited in isotopic equilibrium with the surrounding seawater [Epstein et al., 1953; Weidman et al., 1994].

Growth increment widths in *A. islandica* have been shown to respond to: food availability associated with phytoplankton and zooplankton abundance [Witbaard et al., 2003; Wanamaker et al., 2009], and local seawater temperatures [Butler et al., 2010; Butler et al., 2013; Lohmann and Schöne, 2013; Schöne et al., 2003]. Master composite growth chronologies compiled from multiple nearby specimens demonstrate synchronous shell growth [Butler et al., 2009; Marchitto Jr et al., 2000], and can extend back in time for several centuries - with the longest record to date spanning the last 1357 years [Butler et al., 2013]. Multi-centennial master growth chronologies have demonstrated decadal to century-scale sea-level pressure anomalies associated with the North Atlantic Oscillation (NAO) [Butler et al., 2010; Lohmann and Schöne, 2013; Schöne et al., 2003], and sea surface temperature oscillations in phase with the Atlantic Multidecadal Oscillation (AMO) [Butler et al., 2013]. In addition to growth increment analyses, a number of geochemical methods have been applied in the field of *A. islandica* sclerochronology for the reconstruction of various climate and environmental signals such as: ocean circulation patterns – based on radiocarbon data ($\Delta^{14}$C); primary productivity - based on carbon isotopes ($\delta^{13}$C) and barium-to-calcium element ratios (Ba/Ca); water temperature - based on oxygen isotopes ($\delta^{18}$O), carbonate clumped isotopes ($\Delta^{47}$), calcium isotopes ($\delta^{44/40}$Ca), strontium-to-calcium (Sr/Ca), and magnesium-to-calcium (Mg/Ca) element ratios; continental weathering - based on lithium-to-calcium element ratios (Li/Ca); and pollution by human activities - based on heavy metal concentrations such as Lead (Pb), Cadmium (Cd), Zinc (Zn), and Copper (Cu), etc. as reviewed in Schöne [2013].
Extensive sclerochronological research using the bivalve mollusc *A. islandica* has demonstrated a broad spectrum of applications for past climate and environmental reconstructions in extra-tropical seas; however, interpretation of this biogenic archive may be complicated by: 1) Growth that is influenced by an ontogenetic, asymptotic growth trend (ex. rate of shell growth decreases exponentially with age resulting in narrower increments and decreased resolution in older portions of the shell [Goodwin et al., 2003]) - requiring the application of exponential detrending functions that may also remove low-frequency climate variability; 2) Shell growth occurs during both summer and winter seasonal extremes, but does not occur throughout the calendar year [Schöne et al., 2005b] and exhibits a shutdown of growth once water temperatures drop below 6 °C [Wisshak et al., 2009]; 3) Biogeographical distribution that is mainly limited to the northeast Atlantic and is virtually absent from the subarctic northwest Atlantic [Wanamaker et al., 2008]. Hence, in order to overcome the inherent limitations associated with bivalve growth and physiology, I present herein coralline algae - the subject of this dissertation, as an additional source of paleoenvironmental proxy records that is also long-lived, annually-to-subannually resolved, more widely distributed in cold-water environments, and do not suffer from ontogenetic-related growth declines.

### 1.3.3 Crustose Coralline Algae

Coralline algae are calcareous marine photoautotrophs that commonly exist as nodular free-living rhodoliths or rock-like encrustments on hard substrate, both of which can be used for paleoclimate analysis. Coralline algae are abundant and geographically widespread, occurring in shallow rocky sublittoral zones extending from the tropics to the poles [Freiwald and Henrich, 1994; Steneck, 1986]. Coralline algae form key ecological components to the north and south of the tropical coral reef belt, representing the most important framework builders for maintaining habitat and biodiversity [Foster et al., 2013; Freiwald and Henrich, 1994; Nelson, 2009] - providing structurally complex substrate that act as nursery grounds, habitat, refuge from predation, and food for a variety of infaunal and epifaunal invertebrate species [Chenelot et al., 2011; Kamenos et al., 2013; Kamenos et al., 2004; Steneck, 1982].

Coralline algae of the genus *Clathromorphum* are exceptionally abundant in subarctic regions of the North Atlantic, North Pacific, and Arctic oceans, where they can cover up to 100% of the
shallow seafloor, and form accretions of up to 50 cm in thickness (Figure 3A) [Lebednik, 1976]. With an average vertical extension rate of approximately 380 µm/year, *Clathromorphum* *spp.* coralline algae can exhibit age spans of several hundred years [Halfar et al., 2007]. In fact, a living specimen of *Clathromorphum nereostratum* collected from the Aleutian Islands, Alaska has been U/Th dated to 850 ± 28 years, making it one of the oldest known marine organisms [Frantz et al., 2005; Halfar et al., 2007]. Furthermore, in contrast to bivalve mollusces where the sclerochronological interpretation of shell growth is complicated by the ontogenetic decline in linear growth rates [Goodwin et al., 2003], coralline algae demonstrate constant growth over their lifespan, and do not exhibit a slowdown in growth with increasing age. Growth of coralline algae occurs mainly during the spring and summer months, through the accretion of high-Mg calcite as large and less-extensively calcified cells in the perithallus [Kamenos and Law, 2010]. Whereas during the cold (below 5 °C) and dark winter months, growth slows (or even ceases entirely), producing smaller and more extensively calcified cells [Adey, 1970; Halfar et al., 2008]. This seasonal variation in cell size and cell wall thickness produces conspicuous light and dark couplets, which together form well-developed annual growth banding patterns – similar to tree-rings [Foster et al., 2013; Freiwald and Henrich, 1994; Halfar et al., 2008; Kamenos and Law, 2010; Moberly, 1968]. Growth increments within coralline algae are also delineated by the presence of annually-forming rows of reproductive cavities called “conceptacles”, which form in the fall by the downward decalcification of growth increment material laid down during the previous growing season (Figure 3B) [Adey, 1965]. The annual feature of coralline algal growth increments have been demonstrated independently for both free-living and encrusting species via in-situ field calibration studies in the temperate North Atlantic [Halfar et al., 2008; Kamenos et al., 2008]. Therefore, coralline algae may serve as excellent high-resolution paleoclimate archives for reconstructing past changes in the ocean environment due to their: 1) Abundance and ubiquitous distribution, particularly in mid-to-high latitude oceans; 2) Longevity on multi-decadal to multi-centennial timescales; and 3) Formation of clear and consistent annual growth increments, which allows for precise calendar dating of calcified tissue and do not suffer from ontogenetic declines [Halfar et al., 2008; Kamenos et al., 2008].
Figure 3 | Underwater and cross-sectional image of crustose coralline algae. A) Underwater image of crustose coralline algae *Clathromorphum nereostratum*. B) Digital photomosaic of algal cross-section displaying: epithallus layer (outer protective covering); perithallus layer (where skeletal calcite accumulates and proxy information is stored) directly underneath the epithallus, separated by the meristem (where growth and calcification occurs); annual growth increments (delineated by red lines); conceptacles (reproductive cavities – black holes); and calendar dating of the algal skeleton using layer counting methods – beginning with the year of collection (in 2011).

Early studies have suggested that the cyclic pattern of Mg-content in coralline algae was related to seasonal variations in the seawater temperatures in which the carbonates formed [Chave, 1954; Chave and Wheeler, 1965], and algal growth - which are influenced by a combination of factors such as light intensity, physiological cycles, and seawater temperature [Moberly, 1968]. Since then, the Mg-temperature relationship was confirmed for temperate (*Lithothamnion glaciale* - Strait of Belle Isle, Canada, and Loch Sween, Scotland; and *Phymatolithon calcareum* - Isle of Arran, Scotland), subtropical (*Lithothamnion crassiusculum* - Gulf of California, Mexico), and tropical (*Lithophyllum kotschyanum f. affine* - Gulf of Aden, Yemen, and *Sporolithon durum* - Nouméa, New Caledonia) rhodolith species through the comparison of magnesium-to-calcium (Mg/Ca) trace element ratios and Mg-content (mol% MgCO₃) to local SST [Caragnano et al., 2013; Darrenougue et al., 2014; Halfar et al., 2000; Kamenos et al., 2008]. Significant positive correlations were also demonstrated between Mg/Ca ratios and instrumental station-based and gridded SST data for encrusting species of coralline algae off
Newfoundland, Canada (Clathromorphum nereostratum) [Gamboa et al., 2010], and off the Aleutian Islands, Alaska, USA (Clathromorphum nereostratum) [Chan et al., 2011; Chan, this thesis; Hetzinger et al., 2009; Hetzinger et al., 2012; Williams et al., 2014]. Additional calibration studies based on synchrotron Mg-X-ray absorbance near edge structure (XANES), and electron and ion microprobes further confirmed the use of coralline Mg as a paleotemperature proxy; demonstrating that Mg is associated with the calcite lattice (and therefore not organically-bound) [Kamenos et al., 2009], and that Mg contents (mol% MgCO₃) were positively and linearly related to in-situ and gridded SST [Kamenos et al., 2008].

The oxygen isotopic composition (δ¹⁸O) of Lithothamnion spp. have been shown to be negatively offset from isotopic equilibrium (likely due to species-specific vital effects) [Halfar et al., 2000; Halfar et al., 2007; Wefer and Berger, 1991], with values exhibiting a strong temperature dependence. In fact, centennial scale records of δ¹⁸O have been linked to large-scale patterns of climate variability such as the El Niño Southern Oscillation (ENSO) and Pacific Decadal Oscillation (PDO) [Halfar et al., 2007]. Conversely, others found no such offset, suggesting that carbonate δ¹⁸O is deposited in isotopic equilibrium with ambient seawater [Kamenos et al., 2012; Rahimpour-Bonab et al., 1997]. A Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) analysis of various trace element ratios (Mg/Ca, Ba/Ca, Sr/Ca, U/Ca) have demonstrated high inter- and intra-specimen data reproducibility [Hetzinger et al., 2013], and found that Sr/Ca also shows a relationship correspondence to SST (slightly weaker in comparison to the Mg/Ca-temperature relationship) [Hetzinger et al., 2011], which agrees with a previous calibration study of mol% SrCO₃–temperature relationships [Kamenos et al., 2008]. δ¹³C in the coralline algae have also been investigated, displaying a larger than expected decline as a result of combined influences of the anthropogenic Suess effect, with upwelling of isotopically light carbon (¹²C) enriched deep waters driven by the Aleutian Low pressure system (AL) [Williams et al., 2011]. In addition to geochemical analyses of the coralline algal skeleton, growth increment widths have been interpreted to reflect shallow marine light dynamics driven by the AL [Halfar et al., 2011b], with low frequency variability linked to the North Atlantic Oscillation (NAO) and Atlantic Multidecadal Oscillation (AMO) climate indices [Halfar et al., 2011a]. Meanwhile, percent calcification (degree of cellular carbonate infilling in randomly placed digital quadrats) within the algal skeleton have been interpreted to
reflect historic changes in marine cloud cover [Burdett et al., 2011], and SST - with a negative relationship to the AMO [Kamenos and Law, 2010].

Geochemical and physical growth signals extracted from growth bands of coralline algae continue to provide a wealth of annually to seasonally resolved proxy climate information from extratropical marine settings where other archives are not present, revealing connections to large-scale multi-decadal climate oscillation patterns that were previously unresolvable by the short record of oceanographic observations. Recent advancements in the study of coralline algae have focused on developing novel proxies, such as: Barium-to-calcium (Ba/Ca) element ratios – for reconstructing coastal freshwater variability associated with seasonal glacial melt [Chan et al., 2011; Chan, this thesis], and ice melt-induced stratification of the surface ocean [Hetzinger et al., 2013]; Algal Mg/Ca and growth as a combined proxy for temperature and solar radiation associated with sea-ice variability [Halfar et al., 2013]; and Algal Mg/Ca and δ¹⁸O as a combined proxy for temperature and glacial runoff [Kamenos et al., 2012].

There is also a growing interest in examining and quantifying the physiological response of high-Mg calcite coralline algal skeletons to ocean acidification in coastal marine environments. Current models suggest that much of the Southern Ocean, Arctic Ocean and subarctic north Pacific will become undersaturated with respect to aragonite by 2100 [Orr et al., 2005]. In fact, seasonal aragonite undersaturation has already been observed in some northern polar seas [Bates et al., 2013]. Solubility experiments conducted on biogenic Mg calcites have found that calcareous marine organisms containing significant (> 4 mol% MgCO₃) termed “high Mg-calcite” exceeds the solubility of both calcite and aragonite at 12 mol% MgCO₃, with increasing solubility at higher Mg concentrations [Andersson et al., 2008; Morse et al., 2006]. The substitution of calcium ions by much smaller magnesium ions within the calcite lattice leads to a distortion of the mineralogical structure, resulting in metastable form of carbonate that is more readily dissolved at reduced saturation states [Andersson et al., 2008].

The response of various tropical-to-polar species of coralline algae to declining ocean pH has been tested in a number of short-term pCO₂–enrichment studies - and has only recently been observed in the field [McCoy and Ragazzola, 2014]; demonstrating adverse effects on coralline algal calcification [Büdenbender et al., 2011; Gao and Zheng, 2010; Johnson and Carpenter, 2012; Johnson et al., 2012; Martin and Gattuso, 2009; Ragazzola et al., 2012]; photosynthesis
[Anthony et al., 2008; Gao and Zheng, 2010]; juvenile recruitment rate [Kuffner et al., 2008]; percentage cover (abundance) [Hall-Spencer et al., 2008; Kuffner et al., 2008]; competitive ability [McCoy and Pfister, 2014; McCoy and Ragazzola, 2014]; survivorship [Martin and Gattuso, 2009], and a community shift towards non-calcifying taxa [Johnson et al., 2012; Porzio et al., 2011]. However, despite their importance for supporting diverse reef-like communities in benthic subarctic ecosystems, field observations of the in-situ physiological response of coralline algae to changing seawater chemistry are scarce and lacking. Furthermore, it has been hypothesized that high-latitude marine calcifiers would be even more vulnerable to undersaturated conditions owing to the naturally depressed Ω resulting from increased CO$_2$ solubility in colder water temperatures [Andersson et al., 2008; Büdenbender et al., 2011; Feely et al., 2004; Orr et al., 2005; Ragazzola et al., 2012]. Therefore, coralline algae composed of highly soluble high Mg-calcite residing in Subarctic regions where the carbonate saturation state is exceptionally low, will likely be amongst the first responders to changes associated with ocean acidification.

### 1.4 Aims and Objectives

The objectives of this dissertation is threefold; (1) explore new applications to existing Mg/Ca and Ba/Ca trace element proxies used in mid-to-high latitude coralline algal paleoclimatology; (2) develop a novel method for the visualization and quantification of internal structural changes within the coralline algae skeleton; and (3) apply this method for investigating the physiological response of coralline algae to changing seawater chemistry in cold-water regions of the Subarctic North Pacific. These three components will allow us to further develop proxy records used for coralline algal sclerochronology, and make inferences on the anticipated consequences of climate and environmental changes for coralline algal-dominated ecosystems in the North Pacific and North Atlantic oceans.
1.5 Structure of Thesis

This thesis consists of six chapters. **Chapter 1** began with modern climatological changes in Arctic and Subarctic regions, followed by a general introduction to a variety of mid-to-high latitude paleoclimate archives (including the subject of this study - crustose coralline algae), used for understanding long-term climate and environmental variability. **Chapters 2 – 5** are comprised of four stand-alone manuscripts structured in a format intended for publication in peer-reviewed scientific journals - thus each containing its own introduction, methods, results, discussion, and conclusion sections. The following paragraphs provide a brief overview of the hypotheses and thematic content addressed in each respective chapter:

In **Chapter 2**, “Micro computed tomography: Applications for high-resolution skeletal density determinations in annually-resolved crustose coralline algae”, the application of high-resolution micro-computed tomography (micro-CT) for the precise measurement of subannual-to-annual scale changes in skeletal density and calcification rates of crustose coralline alga *Clathromorphum nereostratum* is demonstrated. The potential for micro-CT as a high resolution method for two and three-dimensional visual and quantitative analysis of internal skeletal features is evaluated in a sensitivity analysis. Based on the high-resolution data derived from micro-CT, seasonal variations in skeletal density of coralline algae are linked to structural changes in the skeletal matrix captured using scanning electron microscopy (SEM), highlighting differences between summer vs. winter calcification. This paper will be submitted to *Geochemistry, Geophysics, Geosystems*.

In **Chapter 3**, “Recent decline in skeletal density and calcification rates of subarctic crustose Coralline algae”, annually-resolved multidecadal records of algal growth, skeletal density, and calcification rates obtained based on the methods introduced in Chapter 2 are used to reconstruct the long-term in-situ response of *Clathromorphum nereostratum* to the decline in ocean pH in the Aleutian Islands, Alaska since the late 19th century. Warming surface ocean temperatures, combined with the continued invasion of atmospheric CO₂ into seawater are expected to increase physiological stresses on the high-Mg calcite skeletons of coralline algae. In this chapter, the feasibility of using algal structural growth characteristics as proxies for changing marine climate and seawater chemistry are assessed. Trends in algal skeletal densities are discussed in relation to long-term trends in local and global pH levels. In addition, multi-decadal relationships between
algal skeletal density and regional SST variability, and between calcification rates and sunlight availability reveals that algal calcification responds not only to acidification, but also to regional and large-scale climate oscillation patterns in the North Pacific. This paper will be submitted to *Geology*.

In Chapter 4, “*Freshening of the Alaska Coastal Current recorded by coralline algal Ba/Ca ratios*”, coralline algal Mg/Ca and Ba/Ca trace element ratios are utilized to reconstruct temperature and salinity variability respectively, within the Alaska Coastal Current (ACC). Multi-decadal variability within the 60-year algal record reveals a pattern of SST variability associated with Pacific Decadal Oscillation (PDO), and unprecedented freshening of the ACC related to glacial melt and precipitation runoff from mountainous (clay, thus Ba-rich) regions of coastal Alaska. This paper has been published in *Journal of Geophysical Research – Biogeosciences* 116 (G1), G01032 (2011).

In Chapter 5, “*Multicentennial record of North Atlantic primary productivity and sea-ice variability archived in coralline algal Ba/Ca*”, a barium-to-calcium trace element record from *Clathromorphum compactum* collected from northern Labrador, Canada, is utilized for the first time as a proxy for North Atlantic primary productivity associated with climate driven sea-ice variability over the past 360 years - extending well into the Little Ice Age (LIA). In a region where intense seasonal phytoplankton blooms can effectively scavenge trace nutrients from the surface water column, Ba/Ca taken up by shallow benthic coralline algae may be used as a proxy for phytoplankton productivity. The relationship between algal Ba/Ca and various instrumental and proxy sea-ice records are examined. Furthermore, spectral analysis of this multi-centennial record reveals the dominant role of the Atlantic Multidecadal Oscillation (AMO) on influencing sea-ice melt/formation, and thus productivity in the Subarctic North Atlantic. This paper will be submitted to *Nature Communications*.

In Chapter 6, the main results and conclusions of the dissertation are summarized along with recommendations for areas of future research. Lastly, the paleoclimatic significance of the findings are discussed in the context of understanding current, and predicting future climate and environmental changes.
2 Abstract
The world oceans are changing at an unprecedented rate, and is expected to have widespread consequences for marine biodiversity and ecosystem functioning. However, the relatively short record of instrumental observations has hindered our ability to properly evaluate currently observed changes in the context of long-term variability. Thus, we have relied upon geochemical and physical proxy information stored in biomineralized shells and skeletons of calcareous marine organisms as in-situ recorders of past climate and environment. This study will demonstrate the application of micro-computed tomography (micro-CT) for three-dimensional visualization and analysis of structural growth characteristics (ex. growth, skeletal density, and calcification rates) in a slow-growing, annually-banded crustose coralline algae *Clathromorphum nereostratum* (average increment width ~380 µm). Imaging at 20 µm resolution produces seasonal cycles in skeletal density that correspond to summer / winter variations in skeletal structure. X-ray images, and timeseries of skeletal density and calcification rates were generated at various spatial resolutions (20, 40, 60, 80, and 100 µm) in a sensitivity analysis. Results indicate that while both skeletal density and calcification rates do not significantly differ at varying spatial resolutions, clear visualization of micron-scale growth features and quantification of structural changes on subannual timescales would require higher scanning resolutions. Therefore, micro-CT can be used as a fast, non-destructive, and high-resolution technique for structural and morphometric analyses of paleoclimate archives, particularly those that are microscopic in size, or exhibiting slow or compressed growth.

2.1 Introduction
Since the initial discovery of annual density banding patterns in skeletons of hermatypic corals, the analyses of coral growth parameters have enabled paleoclimate scientists to derive
information about the seawater environment in which the corals grew [Buddemeier et al., 1974; Knutson et al., 1972]. This has opened the door to the field of sclerochronology – the study of marine organisms that form incremental growth patterns in biomineralized shells and skeletons as recorders of past climatic and environmental conditions [Buddemeier et al., 1974]. The formation of banding patterns in a multitude of long-lived biogenic archives (ex. corals, coralline algae, bivalves) occur annually to subannually, and can extend back in time on multidecadal and even multicentennial timescales. This allows for long records of proxy climate information to be obtained at high temporal resolution. Information about past oceanographic conditions can be gathered through geochemical analyses and physical measurements of structural changes (ex. growth increment widths, skeletal density, calcification rates). These structural aspects have recently been receiving growing attention in ocean acidification research, as changes in ocean chemistry are expected to have direct and observable impacts on the physical growth characteristics of carbonate-secreting organisms [D'Oliveiro et al., 2013; De'ath et al., 2009; Lough and Cantin, 2014].

This paper will briefly review the traditional methods used for quantifying structural growth characteristics (primarily developed for coral research), and discuss micro-computed tomography (micro-CT) as a novel, high-resolution, efficient, and non-destructive approach - with the potential for broad applications in the field of paleoclimatology. The application of this technique for viewing internal ultrastructure, and the precise measurement of subannual-scale changes in the slow growing, annual increment forming crustose coralline alga *Clathromorphum nereostratum* will be discussed. Crustose coralline algae are a dominant group of high Mg-calcite secreting marine photoautotrophs that are widely distributed in shallow coastal regions extending from the tropics to the poles (Figure 4A) [Adey et al., 2013; Freiwald and Henrich, 1994]. Proxy climate information obtained from these annually-banded carbonate organisms has recently yielded multicentennial records from high-latitude oceans [Halfar et al., 2013], and have been hypothesized to undergo rapid structural changes with ongoing acidification due to its metastable mineralogical structure [Chan, this thesis; Andersson et al., 2008; Büdenbender et al., 2011; Ragazzola et al., 2012]. Structural characteristics such as skeletal density and calcification rates of the coralline alga will be examined through micro-CT imaging techniques. In addition, a sensitivity analysis of the method will be performed comparing: 1) Digital two-dimensional tomographic x-ray images; and 2) Timeseries of structural parameters; generated at different
spatial resolutions. This sensitivity analysis will enable investigators to properly determine the spatial resolution necessary for accurate visualization and quantification of specific growth features within their archive, with the purpose of broadening the applications of micro-CT to other areas of paleoclimate or sclerochronological research.

Figure 4 | Crustose coralline alga *Clathromorphum nereostratum*. (A) Underwater image of crustose coralline algae living on shallow seafloor off Aleutian Islands, Alaska. (B) Cross-section of coralline algal specimen with annual growth increments. Red lines delineate annual growth increments and annually-forming conceptacles (black holes = reproductive cavities). Calendar years assigned beginning with year of collection in 2004, and going back in time at one year intervals. Yellow shaded area represents region of interest (ROI) where growth increment widths were measured (green lines), and subsequent micro-CT analyses were performed.

2.1.1 Techniques for Skeletal Density Determinations

A variety of techniques have been established for visualizing and quantifying density banding patterns in skeletons of massive corals species, each with inherent advantages and limitations.
Some have applied Archimedes’ Principal to measure density based on buoyant weight techniques [Davies, 1989; Jokiel et al., 1978], determined by water [Brown et al., 1990; Bucher et al., 1998; D'Olivo et al., 2013; Edinger et al., 2000; Johnson and Carpenter, 2012] or mercury displacement [Dustan, 1975]. This procedure has also been adapted for density determinations based on the differences between the dry and frozen weight of specimens [Carricart-Ganivet et al., 2000]. While these methods generally do not require the use of specialized and expensive equipment, procedures typically involve the excision of a block of coral containing 1-5 years of growth [Lough and Cantin, 2014]. This can greatly reduce the temporal resolution of the technique. For example, a five-year block of *Porites* spp. coral used for a single density determination can range from 3.6 – 8.3 cm in vertical thickness [D'Olivo et al., 2013]. Therefore, applications of the buoyant weight method may be limited due to its destructive nature (making it difficult to obtain continuous records), and low resolution, precluding the analysis of slow-growing organisms that form sub-centimeter scale growth increments.

Alternatively, optical or photo x-ray densitometry techniques can provide continuous measures of density along a core or slice of the coral skeleton [Carricart-Ganivet and Barnes, 2007]. Samples are first x-rayed along with a calcium carbonate or aluminum wedge (or step wedge) standard of known dimensions, thickness, and density for calibration of film optic density to skeletal density [Buddemeier et al., 1974]; and to correct for variations in the intensity of the x-ray beam known as the “heel effect” [Carricart-Ganivet and Barnes, 2007; Chalker et al., 1985; Lough and Cooper, 2011]. X-ray negatives are then scanned in a straight line along the axis of maximum growth using a scanning densitometer, which shines a light beam through the x-radiograph to obtain quantitative measures of skeletal density based on the attenuation of the light beam [Buddemeier et al., 1974; Dodge and Brass, 1984; Dodge and Thomson, 1974]. Gamma densitometry techniques have also been used based on a similar approach [De'ath et al., 2009; Fabricius et al., 2011], replacing the light beam with a gamma ray [Chalker and Barnes, 1990]. While both densitometry methods are able to generate continuous measurements of absolute density, pre-selected tracks are often limited to linear configurations [Lough and Cooper, 2011].

Computed tomography (CT) is an x-ray based technique originally developed for the medical sciences, but has recently been adapted for sclerochronological studies as a method for accurately determining skeletal density [Crook et al., 2013; DeCarlo et al., 2015; Saenger et al.,
CTs are classified into three main groups based on their resolution (detection ranges): nanoCT (150 – 1000 nm), micro-CT (3 – 300 µm), and CT (1 – 2.5 mm); with maximum analyzable specimen diameters of < 11 mm, < 10 cm, and < 200 cm, respectively [Briguglio et al., 2014]. A distinct advantage of computerized tomography over the abovementioned methods is the ability to digitally image an entire core or specimen without any prior sample preparation, or having to remove blocks or slices from the original sample. This rapid, non-destructive technique is particularly important for the preservation of rare and pristine specimens (those with clear and uninterrupted growth, and/or lifespans on multicentennial timescales) for additional physical or geochemical analyses. High-resolution, digital, three-dimensional (3D) rendering of the scanned specimen permits clear viewing of internal and external structures [Teichert, 2014], allowing the operator to measure growth parameters in two-dimensional layers or three-dimensional volumes. 3D digital reconstructions of the specimen can be freely rotated during data analyses to ensure that measurements are carried out along regions containing the longest possible record (or axis of maximum growth [Saenger et al., 2009]), while avoiding areas with distorted or unclear growth.

The micro-CT method has potential for broad applications in paleoclimate research, and can be widely applied in a variety of archives - particularly those that are small in size (ex. plankton, fish otoliths) and/or exhibit ultraslow or compressed growth (10s -100s of microns/year). Micro-CT methods have proven to be a valuable tool for the visualization and morphometric analyses (volume, surface area, shape, density, etc.) of microorganisms (ex. foraminifera) and microstructures (ex. fish otoliths); allowing for precise taxonomical identification of organisms for phylogenetic, biostratigraphic, and paleoenvironmental studies [Bignami et al., 2013; Briguglio et al., 2014; Ferrandez-Canadell et al., 2014; Gorog et al., 2012; Speijer et al., 2008]. Computerized tomography has also been gaining popularity in ocean acidification research as a method for visualizing and quantifying biological erosion (bioerosion) and predation by boring organisms (associated with weakening of the carbonate skeleton as a result of acidifying seawaters) [Crook et al., 2013; DeCarlo et al., 2015; Silbiger et al., 2014]; and the examination of biodiversity through the reconstruction of characteristically-shaped boring cavities [Beuck et al., 2007; Beuck et al., 2008; Teichert, 2014]. More recently, micro-CT has been used for analysing the skeletal density and calcification rates of subarctic crustose coralline algae to
investigate the response of algal calcification to the changing ocean environment [Chan, this thesis].

2.2 Methods

2.2.1 Sample Collection and Growth Increment Mapping

A living specimen of the coralline alga *Clathromorphum nereostratum* was collected in 2004 from shallow rocky substrate off Attu Island in the Aleutian archipelago, Alaska at 10 m water depth via SCUBA (Figure 4A). This long-lived sample displays pristine and continuous growth, and has therefore been analysed using multiple geochemical methods for understanding SST variations [Hetzinger et al., 2009]; multidecadal climate oscillations and teleconnection patterns [Halfar et al., 2007]; ocean pH [Fietzke et al., 2015]; and the combined influences of climatological and chemical changes of surface seawater on algal calcification [Chan, this thesis].

The algal specimen was vertically thick-sectioned into 1.5 - 2 cm slabs, parallel to the algal growth axis (direction of algal growth - oldest growth record at bottom, youngest on top), using a Buehler IsoMet 1000 precision saw. The thick-section with the longest growth record was then glued to a petrographic glass slide using epoxy resin and first manually, then machine polished using 9 µm, then 3 µm diamond suspension on a Logitech CL50 compact polishing machine. High-resolution digital photomosaics of the polished specimen surface (generated by a series of 2D images that are automatically stitched together) was scanned using an Olympus reflected light microscope (VS-BX) attached to an automated sampling stage imaging system equipped with Geo.TS software (Olympus Soft Imaging Systems); for details, see Hetzinger et al., [2009]. This optical microscope scanning setup enabled detailed viewing with zoom and panning functions using a pyramidal file format. The resulting photomosaics facilitated the identification of annual growth increments, which were clearly delineated by couplets of dark and light banding patterns, and coincide with the occurrence of rows of annually forming conceptacles (black holes; Figure 4B). Conceptacles are uniformly-sized cavities (~150 - 200 µm in diameter) that enclose the algal reproductive structures, and form seasonally in fall (September – October) by the downward decalcification of skeletal material [Adey et al., 2013]. Annual growth
increments were manually traced out using Geo.TS software to identify annual growth boundaries (red lines; Figure 4B) which facilitated the precise measurement of annual growth increment widths (green lines; Figure 4B). Special care was taken to ensure that growth records were taken directly within the region of interest (ROI - yellow region; Figure 4B) where skeletal density measurements were later obtained for analysis using micro-CT. Lastly, the algal growth timeseries were generated by matching the growth increment width data to the corresponding calendar year of formation assigned using layer counting methods (beginning with the top layer representing the year of collection ex. 2004, and extending back in time at one year intervals).

2.2.2 X-Ray Micro-CT Imaging

Imaging of the coralline alga was conducted at the Robarts Research Institute, in Western University using a micro-CT scanner (GE Healthcare, eXplore Locus RS-9) at a peak x-ray energy of 80 kVp and tube current of 450 uA. The sample were oriented such that the CT scan slice-plane was as perpendicular as possible to the growth axis of each alga. 900 two-dimensional x-ray projections or views (image matrix size 2010 x 1920 pixels) were collected at 0.4 degree angular increments over a stepwise 360 degree rotation around the central axis of the sample. At each of the 900 view angles, 2 frames, 4500 ms in duration, were acquired and averaged to reduce image noise, resulting in a total scan time of 2.75 hours. Each scan was reconstructed into a 3D volume data set with isotropic 20 µm voxel spacing by using a conebeam-geometry, filtered-backprojection reconstruction algorithm. Any samples longer than the axial extent of the field of view (FOV) of the scanner, were scanned in two sections and reconstructed into two 3D volume data sets. These two 3D volumes were then aligned and digitally stitched into a single, contiguous 3D volume. Sample vials containing air, water, SB3 cortical bone mimic (Gammex RMI hydroxyapatite, Ca(PO₄)₃, 1.1 g/ml), and calcite crystal (pure calcium carbonate, CaCO₃, 2.71 g/cm³,) were included in the scanner field of view (FOV) during acquisition and used for internal greyscale calibration purposes (Figure 5). The final 3D data set was first linearly rescaled into Hounsfield units (HU) so that air and water were represented by mean voxel (3D pixel) greyscale intensities of -1000 and 0 respectively, and was later linearly rescaled to units of absolute density (g/cm³).
Figure 5 | 3D reconstruction of coralline alga compiled from array of 2D micro-CT x-ray slices along X, Y and Z-axes. External standards were scanned alongside specimen (top of image in sample vials) for calibration purposes: (1) Water; (2) Hydroxyapatite SB3 (density 1.1 g/cm³); (3) Air - in empty space; and (4) Pure crystal calcite (density 2.71 g/cm³). Yellow square-based prism represents region of interest (ROI) used for micro-CT analyses. X-ray image slices were taken along: X-axis (green), Y-axis (blue), and Z-axis (red). Z-axis represents the axis of algal growth (oldest record at bottom, youngest on top).
2.2.3 3D Visualization and Skeletal Density Analysis

Micro-CT imaging produced a three-dimensional distribution of linear attenuation coefficients that was stored as stacks of 2D x-ray image slices along the X, Y, and Z-axis plane of the sample for quick viewing and analysis using MicroView Standard 2.5.0-2799 (Parallax Innovations Inc., 2015) (Figure 5). This 3D analysis software produces a 3D reconstruction of the specimen based on the series of parallel 2D x-ray slices along each axis, enabling the user to scroll through and rotate individual slices.

MicroView software also allows for a region of interest (a core delineating the region to be analysed) to be freely selected, tilted, split, and stitched together to ensure that the longest possible record can be obtained from each specimen, and that the core always remains perpendicular to growth increments. In the example of the coralline alga, the ROI was positioned in an area where there was the least interruption and distortion of the growth banding pattern by macro-architectural skeletal features such as bore holes, cracks and other imperfections. Careful selection of ROI dimensions should constrain the X-Y slice cross-sectional area used for analysis in order to minimize the possibility of individual x-ray slices cutting through multiple growth layers; but at the same time needs to be large enough to capture as many structural characteristics of interest (ex. conceptacles) as possible (Figure 6A). This is especially important in archives that exhibit subconcentric or concave growth formations (ex. fish otoliths, bivalves; Figure 6B). The ROI for the algal specimen was plotted as a tall, thin, parallelepiped ROI (square base along X-Y slice plane: 81 x 81 pixels or 1.59 mm x 1.59 mm) including approximately 3-4 conceptacles across, and spanning the entire Z-range (growth axis of algae). ROI dimensions are not limited to square prism configurations in MicroView, but can also be custom selected to be rectangular or cylindrical in shape. However, it is important that ROI dimensions are kept constant when imaging multiple specimens to ensure that analyses are performed in a consistent manner. Once selected, the ROI core was cropped out from the complete volume, and analyzed on a 20 µm thick slice-by-slice basis as perpendicular as possible to the direction of algal growth on the Z-axis (Figure 7).
Figure 6 | Region of interest (ROI) selection. (A) View from top looking down a horizontal x-ray slice taken along Z-axis of algal growth. X-ray slice cuts through multiple growth years as indicated by presence of numerous annual growth bands (cloudy white lines) interspaced with conceptacle cavities. (B) Diagram showing same x-ray slice (blue) from side cutting through algal cross section with conceptacles. Note how x-ray slice cuts through multiple growth layers. ROI selection (yellow column) allows data to be collected from each annual layer while minimizing chances of cutting through multiple growth layers. X, Y, Z-axis on top right corner shows orientation of viewing plane.
2.2.4 Removal of Cavity Structures from Skeletal Density Analyses

Greyscale values measured in the voxels of each slice are directly related to density, with lighter (darker) regions exhibiting higher (lower) density values. Greyscale measurements along a line transect cutting through the center of two conceptacle cavities and their surrounding skeletal matrix demonstrate that there are no significant changes in density at conceptacle wall boundaries, and therefore do not bias overall skeletal density calculations (Figure 8). A threshold greyscale value of 1600 HU was selected (Dashed line - approximately halfway between the highest and lowest density values measured; Figure 8) to digitally remove conceptacle cavities from the algal skeleton prior to constructing the skeletal density timeseries. Voxels with a greyscale value below the threshold were deemed to be empty space (ex. conceptacle) and excluded from the skeletal density calculation. This approach assured self-consistent sampling
focused on skeletal calcite only. This procedure can also be used for the visualization and removal of empty spaces and/or cavities created by boring organisms. However, features that are smaller than the voxel size (20 µm), such as the small pore-like spaces of uncalcified cell interiors (~ 3 – 6 µm) could not be distinguished in the reconstructed dataset (thus were included in the skeletal density calculation).

Figure 8 | Greyscale intensity values of conceptacle cavities and surrounding calcite skeletal matrix. Greyscale measurements in Hounsfield Units (HU) along white line transect extending from A to B. Transect was plotted through center of two conceptacle cavities and surrounding calcite skeletal matrix to demonstrate no significant changes in skeletal density at conceptacle wall boundaries. Greyscale value in each voxel is directly related to density, with lighter (darker) regions exhibiting higher (lower) density values. Dashed horizontal line delineates threshold of 1600 HU used to exclude conceptacle cavity areas from skeletal density calculations.
The skeletal density for each slice was determined based on the fractional mineral content of each voxel above the threshold measured in HU and linearly rescaled to units of pure crystal calcite (density = 2.71 g/cm³), and integrated over all the voxels in that slice. The total mineral content of each slice was plotted against the slice number (numbered consecutively beginning with 0 for the very first scan along the Z-axis) to construct the raw skeletal density data. The 20 µm data was later downsampled to lower spatial resolutions of 40 µm, 60 µm, 80 µm, and 100 µm voxels for subsequent sensitivity analysis.

2.2.5 Skeletal Density and Calcification Rate Timeseries Development

Annual linear growth (average 380 µm/year) was divided by the resolution of the micro-CT scan (ex. 20 µm, 40 µm, etc.) to calculate the number of horizontal x-ray slices included in each annual growth increment in vertical succession along the Z-axis (Figure 7). A weighted average was then applied to the greyscale values of all the slices within each annual growth increment to generate the annual skeletal density value for each year of growth (average 1.22 g/cm³). Annual calcification rates (average 0.044 g/cm²/yr) were subsequently calculated as the product of annual growth rates (cm/yr) and annual skeletal densities (g/cm³; Figure 9).
Figure 9 | Annual coralline algal growth, density and calcification rate time series.
Annually averaged time series for growth (µm; light grey), density (g/cm³; medium grey), and calcification rate (g/cm²/yr; black) from 1950 - 2000.
2.3 Results and Discussion

2.3.1 Potential for Subannual/Seasonal Reconstructions

20 µm scanning of the algal skeleton produces on average 17.73 x-ray slices for each annual growth increment (average 380 µm/year). This high-resolution sampling captures anomalously thin or compressed layers, and even enables subannual sampling of the algal skeleton, allowing for seasonal changes in skeletal density to be interpreted (Figure 10). Peaks (troughs) in skeletal density values represent the warmest - August (coldest - March) months of the year in the Aleutian Archipelago. Seasonal variations in skeletal density have been hypothesized to result from higher concentrations of deltoid-shaped interfilament calcite crystals (formed in cavities between cell walls) during summer and fall months (Figure 11A – D), resulting in considerably denser tissue in comparison to winter tissue (Figure 11E and F) [Adey et al., 2013].

![Figure 10](image)

**Figure 10 | Seasonal cycles in skeletal density (g/cm³).** Micro-CT derived density variations displaying maximum (minimum) values associated with growth during summer (winter) months of algal growing season. One full cycle represents one calendar year.
Figure 11 | SEM of pore-like uncalcified cell interiors and surrounding skeletal matrix comparing summer versus winter cells. (A) View of upper epithallus layer on top of perithallial cells shown via transverse fracture (downward view) and vertical fracture (side view). (B) Size and distribution of perithallial cells in relation to conceptacle. (C, D) Summer cells with large deltoid-shaped interfilament calcite crystals between smaller radial inner cell wall crystals; (C) Transverse fracture view; (D) Vertical fracture view. (E, F) Close-up of winter cells with mostly radial inner cell wall calcite crystals and small interfilament crystals only present at cell junctions. (E) Transverse fracture view. Starch grains (tiny spheres) are sometimes present in cell spaces; (F) Vertical fracture view. Figure adapted from (Adey et al., 2013).
2.3.2 Sensitivity Analysis

A visual comparison was performed on a single horizontal 2D x-ray slice taken along the vertical axis of algal growth over a range of resolutions from 20 µm to 100 µm. To investigate the effect of spatial resolution on measured quantities, the 20 µm isotropic voxel 3D data was re-binned 2 x 2 x 2, 3 x 3 x 3, 4 x 4 x 4, and 5 x 5 x 5 to increase the voxel spacing and reduce resolution to 40 µm, 60 µm, 80 µm and 100 µm respectively (Figure 7B). Hence the larger the inter-voxel spacing, the less x-ray data is generated within a given growth increment (as indicated by progressively pixelated images with decreasing spatial resolution; Figure 12A-E).

Figure 12 | Sensitivity analysis comparing x-ray images generated at different spatial resolutions. (A-E) A single 2D tomographic x-ray slice of coralline alga taken along the Z-axis of algal growth imaged at: (A) 20 µm; (B) 40 µm; (C) 60 µm; (D) 80 µm; and (E) 100 µm resolution. Increasingly pixelated images is observed with decreasing spatial resolution (larger pixel size).
Skeletal density timeseries were generated for each scan resolution (ex. 20 µm, 40 µm, etc.) using data obtained from an ROI core plotted with identical coordinates (Figure 13A). The density timeseries were not significantly different between resolution treatments (Figure 13B); however a trend toward lower density values and smoother curve shape (low-pass filtered or “lower” peaks) is observed at lower image resolutions. Calcification rates were subsequently calculated as the product of skeletal density (generated at each spatial resolution) and previously measured growth. Similarly, a plot of calcification rates showed no significant difference between higher and lower resolutions (Figure 13C and D). This indicates that although x-ray images appear to be more pixelated with higher image spacing, the slight differences in skeletal density have no overall effect on calcification rates. The nearly identical curves indicate that calcification rates in coralline algae are likely to be dominated by variations in growth rate (Figure 9). This finding is in good agreement with previous investigations of coral calcification rates from the Great Barrier Reef [D'Olive et al., 2013; De'ath et al., 2009]. Similarly, a recent study of coralline algae from the Aleutian Islands found that the long-term decline in calcification rates were largely driven by a gradual slowdown in growth [Chan et al., submitted].
Figure 13  | Sensitivity analysis comparing skeletal density and calcification rate timeseries generated at different spatial resolutions. (A) Skeletal density timeseries (g/cm$^3$) and (B) Skeletal density standard error; (C) Calcification rate (g/cm$^2$/yr) timeseries and (D) Calcification rate standard error; generated using scans taken at: 20 µm (red); 40 µm (orange); 60 µm (yellow); 80 µm (green); and 100 µm (blue) resolution. Both the skeletal density and calcification rate timeseries are not significantly different between resolutions; but a step-wise smoothing of skeletal density curves is evident at each successive scan from highest to lowest resolution. Conversely, calcification rates show nearly identical curves regardless of scanning resolution, demonstrating the influence of growth on calcification rates.

2.3.3 Optimizing Scan Resolution

The here documented example of the micro-CT technique applied to crustose coralline algae suggests that while calcification rates do not appear to be sensitive to variations in scan resolution, the selection of 40 µm image spacing would be most suitable for the purposes of viewing internal ultrastructure and calculating subannual skeletal density in *C. nereodstratum*
(where conceptacle cavities are ~ 150 µm in width, and annual growth rates average ~ 380 µm/year).

The selection of scan resolution varies depending upon the size and scale of the characteristics of interest (ex. growth features, boring cavities) within a given paleoclimate archive. While the lowest resolution was acceptable for the calculation of growth-driven calcification rates in coralline algae, 100 µm would not be sufficient resolution for the analyses of extremely slow growing organisms such as Arctic specimens of coralline algae (~60 µm/year; [Halfar et al., 2013], or for the analysis of later growth stages in specimens exhibiting ontogenetic declines in growth rate (ex. as little as 10 – 25 µm in bivalves; [Beierlein et al., 2015; Schöne et al., 2005a].

During the imaging process, a large amount of greyscale information is recorded as a series of image files (ex. “tiff”, “vff”, etc.), which are subsequently processed to obtain a three-dimensional reconstruction of the scanned object. High-resolution and/or larger specimens require considerably more time and larger computer storage capacity for the recording, reconstruction, and manipulation of CT data, thus resulting in higher costs for the analyses. At the highest spatial resolution (ex. 20 µm), micro-CT is capable of capturing minute changes in structural characteristics (ex. skeletal density). However, these high-resolution scan protocols require significantly longer acquisition times in order to reduce the noise to an acceptable level – and a poor signal-to-noise ratio can make it difficult to choose a threshold that clearly delineates boundaries between calcite material and empty space. Conversely, lower resolution scans (ex. 100 µm) have larger spacing distance between scans, producing a higher signal-to-noise ratio and allowing for clearer distinction between calcite material and empty space (ex. conceptacles, boring cavities). However, this comes at the expense of reduced spatial resolution (ex. more pixelated images, and smoothing of density values; Figure 12 and Figure 13). Therefore careful selection of the appropriate scanning resolution is necessary in order to ensure an optimal balance to between maximizing optical resolution and simultaneously minimizing noise, scanning time, and cost.
2.4 Conclusions and Future Applications

The application of high-resolution micro-CT for reconstructing seasonal changes in skeletal density and calcification rates in an annually-banded species of slow-growing crustose coralline algae has been demonstrated. Precise determination of subannual-scale changes in skeletal densities allow for more accurate differentiation between seasonal and long-term variability, which is key for interpreting covarying climatic and environmental influences on growth and calcification of carbonate organisms.

Micro-CT is a non-destructive x-ray technique that allows for direct imaging and quantification of 3D volumes, and preserves pristine specimens for further analyses so that additional parameters may be examined from the same specimen (providing better insight into physical and chemical changes). Through the use of image analysis software, data can be visualized and freely manipulated as a series of 2D slices and 3D volumes for more accurate quantification of structural and/or growth parameters. A number of user-defined parameters (ROI dimensions and positioning, threshold values, and imaging resolution) allows users to select the best regions and longest records within each specimen for analysis, and remove empty spaces for density evaluation (or conversely quantify boring cavities for bioerosional analysis).

A sensitivity analysis has been conducted here for the purposes of broadening the applications of micro-CT to other areas of paleoclimate and paleoenvironment research. Careful selection of spatial resolution not only depends on the size of specimen, growth features, and characteristics of interest, but should also consider other factors such as the signal-to-noise ratio, scan time, costs for analyses, and computer data storage requirements. High-resolution micro-CT is a powerful tool for non-destructive viewing and quantification of structural changes (ex. linear extension, density, calcification rate) in temporally-banded paleoclimate and paleoenvironmental archives, especially those that are small in size (ex. plankton, fish otoliths), or have slow or declining growth rates (ex. Arctic coralline algae specimens, bivalves). In summary, Micro-Computed Tomography is a novel technique for paleoclimate research, with many more applications that have yet to be explored.
Recent Decline in Skeletal Density and Calcification Rates of Subarctic Crustose Coralline Algae

3 Abstract

Warming surface ocean temperatures combined with the continued invasion of atmospheric CO$_2$ into seawater are increasing physiological stresses on calcareous marine organisms. An annually-resolved growth, density, and calcification rate record was obtained from crustose coralline algae of the genus *Clathromorphum* collected in the Subarctic North Pacific, a region that has undergone a long-term decline of 0.08 ± 0.01 pH units since the late 19$^{th}$ century. Coralline algal skeletal densities have entered an unprecedented decline since 1980. The metabolic trade-offs associated with weakening algal skeletons may offset the benefits of continued growth and calcification, and expose skeletons to bioerosional processes, with anticipated consequences for the diverse reef-like communities associated with algal structures. The exact cause(s) for this decline are uncertain; however, correlations to regional climate and warming sea surface temperatures indicate that in addition to acidification, sunlight availability and temperature stress may also be factors that influence the ability of corallines to calcify.

3.1 Introduction

As oceans become more acidic, changes in surface seawater carbonate chemistry lead to a reduction in available carbonate ions (CO$_3^{2-}$), thereby lowering the calcium carbonate saturation state (Ω) [Doney et al., 2009; Orr et al., 2005]. Numerous mesocosm studies conducted over the past years have shown that this can compromise the ability of marine organisms to calcify, which may lead to the production of weaker skeletons that are more vulnerable to biological and or physical erosion [Feely et al., 2009; Ragazzola et al., 2012]. Aragonitic coral cores from the Great Barrier Reef have revealed a dramatic decline in calcification rates since 1990, which is unprecedented in the past 400 years [De'ath et al., 2009]. However, this reduction in calcification rates has largely been attributed to a bias towards inshore reefs that are influenced by thermal stress and high seasonal discharge of sediment and nutrients [D'Olivo et al., 2013; Ridd et al., 2013]. Alternatively, it has been hypothesized that high-latitude marine calcifiers would be even
more vulnerable to undersaturated conditions owing to the naturally depressed $\Omega$ resulting from increased $CO_2$ solubility in colder water temperatures [Andersson et al., 2008; Büdenbender et al., 2011; Orr et al., 2005; Ragazzola et al., 2012]. In fact, seasonal aragonite undersaturation has already been observed in the surface waters of some polar oceans [Bates et al., 2013].

Marine calcifiers build their shells and skeletons using a variety of calcium carbonate ($CaCO_3$) polymorphs (aragonite, calcite, and Mg-calcite) which experience different solubilities in seawater [Ries, 2011]. Mineralogical studies have indicated that the solubility of biogenic magnesium calcite containing $> 4$ mol% MgCO$_3$ termed “high Mg-calcite” exceeds the solubility of both calcite and aragonite at $>12$ mol% MgCO$_3$, with increasing solubility towards higher Mg concentrations [Andersson et al., 2008; Morse et al., 2006]. This is because Mg-calcites are formed by the replacement of calcium ions by much smaller magnesium ions within the calcite lattice, leading to a distortion in the mineralogical structure, and resulting in a metastable form of carbonate that is more readily dissolved at lower $\Omega$ [Andersson et al., 2008].

Crustose coralline algae represent an important group of high Mg-calcite calcifiers that are especially abundant in cold-water environments. In fact, coralline algae are possibly the most dominant group of calcifiers in sublittoral zones of the Arctic and Subarctic, forming extensive crusts and mobile rhodoliths that can cover up to 100% of the rocky seafloor [Adey et al., 2013], and even extend northwards into regions that are seasonally covered by sea ice [Halfar et al., 2013]. These shallow marine calcareous plants play a key ecological role in providing structurally complex substrate that can act as stabilizing structures, shelter, and settlement and nursery grounds for rich and diverse benthic invertebrate communities [Adey et al., 2013; Chenelot et al., 2011]. Coralline algae belonging to the family Corallinaceae precipitate high Mg-calcite skeletons ranging from 9 – 25 mol% MgCO$_3$, with the majority of genera containing greater than 12 mol% MgCO$_3$ [Smith et al., 2012]. Therefore, the highly soluble coralline algae residing in cold-water regions with exceptionally low $\Omega$ may be expected to show an even more pronounced response to the effects of ocean acidification than tropical aragonitic corals [Büdenbender et al., 2011].

The response of coralline algal calcification to declining ocean pH has recently been tested in a number of short-term $pCO_2$–enrichment studies, which have found negative effects on coralline algal calcification [Büdenbender et al., 2011; Gao and Zheng, 2010; Johnson and Carpenter,
2012; Johnson et al., 2012; Martin and Gattuso, 2009; Ragazzola et al., 2012]. However, others have reported an increase in calcification rates at moderately elevated experimental pCO₂ levels [Kamenos et al., 2013; Ries, 2011; Ries et al., 2009]. Recent evidence suggests that the calcification rate response of coralline algae is likely to be species-specific [McCoy and Ragazzola, 2014; Noisette et al., 2013], and results from the combined effects from multiple environmental stressors such as ocean temperature [D'Olivo et al., 2013; Johnson and Carpenter, 2012; Martin and Gattuso, 2009], solar insolation [Gao and Zheng, 2010], and benthic community structure [Johnson and Carpenter, 2012; Johnson et al., 2012], rather than ocean acidification alone. Laboratory studies have also demonstrated the adverse effects of elevated pCO₂ on other aspects of coralline algal fitness such as: juvenile recruitment rate [Kuffner et al., 2008]; photosynthesis [Gao and Zheng, 2010]; percentage cover (abundance) [Hall-Spencer et al., 2008; Kuffner et al., 2008]; competitive ability [McCoy and Pfister, 2014; McCoy and Ragazzola, 2014]; and survivorship [Martin and Gattuso, 2009]. Mesocosm experiments have contributed greatly to the understanding of the consequences of ocean acidification (OA) on coralline algal calcification rates; however, the short-duration, highly confined, and controlled nature of these artificial model systems do not allow us to thoroughly examine the full spectrum of environmental and ecological interactions that are found in natural settings [Hall-Spencer et al., 2008; Johnson et al., 2012; McCoy and Ragazzola, 2014]. Therefore in this study, the long-term in-situ response of coralline algal calcification rates to declining pH will be investigated in cold-water regions of the Subarctic North Pacific, a region that experiences one the lowest carbonate saturation states globally (Figure 14A).
Figure 14 | Map of sample collection sites superimposed on GLODAP-based carbonate concentration for the North Pacific. (A) Algal collection sites (starred) along Aleutian Islands, Alaska. GLODAP based sea surface carbonate (CO$_3^{2-}$) concentration (μmol/kg) in the subarctic North Pacific for year 1995 [Feely et al., 2009]. White regions indicate areas with no data. (B) Buildup of living coralline alga Clathromorphum nereostratum.
3.2 Methods

Annually resolved multidecadal reconstructions of coralline algal growth, skeletal density, and calcification rates were generated from seven long-lived specimens of the encrusting *Clathromorphum nereostratum* collected live from shallow subtidal rocky substrate in the Aleutian Islands (Figure 14B). *Clathromorphum* spp. populations are widely distributed throughout the Subarctic and Arctic Northern Hemisphere oceans and are important archives for understanding past climates and environmental changes due to their multicentennial longevity, and their clearly developed annual incremental growth pattern, allowing for the precise calendar dating of hard tissue [Halfar et al., 2013]. Annual growth rates (average 0.38 mm/year) were measured from high-resolution photomosaics (Figure 15A), and skeletal density records (average 1.22 g/cm³) were generated using micro computed tomography (micro-CT) at a resolution of 20 microns (Figure 15B - see supplementary information for micro-CT setup). Annual calcification rates (g/cm²/yr) were calculated as the product of annual growth rates (cm/yr) and annual skeletal densities (g/cm³). Samples were all live-collected from two separate time periods (modern: 2004, 2008 and museum: 1969; Table 2) to verify that *C. nereostratum* do not experience ontogenetic declines in density during the final years of growth prior to collection (Supplementary Figure 19). In addition, measurements from the epithallus (the outer calcified protective layer - analogous to the bark of a tree) and the final year of growth were removed from the record of every sample to avoid inclusion of the youngest growth layers. These outer growth layers were excluded as they may possibly exhibit artificial ontogenetic-related declines due to immature growth [D'Olivo et al., 2013; Ridd et al., 2013].
Figure 15 | Polished specimen surface and 3D reconstruction of micro-CT core. (A) Polished thick section surface of live-collected *Clathromorphum nereostratum*. Yellow shaded area represents region of interest (ROI) used for micro-CT analyses. (B) Three-dimensional reconstruction of ROI using individual two-dimensional micro-CT slices along X, Y and Z axes spaced at 20 μm intervals. Skeletal density measured in Hounsfield Units (HU) was computed based on greyscale values of X-ray slices taken along Z-axis (axis of algal growth).

3.3 Results and Discussion

Distinct multidecadal oscillations are evident in time series of growth, skeletal density, and calcification rates from 1935-2003 (Figure 16). Three-year running means are used to highlight interannual variability in calcification rates, which are more strongly influenced by growth ($n = 66; \ r = 0.94; \ p <0.0001$), than skeletal density ($n = 66; \ r = 0.64; \ p = 0.002$). The finding that calcification rates are largely driven by growth is in good agreement with previous investigations of coral calcification rates from the Great Barrier Reef [D'Olivo et al., 2013; De'ath et al., 2009].
Coralline algal calcification rates display multidecadal variability superimposed on a gradual long-term decline throughout the record, and are inversely related with a one year lag to the Arctic Oscillation Index (AO) (Figure 17; 3 year mean: \( n = 66; \ r = 0.57; \ p = 0.0061 \)). The AO exhibits the largest variability during the cold season (November – April), which is reflected in sea level pressure anomalies of the AO index [Thompson and Wallace, 2000]. However, coralline algae have been shown to exhibit a significant slowdown in growth during winter months when light intensities and water temperatures are at a minimum [Halfar et al., 2007]. Therefore, the influence of the wintertime AO index is most clearly evident during the main growing season of coralline algae beginning in the spring of the following year. Alternations between positive (negative) AO phases are closely linked with strengthening (weakening) of the Northern Hemisphere polar vortex, which regulates the frequency and intensity of storm events [Thompson and Wallace, 2000]. During the positive AO phase, anomalously low pressure dominates the Polar Regions, steering clouds and stormy weather northward. This induces wind mixing and upwelling, increasing surface water nutrients for kelp and plankton production (which intercepts solar insolation available for coralline algal photosynthesis), thereby resulting in reduced algal growth and calcification rates. This light-driven growth pattern in *Clathromorphum* has previously been documented in an Aleutian-wide study, where increased shading resulting from wind mixing and phytoplankton blooms has been shown to negatively impact algal growth [Halfar et al., 2011b].

In contrast, while algal skeletal density also shows pronounced multidecadal variability, values have entered an unprecedented decline since 1980 (Figure 16). A recent study examining boron isotopes (\( \delta^{11}\text{B} \)) from a long-lived *C. nereostratum* alga reveals a long term decline in seawater pH of \( 0.08 \pm 0.01 \) pH units off the Aleutian Islands, Alaska between the late 19\(^{th}\) and 20\(^{th}\) centuries [Fietzke et al., 2015]. The contemporary decline in coralline algal skeletal density with locally acidifying seawaters provides evidence for the effects of changing ocean chemistry on marine calcifiers in shallow coastal regions of the Aleutian archipelago. While studies have shown that tropical species of coralline algae may exhibit a plastic response to ocean acidification by producing less soluble forms of carbonate (i.e., dolomite; [Nash et al., 2013]), or by means of lowering the calcite Mg/Ca ratio within the algal skeleton [Ries, 2011], recent studies of *C. nereostratum* from the Aleutian Islands indicate no decadal-scale or long-term decline in algal Mg/Ca ratios in recent decades [Chan et al., 2011; Williams et al., 2014].
Figure 16 | Coralline algal growth, density and calcification rate time series. Normalized time series for growth (yellow), density (red), and calcification rate (blue). Grey lines represent annual means, colored lines represent 3 year running means. Linear trends for all time series are plotted 1978 onwards. Bars represent number of samples averaged in each year of time series.
Figure 17 | Comparison of algal calcification rates to Arctic Oscillation Index. Black line represents normalized 3 year mean algal calcification rates. Grey line represents normalized 3 year mean Arctic Oscillation Index, with an inverted vertical axis orientation. Running means highlight interannual variability in time series. Note that algal calcification rates lag 1 year behind the Arctic Oscillation Index due to a slowdown in algal growth and calcification during winter months when the Arctic Oscillation is strongest.
A short-term laboratory study of high-latitude cold-water coralline algae has demonstrated the response of algal growth rate and cell wall thickness (related to cell density) to elevated pCO$_2$ conditions; with a decrease in inter- and intra-filament cell wall thicknesses at three months while growth rates were sustained [Ragazzola et al., 2012]. However, when the same experiment was extended to ten months, a reversal in algal response was evident, demonstrating a reduction in growth rates while cell wall thicknesses were maintained [Ragazzola et al., 2013]. This may indicate that coralline algae are capable of rapid morphological responses through the reallocation of energy within the skeletal matrix, thus resulting in a plastic acclimatization response to acidifying conditions. Although slight increases in skeletal density are observed in the early 1990s and 2000 of the *C. nereostratum* record (Figure 16), the continued decline in algal density to the end of the record suggests that a return to pre-1980 densities through a plastic mechanistic response has not taken place.

Similarly, an observational study of skeletal trade-offs of coralline algae from the northeast Pacific demonstrates varying responses of different species of coralline algae to declining ocean pH; with thick crusted species exhibiting reductions in intra-filament cell walls (influencing vertical extension) and thin crusted species experiencing reductions in inter-filament cell walls (influencing lateral growth) [McCoy and Ragazzola, 2014]. *C. nereostratum* from the North Pacific have been reported to form thick crusts of up to 20 cm in vertical thickness [Lebednik, 1976], and are among the oldest living marine organisms with typical ages ranging from multidecadal to centennial timescales (the oldest known alga U/Th-dated at 850 ± 28 years cal BP; [Halfar et al., 2007]. Though it was initially hypothesized that vertical growth rates would be most severely impacted for thick-crusted *C. nereostratum*, this current investigation shows a decline in all three measures of growth, density and calcification rates (Figure 16). While a modest decline is apparent in algal growth and calcification rates, the steeper reduction in skeletal density indicates that algal densities are more sensitive to changing ocean pH than growth (which largely determines calcification rates). The ecological benefits of sustained vertical growth in coralline algae may allow organisms to quickly heal and regenerate skeletal/thallus mass from damaging disturbance events such as wave action and herbivore grazing [Steneck, 1986], both of which are common features of the Aleutian Island nearshore ecosystem. However, the metabolic costs associated with maintaining algal calcification through continued growth may have translated into the production of weaker (less dense) skeletons.
resulting in a loss in structural integrity. Ultrastructural weakening of coralline algae may facilitate the propagation of skeletal fractures, thereby amplifying damage to the algal crust and exposing the algal calcification centers to acidifying ambient seawater conditions [Kamenos et al., 2013; Ragazzola et al., 2012].

Diminishing algal density may also be associated to temperature stress resulting from increasing regional sea surface temperatures (SST) recorded in the Aleutian Island region. Regression analysis between algal density and Hadley Centre SST 1° x 1° reconstruction [HadISST; Rayner et al., 2003] indicates a strong negative relationship (Figure 18; 3 year mean: n = 66; r = 0.64, p = 0.001), such that coralline algal skeletal density increases (decreases) with cooler (warmer) SST. This is particularly evident in the decrease in algal density associated with the 1968 warming event, which was followed by a recovery in density values after seawater temperatures cooled. Similarly, skeletal density values declined with gradually warming SST to 1996, but only show a weak recovery with rebounding SST in 1999. Although previous studies have demonstrated increases in coralline algal growth and calcification rates with warming SST [Kamenos and Law, 2010; Martin and Gattuso, 2009], it was concluded that growth rates of *C. nereocstratum* from the Aleutian Islands were driven mainly by light availability, with a variable relationship to SST [Halfar et al., 2011b].
Figure 18 | Composite chronology of coralline algal skeletal density, pH, and sea surface temperature. Green line represents global ocean pH [Wolf-Gladrow et al., 1999]. Black bar indicates comparable magnitude of change recorded by proxy-based pH reconstruction from Aleutian Islands 1961 – 1992 [Fietzke et al., 2015]. Red line represents normalized 3 year mean algal skeletal density. Blue line represents 3 year mean Hadley center 1° x 1° sea surface temperature reconstruction (HadISST1) [Rayner et al., 2003]. Vertical axis orientation is reversed for SST.
3.4 Conclusions

The exact mechanisms responsible for the here observed declines in coralline algal skeletal density, calcification rate, and growth remain unknown. However, this study reveals that they are likely influenced by the synergistic effects of multiple environmental stressors such as decreased solar insolation (from increased shading), warming ocean temperatures, and increased CO₂ uptake in the coastal regions of the Subarctic North Pacific. Algal skeletal density appears to be the most sensitive to these changes, and may possibly be the best metric for monitoring future impacts of changing climate and ocean chemistry. The increased metabolic cost required for maintaining growth and calcification in continually acidifying seawaters may be the underlying cause for the production of weaker (less dense) skeletons and decreased structural integrity. If these conjectures are valid, changes in climate and ocean chemistry projected for the future will likely lead to additional skeletal weakening, increasing susceptibility of the algal crust to breakage and bioerosion, thereby reducing benthic cover of *Clathromorphum* and other genera of coralline algae. Such an outcome would have serious adverse effects on the abundant and diverse invertebrate species associated with the coralline algal-dominated ecosystems of the Arctic and Subarctic, resulting in ecological shifts in species diversity and benthic community structure [Hall- Spencer et al., 2008; McCoy and Pfister, 2014; Ragazzola et al., 2012].

3.5 Supplementary Material

3.5.1 Sample Collection

Live specimens of *Clathromorphum nereostratum* were collected at 10 - 25 m water depth via SCUBA off Amchitka and Attu Islands of the Aleutian archipelago, Alaska in 1969, 2004, and 2008 (Figure 14A and B). The Aleutian Islands are surrounded by a well-mixed surface water column as a result of wind mixing [Stabeno et al., 2001], upwelling, and the exchange of water to the north and south of the archipelago [Fietzke et al., 2015]. Samples collected in 1969 were obtained from the University of British Columbia Herbarium and the U.S. National Herbarium Collection at the Smithsonian Institution in Washington D.C.; Table 2).
3.5.2 Growth Increment Mapping and Analyses

Samples were vertically thick sectioned into 1.5 - 2 cm slabs parallel to the growth axis of the alga and polished to 3 μm. High resolution digital photomosaics of each polished specimen surface was scanned using an Olympus reflected light microscope (VS-BX) attached to an automated sampling stage imaging system equipped with Geo.TS software (Olympus Soft Imaging Systems; for details, see Hetzinger et al., [2009]. This microscopic scanning setup enabled detailed viewing with zoom and panning functions using a pyramidal file format. The resulting photomosaics facilitated the establishment of a precise age model through the identification of annual growth increments, which are delineated by couplets of dark and light bands, and coincide with the occurrence of rows of annually forming conceptacles. Conceptacles are uniformly-sized cavities that enclose the algal reproductive structures, and form seasonally in Fall (September – October) by the downward decalcification of skeletal material (Figure 15A). Annual growth increment widths ranging from approximately 242 to 567 μm were measured parallel to the direction of algal growth using Geo.TS software. Special care was taken to ensure that growth records were taken directly within the region of interest (ROI) where skeletal density measurements were obtained (Figure 15A).

3.5.3 Micro-CT Setup

Specimens were scanned in a micro-CT scanner (GE Healthcare, eXplore Locus RS) at a peak x-ray energy of 80 kVp and tube current of 450 uA at a resolution of 20 μm. 900 x-ray projections or views (image matrix size 2010 x 1920 pixels) were collected at 0.4 degree angular increments over a single 360 degree rotation. Each projection was 4500 ms in duration and 2 image frames were acquired at each view angle and averaged to reduce image noise resulting in a 2.75 hour long scan. Each averaged x-ray projection was corrected for brightfield, darkfield, and geometric distortions and then a 3D volumetric image was re-constructed from the x-ray projection data using a filtered-back projection algorithm with isotropic 20.171 um voxels. Sample vials containing air, water, SB3 cortical bone mimic (Gammex RMI, 1.1 gm/ml hydroxyapatite, Ca(PO4)3), and calcite crystal (pure calcium carbonate, CaCO3) were included in the scanner field of view during acquisition and used for calibration purposes. The 3D data set was linearly rescaled into Hounsfield Units (HU) so that air was represented by voxel greyscale values near -1000 and water by values near 0. Samples roughly the same size as the scanner field of view or
larger were scanned in multiple sections and digitally concatenated or stitched into a single, larger 3D volume. The samples were oriented such that the CT scan slice-plane was as perpendicular as possible to the growth axis of the coralline algae samples. A series of two dimensional x-ray images were taken of each sample and compiled into a three-dimensional reconstruction using MicroView Standard 2.5.0-1549 (Parallax Innovations Inc., 2013). A square based rectangular prism region of interest (ROI), 81 x 81 pixels (1.59 mm X 1.59 mm) in the X-Y slice-plane, and spanning the entire Z-range (growth axis of algae), was selected such that it avoided areas with cracks, boring cavities or diagenetic alterations as much as possible (Figure 15B). The ROI core was cropped out of the complete volume and digitally divided into a number of slices spaced at 20 μm intervals, and perpendicular to the Z-axis of growth. Ellipsoid-shaped conceptacles are uniformly-sized and randomly distributed in three dimensional space, hence any particular slice cutting through a number of conceptacles show size variations due to the positioning of the slice through the cavity (ex. at the edge vs. through the center). A threshold greyscale value of 1600 HU was carefully selected to remove conceptacle cavities (ranging from 150 - 200 μm in diameter) from the coralline algal skeleton. This approach assured consistent sampling focused on skeletal calcite only. Voxels with a greyscale value below the threshold were deemed to be empty space and excluded from the skeletal density calculation. However, skeletal porosity was included in the skeletal density calculation as small pore spaces (~ 5 μm) were unresolvable at 20 μm resolution. Skeletal density for each slice was determined using the fractional mineral content of each voxel above the threshold measured in HU and linearly rescaled to units of pure crystal calcite (density = 2.71 g/cm³), and integrated over all the voxels in that slice. The total mineral content of each slice was plotted against the slice number, and an age model was established to create an annually-resolved skeletal density time series. While seasonal (summer/winter) changes in algal skeletal density were also observed (Supplementary Figure 20), annual weighted means were used to reduce the level of noise in the data. Annual growth was divided by the resolution of the micro-CT scan (20 μm) to calculate the number of x-ray slices included in each annual growth increment. A weighted average was then applied to the greyscale values of all the slices within each annual growth increment to generate the annual skeletal density value for each year of growth (g/cm³) (Supplementary Figure 19). All records were compiled by normalizing the time series for individual samples to zero mean and unit variance and then averaging all samples, concentrating on periods where three or more samples were available.
Figure 19 | **Skeletal density comparison between modern and museum-collection specimens.** (A) Annual skeletal density for museum samples collected in 1969 normalized to zero mean and unit variance for a common time period (1935 – 1968). (B) Annual skeletal density for modern collected samples normalized to zero mean and unit variance for a common time period (1935 – 2003). Grey lines represent annual data from individual samples, red lines represent annual averages. The upward trend shown in the last years of growth (prior to collection) in museum samples indicate the absence of an ontogenetic decline in skeletal density of coralline algae.
Table 2 | Site information for museum and modern collections of *C. nereostratum* used for growth, density, and calcification rate chronologies.

<table>
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<th>Location</th>
<th>Site</th>
<th>Sample Name</th>
<th>Collection Date</th>
<th>Latitude</th>
<th>Longitude</th>
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<td>E 179° 18.549</td>
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<td>AM-SM-30</td>
<td>December 1969</td>
<td>N 51° 22.439</td>
<td>E 179° 11.589</td>
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<td></td>
<td>Bat Island</td>
<td>AM 4-1</td>
<td>August 2004</td>
<td>N 51° 25.568</td>
<td>E 179° 14.277</td>
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<tr>
<td></td>
<td>Attu Island</td>
<td>Murder Point</td>
<td>Attu 11-4</td>
<td>N 52° 47.787</td>
<td>E 173° 10.796</td>
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<td></td>
<td>Murder Point</td>
<td>Attu 11-5</td>
<td>August 2004</td>
<td>N 52° 47.787</td>
<td>E 173° 10.796</td>
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<tr>
<td></td>
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<td>08-1-25</td>
<td>June 2008</td>
<td>N 52° 56.016</td>
<td>E 173° 15.970</td>
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</tbody>
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Figure 20 | Seasonal variations in skeletal density. Annual skeletal density cycles (g/cm³) displaying maximum (minimum) micro-CT derived density variations associated with summer (winter) months of the algal growing season.
4 Abstract

Arctic Ocean freshening can exert a controlling influence on global climate, triggering strong feedbacks on ocean-atmospheric processes and affecting the global cycling of the world’s oceans. Glacier-fed ocean currents such as the Alaska Coastal Current are important sources of freshwater for the Bering Sea shelf, and may also influence the Arctic Ocean freshwater budget. Instrumental data indicate a multiyear freshening episode of the Alaska Coastal Current in the early 21st century. It is uncertain whether this freshening is part of natural multidecadal climate variability or a unique feature of anthropogenically induced warming. In order to answer this, a better understanding of past variations in the Alaska Coastal Current is needed. However, continuous long-term high-resolution observations of the Alaska Coastal Current have only been available for the last 2 decades. In this study, specimens of the long-lived crustose coralline alga *Clathromorphum nereostratum* were collected within the pathway of the Alaska Coastal Current and utilized as archives of past temperature and salinity. Results indicate that coralline algal Mg/Ca ratios provide a 60 year record of sea surface temperatures and track changes of the Pacific Decadal Oscillation, a pattern of decadal-to-multidecadal ocean-atmosphere climate variability centered over the North Pacific. Algal Ba/Ca ratios (used as indicators of coastal freshwater runoff) are inversely correlated to instrumentally measured Alaska Coastal Current salinity and record the period of freshening from 2001 to 2006. Similar multiyear freshening events are not evident in the earlier portion of the 60 year Ba/Ca record. This suggests that the 21st century freshening of the Alaska Coastal Current is a unique feature related to increasing glacial melt and precipitation on mainland Alaska.

4.1 Introduction

Freshening of the Arctic Ocean can have considerable impacts on ocean stratification and ventilation [Woodgate et al., 2005] affecting the Arctic sea ice budget [Mizobata et al., 2010; Paquette and Bourke, 1981; Shimada et al., 2006]. Furthermore, Arctic freshening can disrupt the flow of large-scale circulation features such as the Atlantic thermohaline circulation [Kuhlbrodt et al., 2009; Peterson et al., 2002; Rahmstorf, 1995; Woodgate et al., 2006]. Recent studies indicate a significant warming and freshening of the Bering Strait throughflow into the Arctic Ocean [Ladd and Stabeno, 2009; Mizobata et al., 2010; Royer and Grosch, 2006; Woodgate and Aagaard, 2005; Woodgate et al., 2006; Woodgate et al., 2010]. The Bering Strait throughflow accounts for approximately one third of the Arctic Ocean freshwater budget, a quarter of which is determined by freshwater influx from the Alaska Coastal Current, which originates in the Gulf of Alaska and later combines with freshwater from the rivers of western Alaska [Aagaard et al., 2006; Woodgate and Aagaard, 2005; Woodgate et al., 2006]. The Alaska Coastal Current is a narrow coastal jet fed by terrestrial freshwater runoff from British Columbia and mainland Alaska [Weingartner et al., 2005]. It flows westward along the southern coastline of Alaska, through the narrow Unimak Pass and into the Bering Sea (Figure 21A) [Schumacher et al., 1982; Stabeno et al., 2002]. A recent increase in glacial melt in southeast Alaska suggests a growing freshwater contribution of the Alaska Coastal Current to Bering Strait throughflow [Arendt et al., 2009; Arendt et al., 2002; Royer and Grosch, 2006]. While a 20th century runoff record from Gulf of Alaska rivers permits retrospective examination of the Alaska Coastal Current, coastal observations of salinity appear to be better predictors of ACC freshwater variability [Weingartner et al., 2005]. However, long-term high-resolution nearshore oceanographic observations of Alaska Coastal Current freshwater variability are absent prior to the 1970s.

In this study, we use the nearshore crustose coralline algae *Clathromorphum nereostratum* as a long-term archive for past temperature and salinity variations in the Alaska Coastal Current. High-Mg calcite secreting photosynthetic coralline algae are well-suited for mid-to-high latitude century-long oceanographic reconstructions on high-resolution timescales [Halfar et al., 2007; Hetzinger et al., 2009; Kamenos et al., 2008]. Stable oxygen isotopes and Mg/Ca ratios from different species of *Clathromorphum* track in-situ sea surface temperatures as well as large-scale
climate patterns such as the El Niño Southern Oscillation, North Atlantic Oscillation and Pacific Decadal Oscillation [Gamboa et al., 2010; Halfar et al., 2007].

Figure 21 | Map of North Pacific/Bering Sea region and Aleutian archipelago. (A) Arrows depict pathway of Alaska Coastal Current based on data from Schumacher et al. [1982]. Star represents location of GAK 1 oceanographic station. (B) Inset figure shows sample collection site in Akun Bay, west of Unimak Pass.

In contrast, Ba/Ca ratios in Clathromorphum display weak correlations with temperature [Hetzinger et al., 2011], but respond to variability in surface ocean Ba content. Upper ocean Ba concentrations are influenced by upwelling of cold Ba-rich deep waters, or by coastal influx of suspended sediments [Alibert et al., 2003]. In open ocean settings, Ba is often depleted in surface waters and enriched in deep waters due to the uptake of Ba as barite (Ba SO₄), associated with
the formation of biological particulate matter [Bishop, 1988; Chan et al., 1977; Collier and Edmond, 1984; Dehairs et al., 1980; Dehairs et al., 1987; Falkner et al., 1993; Lea and Boyle, 1989; Lea and Boyle, 1991; Lea et al., 1989]. However, corals growing close to shore experience higher levels of Ba compared to those in the open ocean [Shen and Boyle, 1988]. Ba is abundant in estuarine environments due to high terrestrial freshwater and sediment influx into coastal waters [Coffey et al., 1997]. Ba previously absorbed by riverine clay minerals is released into the ocean upon encountering more alkaline elements (i.e. Ca, Mg, and Sr) present in the seawater [Falkner et al., 1994; Guay and Falkner, 1998; Hanor and Chan, 1977; Li and Chan, 1979; Shen and Boyle, 1988; Sinclair and McCulloch, 2004]. Thereafter, Ba commonly behaves as a conservative dissolved tracer, and is incorporated into the carbonate skeleton of calcifying organisms in proportion to ambient seawater concentrations [Lea et al., 1989]. Skeletal Ba/Ca ratios have proven to be a valuable proxy for seawater Ba/Ca, providing information on coastal sediment transport, freshwater discharge, salinity, as well as nutrient and alkalinity distributions [Fleitmann et al., 2007; Grove et al., 2010; Lea and Boyle, 1989; Lea and Boyle, 1991; McCulloch et al., 2003; Sinclair et al., 1998; Sinclair and McCulloch, 2004]. However, these Ba/Ca-based proxy studies have typically been conducted in tropical, low-latitude regions. In the North Pacific where historical records are sparse, a proxy for long-term changes in freshwater variability of the Alaska Coastal Current would be highly valuable. Here we employ coralline algal Ba/Ca and Mg/Ca ratios to reconstruct temperature and salinity variability of the Alaska Coastal Current in order to demonstrate that a recent freshening episode of the Alaska Coastal Current is unprecedented during the last 60 years.

4.2 Methods

In June 2008, living specimens of the coralline alga Clathromorphum nereostratum were collected at 10 m water depth using SCUBA from Akun Bay (54.2°N, 165.5°W) on the western shore of Unimak Pass, the main conduit for flow of the Alaska Coastal Current from the North Pacific into the Bering Sea (Figure 21B) [Stabeno et al., 2002]. Samples were sectioned vertically and polished to 1 μm. High-resolution digital photomosaics of the polished specimens were scanned using an automated sampling stage-imaging system equipped with Geo.TS (Olympus Soft Imaging Systems) software (for details see Hetzinger et al., [2009]). The resulting
photomosaics enabled the identification of annual growth increments which are identified by the occurrence of rows of annually forming conceptacles (cavities containing reproductive structures) (Figure 22A and B). Laser-line transects were digitized along the growth axis of each sample using Geo.TS (Figure 22A and Figure 23), carefully avoiding conceptacle cavities and areas of discontinuous growth. The resulting x, y coordinates were used for positioning line transects for Laser Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICP-MS) analysis.

Figure 22 | Overview cross-sectional image of coralline alga with laser ablated transects. A) Overview image of polished *C. nereostratum* thick section with overlapping laser transects (lines 1, 2 and 3). Element ratios obtained from overlapping sections were averaged; B) Magnified view of alga depicting annual growth increments and conceptacle cavities.

LA-ICP-MS analysis of Mg/Ca and Ba/Ca elemental ratios were conducted at the Earth System Science Research Centre at the University of Mainz Germany, using an Agilent 7500 ce Quadrupole ICP-MS attached to a New Wave Research UP-213 laser ablation system equipped with a large format cell. Laser measurements were performed using laser energy densities of 6 J/cm², and helium as a carrier gas. Transect lengths were limited to 6000 µm in order to minimize the effect of ICP-MS drift and were analyzed at a scan speed of 10 µm/second, with a 65 µm spot size and 10 Hz pulse rate. NIST SRM 610 (US National Institute of Standard and Technology Standard Reference Material) glass reference material was used as an external standard. Data for NIST SRM 610 were extracted from the GeoReM database available from
Detection limits were: Mg = 0.02 ppm, Ca = 5.47 ppm, Ba = 0.01 ppm. Additional details on the analytical setup used can be found in Jacob [2006] and Hetzinger et al. [2011].

Figure 23 | Algal Mg/Ca cycles obtained from LA-ICP-MS superimposed on photomosaic of sample cross-section with laser transect. Mg/Ca measurements display distinct seasonal cycles. Areas with high (low) Mg/Ca ratios are interpreted to be summer (winter) periods of algal growth, with one full cycle representing one calendar year. Calendar years were assigned by counting annual growth increments.
Subannually-resolved age models were constructed using annual variations in algal Mg/Ca. Calendar years were assigned to annual growth increments starting from 2008 (year of collection) and extending back in time. The Mg/Ca cycles were then graphically superimposed on a photomosaic of the sample surface depicting the laser transects where maxima in Mg/Ca were aligned with the center of each growth increment, and minima with growth increment transitions (Figure 23). Using the compiled information from the Mg/Ca cycles and photomosaics, sub-annual markers were assigned to the maxima (representing the warmest month of the year) and minima (representing the coldest month of the year) of each annual Mg/Ca cycle. Based on monthly sea surface temperature averages from bias-corrected satellite measurements obtained from Reynolds Optimum Interpolation sea surface temperature analysis (1982 – 2008), the month exhibiting the warmest (coldest) temperature at Akun Bay was August (March) [Reynolds et al., 2002]. Markers were assigned beginning at the first minimum (i.e. March 2007), followed by the first maximum (i.e. August 2006), and continuing back in time. The algal time series was linearly interpolated between these anchor points using AnalySeries software [Paillard et al., 1996] to obtain a proxy time series with monthly resolution. Since algal Ba/Ca ratios do not exhibit annual cyclicity, the anchor points derived from the Mg/Ca age model were adapted to the Ba/Ca data. In addition, given that coralline growth rates vary throughout the year (e.g. highest rates in summer, lowest in winter, insignificant or no growth between January and April) the 12 equidistant data points were assigned to growing-season months according to a growth model established by Halfar et al [2008].

The final algal record was constructed from the average of two samples: Sample 24 from 1971 – 2006; and Sample 27 from 1946 – 2006 (Figure 24A). The resulting algal Mg/Ca and Ba/Ca time series were correlated to instrumental temperature and salinity observations. Since long-term, high-resolution in-situ oceanographic data are nonexistent from Unimak Pass, sea surface temperature and salinity observations from oceanographic station GAK 1 (located within the flow of the narrow Alaska Coastal Current at the mouth of Resurrection Bay, Alaska; 59.8°N, 149.5°W; Figure 21A), were used for correlations with algal elemental ratios (GAK 1 data are available from http://www.ims.uaf.edu/gak1/). GAK 1 observations from the early 1970s and 1980s are sparse and irregular [Janout et al., 2010]. However, since 1991 more frequent quasi-monthly measurements of temperature and salinity have been made available using conductivity,
temperature, depth recorder casts with accuracies of ± 0.1 ºC and ± 0.2 psu for temperature and salinity, respectively [Royer and Grosch, 2006].

4.3 Results and Discussion

4.3.1 Mg/Ca – Temperature Relationship

Linear regression analysis reveals that annually averaged algal Mg/Ca ratios are weakly correlated to annual mean sea surface temperatures at GAK 1, 1400 km to the east of the coralline algal collection site at Unimak Pass (Figure 24B; n = 35, r = 0.32, p = 0.06). In the absence of longer nearby multidecadal instrumental temperature time series the algal Mg/Ca – temperature relationship was also compared with the large-scale pattern of the summer Pacific Decadal Oscillation (May – September) (Figure 24C; n = 60, r = 0.41, p = 0.001). Algal Mg/Ca ratios closely track the decadal-to-multidecadal variability of the Pacific Decadal Oscillation, the dominant pattern influencing North Pacific climate on 20-30 year periodicities [Mantua et al., 1997]. While the expression of the Pacific Decadal Oscillation is strongest in the Gulf of Alaska, the Pacific Decadal Oscillation signal is likely advected from the Gulf of Alaska to Unimak Pass via the Alaska Coastal Current [Weingartner et al., 2005]. This is consistent with a 117-year long C. nereostratum oxygen isotope record from the western Aleutian Islands located within the pathway of the Alaskan Stream, which also exhibited positive Pacific Decadal Oscillation correlations [Halfar et al., 2007]. Here, algal Mg/Ca ratios closely track sea surface temperature patterns, although correlations with both instrumental temperatures and the Pacific Decadal Oscillation are significantly diminished after 2002. This divergence could reflect a salinity effect on the incorporation of Mg into the algal skeleton caused by strong freshening of the Alaska Coastal Current beginning in 2001. In fact, recent studies of high-Mg calcite in echinoderm skeletons and foraminiferal tests field-collected or cultured in different salinities have demonstrated a clear negative effect on Mg/Ca ratios [Borremans et al., 2009; Ferguson et al., 2008; Kisakuerek et al., 2008].
Figure 24 | Relationship between algal Mg/Ca and temperature. A) *C. nereostratum* Mg/Ca time series from Sample 24 and 27. Overlapping regions in laser transects were averaged to create the final Mg/Ca time series. B) Annual algal Mg/Ca time series (averaged) compared to annual mean instrumental temperature from oceanographic station GAK 1 (1971 – 2006; n = 35, \( r = 0.32, p = 0.06 \)). C) Annual algal Mg/Ca correlated to Pacific Decadal Oscillation (May – Sep) index (n = 60, \( r = 0.41, p = 0.001 \)).
4.3.2 Timing of Alaska Coastal Current freshwater signal at Unimak Pass

Annually averaged Ba/Ca ratios were compared to monthly instrumental GAK 1 salinities for the period of quasi-monthly instrumental observations (1991 – 2006) in order to determine the seasonal timing of the Alaska Coastal Current freshening at Unimak Pass. The annual cycle of sea surface salinity observed at GAK 1 is characterized by high values spanning from winter to spring (December – July), declining to the lowest values in the fall (August – November) associated with the period of maximum discharge due to fall storm activity [Royer, 1982] and enhanced glacial melt (dotted line; Figure 25). Satellite-tracked drifter measurements have revealed an average transport time of ~2.5 months from the head of the Gulf of Alaska (approximate location of GAK 1) to Unimak Pass, located 1400 km to the west [Stabeno et al., 2002]. Therefore, the significant negative correlations between annual Ba/Ca and monthly GAK 1 salinity from mid to late fall demonstrates the propagation of the September – November freshening signal at GAK 1 to the algal collection site, resulting in an increase in algal Ba/Ca values from October – December (grey bars; Figure 25). Significant Ba/Ca – GAK 1 salinity correlations during August are attributed to late spring snowmelt [Royer, 2005] in mainland Alaska. Correlations in late winter and early spring were not included in the analyses, as the algae do not to exhibit significant growth during these months [Halfar et al., 2008]. In the following we focus on comparing the fall maximum discharge (September to November) recorded by GAK 1 salinities with the lagged October – December algal Ba/Ca ratios.
Figure 25 | Correlation of annually averaged Ba/Ca values of *C. nereostratum* with monthly GAK 1 salinity (1991-2006). Black line indicates monthly salinity average at GAK 1 for the same time period. Arrows depict recording lag of algae due to transport time between GAK 1 and Unimak Pass. Note that algal growth is insignificant from January through April.

### 4.3.3 Ba/Ca – Freshwater Relationship

An algal Ba/Ca time series was constructed from the average of samples 24 and 27 (Figure 26A). Algal Ba/Ca (October – December) ratios are significantly correlated with GAK 1 (September – November) salinities (0-20 m) from 1971 to 2006 (Figure 26B; n = 35, r = 0.65, p < 0.0001). Furthermore, a brief freshening event recorded by GAK 1 in 1990 is recorded by both algal samples, with sample 27 showing a strong increase in Ba/Ca. This demonstrates that algal Ba/Ca ratios at Unimak Pass provide a continuous and reliable record of the Alaska Coastal Current freshwater signature. Algal Ba/Ca ratios can therefore be used to reconstruct Alaska Coastal Current freshwater transport prior to 1970 when instrumental observations are non-existent.
A recent freshening episode of the Alaska Coastal Current is represented by a decrease in GAK 1 salinities and an increase in algal Ba/Ca from 2001 to 2006 (grey box; Figure 26B). The multi-year episode of 21st century freshening of the Alaska Coastal Current recorded by algal Ba/Ca ratios and GAK 1 observations was also detected in mooring-based studies of Bering Strait throughflow, which shows an incremental freshening of water masses with a combined Alaska Coastal Current and western Alaska river outflow signature along the eastern Strait from 2002 to 2004 [Woodgate et al., 2006]. In addition, a long-term decrease in salinity of about 0.06 psu in the upper 100 m at GAK 1 since 1970 is consistent with increasing algal Ba/Ca ratios and an increase in freshwater runoff off mainland Alaska over the same time period [Royer, 2005]. The observed freshening of the Alaska Coastal Current is likely due to an increase in annual mean air temperatures – generating a longer warm season and a shorter ice season [Stroeve et al., 2005; Woodgate et al., 2006], resulting in an increased rate of glacial thinning in southeast Alaska since the mid-1990s [Arendt et al., 2009; Arendt et al., 2002]. In addition, warmer air temperatures lead to enhanced moisture transport and coastal precipitation along the Gulf of Alaska [Janout et al., 2009; Weingartner et al., 2005]. In mountainous coastal regions such as the Gulf of Alaska where there is a steep weathering terrain, high suspended sediment loads in seasonal runoff likely deliver large volumes of barium-enriched freshwater to coastal regions [Coffey et al., 1997; Guay and Falkner, 1997]. In fact, extremely high Ba concentrations were detected on the eastern side of the Bering Strait associated with the Alaska Coastal Current [Guay and Falkner, 1997]. Although upwelling could potentially be a significant source of barium in surface water masses, it is unlikely to occur during the fall when the coastal environment in the Gulf of Alaska is dominated by freshwater discharge and downwelling-favorable winds. The resulting freshwater cap enhances vertical stratification, thus preventing mixing of deep nutrient-rich water with the surface [Weingartner, 2007]. Both the instrumentally-measured elevated barium concentrations of the Alaska Coastal Current and the subsequent depletion of barium by biological scavenging after passage of the Alaska Coastal Current into the Arctic Chukchi Sea [Guay and Falkner, 1997] likely point to a largely conservative behavior of Ba in the lower reaches of the Alaska Coastal Current. This, however, does not preclude that at least some of the barium signal recorded by the Unimak Pass algae is influenced by non-conservative behavior of barium. Non-conservative behavior of barium would result in the coralline algal Ba/Ca signal to be dampened with respect to the amount of barium introduced into the Gulf of Alaska.
In contrast to the Unimak Pass algal Mg/Ca time series, multidecadal variability (e.g. Pacific Decadal Oscillation) is not evident in the 60-year Ba/Ca record. This is similar to instrumental observations at GAK 1, where interannual temperature variability follows the Pacific Decadal Oscillation but salinity anomalies show no relationship to the Pacific Decadal Oscillation [Royer, 2005]. Hence, 21st century freshening of the Alaska Coastal Current as indicated by algal Ba/Ca ratios and instrumental observations is not part of multidecadal North Pacific climate variability and is not observed in the earlier portion of the 60-year long algal Ba/Ca time series. This suggests that recent freshening of the Alaska Coastal Current is unprecedented in the past 60-years.
Figure 26 | Relationship between algal Ba/Ca and salinity. A) *C. nereostratum* Ba/Ca time series from Sample 24 and 27. Overlapping regions in laser transects were averaged to create the final Ba/Ca time series (shown in B). B) Subannual GAK 1 (Sep-Nov) highly correlates to (Oct-Dec) algal Ba/Ca (1971 to 2006) \((n = 35, r = 0.65, p < 0.0001)\). The period of scarce oceanographic measurements (prior to 1991, where observations in five or more months of the year are missing) is indicated by dotted grey line. Period of quasi-monthly instrumental measurements (post 1991) is indicated by solid line. Grey bar highlights the significant multi-year freshening of the Alaska Coastal Current from 2001-2006 recorded by both instrumental and algal records.
4.4 Conclusions

We use Mg/Ca and Ba/Ca ratios of the long lived coralline alga *Clathromorphum nereostratum* collected from within the flow path of the Alaska Coastal Current to provide a 60-year record of temperature and freshwater variability. While Mg/Ca ratios track temperature variability associated with the Pacific Decadal Oscillation, Ba/Ca ratios exhibit no multidecadal variability, but closely relate to instrumental salinity observations for the period of overlap between the time series. Based on this relationship, algal Ba/Ca ratios are used as a tracer of coastal freshwater runoff into the Gulf of Alaska for the past 60 years. While it remains to be tested if this relationship has been robust over longer time frames, both instrumental data and algal Ba/Ca ratios indicate a period of enhanced freshening in the 21st century superimposed on a long-term trend of decreasing salinities of the Alaska Coastal Current since the 1970s. Recent freshening of the Alaska Coastal Current, which plays an important role in delivering freshwater into the Bering Sea and possibly even the Arctic Ocean, is attributed to enhanced coastal freshwater and sediment discharge. Enhanced coastal discharge in turn is a likely result of warming air temperatures resulting in an increase in coastal precipitation in the Gulf of Alaska, as well as an increase in the rate of glacial thinning in Southeast Alaska. Episodes similar to the 21st century freshening are not observed in the earlier portion of the 60-year long algal Ba/Ca time series, indicating that the recent Alaska Coastal Current freshening is unique in the last 60-years.

4.5 Acknowledgements

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5 Abstract

Accelerated warming and melting of Arctic sea-ice has been associated with significant increases in phytoplankton productivity. Long-term records of productivity are needed in order to properly evaluate currently observed changes in the context of long-term climate variability. However, satellite-based chlorophyll estimates and in-situ instrumental observations of marine productivity have only been available for the last few decades. Barium has previously been shown to be severely depleted in surface waters due to biological scavenging during periods of intense phytoplankton blooms. Here, an annually-resolved multicentennial record of coralline algal barium-to-calcium ratios (Ba/Ca) is presented and utilized as a proxy for North Atlantic phytoplankton productivity associated with sea-ice variability in Labrador, Canada that extends well into the Little Ice Age (LIA) (1646 AD). Coralline algal Ba/Ca demonstrates significant correlations to observational and proxy records of sea-ice variability, and shows a persistent pattern of covariability that is broadly consistent with the timing and phasing of the Atlantic Multidecadal Oscillation (AMO). Higher (lower) algal Ba/Ca values are interpreted as decreased (increased) primary productivity coinciding with cooling (warming) sea surface temperatures and the expansion (melting) of sea-ice. More frequent positive Ba/Ca excursions (indicating reduced productivity) are observed to be associated with AMO cool phases during the Little Ice Age, followed by a decline in Ba/Ca beginning in 1910 to present. The long-term decrease in coralline algal Ba/Ca coincides with an increasing trend in cod catches off Newfoundland up until 1960, indicative of rising marine productivity levels. This multicentennial record of coralline algal Ba/Ca ratios indicates that the recently observed productivity increase in the Subarctic North Atlantic is unprecedented in the last 360 years. Increasing phytoplankton productivity is expected to fundamentally alter marine biodiversity and ecosystem functioning as warming and freshening of the surface layer is projected to intensify over the coming century.
5.1 Introduction

The Subarctic North Atlantic is one of the most seasonally productive marine environments in the world, accounting for roughly 50% of global ocean productivity [Tilstone et al., 2014; Wassmann, 1990]. The seasonal melting of sea-ice in spring results in an increase in incident solar radiation, shoaling of the mixed layer, and release of nutrients and trace elements into the water column, triggering the onset of the spring phytoplankton bloom [Cherkasheva et al., 2014; Frajka-Williams and Rhines, 2010; Sverdrup, 1953]. Phytoplankton abundance not only influences fisheries production and marine species diversity, but also plays an important role in atmosphere-ocean carbon exchange, and the export of carbon into the deep sea [Alkire et al., 2012; Chassot et al., 2010; Siegel et al., 2014; Takahashi et al., 2009]. This blooming process is dependent upon physical properties of the surface seawater (ex. temperature, mixed-layer depth, stratification, light levels, and nutrient availability) that are directly modified by climatological factors (ex. solar radiation, cloud cover, wind mixing, upwelling, etc.) [Behrenfeld, 2014; Behrenfeld et al., 2008]. Therefore, continued warming and freshening of the surface layer is expected to have significant impacts on primary productivity [Sarmiento et al., 2004].

Arctic sea-ice thickness and concentration have dropped by approximately 9% per decade since 1978 [Comiso, 2012; Perovich and Richter-Menge, 2009]. Concurrent with this sea-ice decline is an increase in rates of phytoplankton productivity, driven by the enhanced transmittance of solar radiation into the surface ocean [Arrigo et al., 2012; Arrigo et al., 2008; Arrigo and van Dijken, 2011; Arrigo and van Dijken, 2015]. This ongoing decline in Arctic sea-ice cover has also led to an increased export of drift ice and freshwater out of the Arctic Ocean through the Fram Strait, and into the North Atlantic [Serreze et al., 2007; Stroeve et al., 2012; Wang et al., 2009]. Since the mid-1960s, large pulses of polar freshwater (as evidenced by negative anomalies in ocean salinities) have been transported into the Nordic and Labrador Seas [Belkin et al., 1998; Curry et al., 2003; Curry and Mauritzen, 2005; Dickson et al., 2002]. Phytoplankton growth in mid-to-high latitude regions is often limited by low light levels due to deep vertical mixing [Doney, 2006; Sarmiento et al., 2004]. The continued freshening associated with warming and sea-ice melt can supply additional buoyancy to the water column, reducing the mixed layer depth and leading to higher levels of productivity [Arrigo et al., 2012; Behrenfeld et al., 2006; Doney, 2006]. This has been recently supported by phytoplankton studies in Arctic and Subarctic basins that have indicated earlier timing [Arrigo and van Dijken, 2015; Harrison et al., 2013; Head and
Pepin, 2010], prolonged duration [Arrigo et al., 2008; Arrigo and van Dijken, 2015; Drinkwater and Pepin, 2013; Wu et al., 2007], and increased primary productivity [Arrigo et al., 2008; Arrigo and van Dijken, 2015; Brown and Arrigo, 2012] of the spring phytoplankton bloom. In fact, the most recent estimate indicates a 30% increase in net primary productivity in the Arctic Ocean between 1998 and 2012 [Arrigo and van Dijken, 2015]. Enhanced biological productivity can significantly alter patterns of nutrient cycling in the ocean by stripping nutrients from the surface layer [Guay et al., 2009], and can also influence the life history and abundance of zooplankton (ex. Calanus finmarchicus) in the North Atlantic [Head et al., 2013]; with implications on fishery yields and higher level trophic interactions [Drinkwater and Pepin, 2013; Townsend et al., 1994; Wassmann, 2011]. Thus, ocean warming, driving variations in sea-ice extent, volume, and freshwater transport can play an important role in determining the timing, magnitude, and duration of the spring bloom. However, difficulties of navigating in remote ice-laden waters and harsh polar climates have resulted in short, and often incomplete records of in-situ plankton abundance in the northern Labrador Sea [Richardson et al., 2006]. The Arrigo and van Dijken [2015] satellite-derived productivity record represents the longest time series of primary productivity associated with changes in Arctic sea-ice cover, allowing for analyses of interannual oceanographic processes and their impacts on primary productivity to be made. While this represents a significant step forward in uncovering the driving mechanisms behind currently observed changes in ocean productivity, the relatively short record (~15 years) precludes analyses of long-term climatic variability prior to the period of satellite observation.

Investigations of dissolved barium (Ba) concentrations in the Arctic have revealed significant depletions of Ba in surface seawaters shortly following the spring phytoplankton bloom [Falkner et al., 1994; Guay and Falkner, 1997]. Barium is a naturally occurring biointermediate element that exhibits nutrient-type behaviour in the open ocean, characterized by depletions in the surface layer and enrichment with depth [Chan et al., 1977; Falkner et al., 1994; Wolgemuth and Broecker, 1970]. This pattern has been attributed to the uptake of Ba in the surface ocean associated with the formation of biological particulate matter, and the subsequent regeneration at depth due to the respiratory breakdown of the organic matter [Bishop, 1988; Collier and Edmond, 1984; Dehairs et al., 1980; Dehairs et al., 1987; Dymond et al., 1992; Edmond et al., 1978]. Barium commonly behaves as a conservative dissolved tracer, and is incorporated into the carbonate skeleton of calcifying organisms in proportion to ambient seawater concentrations.
Barium-to-calcium trace element ratios (Ba/Ca) obtained from coralline algal skeletons have previously been employed in the reconstruction of freshwater variability associated with seasonal glacial melt [Chan et al., 2011]; and ice melt-induced stratification of the surface ocean [Hetzinger et al., 2013] in subarctic regions of the North Pacific and North Atlantic oceans.

In this study, a multicentennial record of Ba/Ca is obtained from an exceptionally long-lived specimen of the crustose coralline alga *Clathromorphum compactum* collected from northern Labrador Canada, as a proxy for North Atlantic primary productivity associated with climate driven sea-ice variability. The algal Ba/Ca proxy is interpreted such that: (1) During warm periods - increased sea surface temperatures (SSTs) and sea-ice melt increase plankton productivity, thus scavenging barium from the water column and resulting in a decrease in algal Ba/Ca; (2) During cool periods - reduced SSTs and sea-ice expansion decreases primary productivity, thus promoting barium uptake by coralline algae. This Ba/Ca proxy record will be used to examine how long-term climate oscillations (warming/cooling) driving sea-ice (melt/formation) in the Subarctic North Atlantic have influenced primary productivity over the past 360 years.

**Figure 27 | Location of crustose coralline alga collection site.** Sample collection site located off Kingitok Island in Labrador, Canada marked with star.
5.2 Methods

5.2.1 Sample Collection, Preparation, and Scanning

A living massive specimen of the encrusting coralline alga *Clathromorphum compactum* was collected in July 2011 at 17 m water depth via SCUBA off the coast of Kingitok Island in Labrador, Canada (55.3983° N, 59.8467° W) (Figure 27, 28A). The sample was vertically thick-sectioned into 1 cm thick slabs along the axis of algal growth (perpendicular to growth increments), and sample surfaces were polished to a 3 µm finish. High-resolution digital photomosaics (a series of digital images stitched together to form a complete image of the polished specimen surface) were generated using an Olympus reflected light microscope (VS-BX) attached to an automated sampling stage imaging system equipped with Geo.TS (Olympus Soft Imaging Systems) software (for details, see [Hetzinger et al., 2011]). This microscopic scanning setup enabled detailed viewing with zoom and panning functions using a pyramidal file format. The resulting photomosaics facilitated the establishment of a precise age model through the identification of annual growth increments, which are delineated by couplets of dark and light bands; Figure 28B). Digitized paths were traced along each annual growth increment boundary using Geo.TS for clear identification of growth layers. Calendar years were assigned to each annual growth increment, starting from the year of collection in 2011 and extending back in time at one year intervals. Lastly, laser-line transects were digitized along the growth axis of the sample using Geo.TS, carefully avoiding conceptacle cavities and areas of discontinuous growth. The laser-line transects were plotted in segments to minimize drift, and allowed for the selection of the most pristine regions of the sample surface for laser analyses (ex. least disruptions in growth due to bioerosion or abnormal growth). In addition, laser-lines were overlapped by 3-5 years to ensure reproducibility and continuity of the barium record. A series of (x, y) coordinates were generated from each of the laser-line transects and was used for positioning of the laser-lines for Laser Ablation Inductively Couple Plasma Mass Spectrometry (LA-ICP-MS) analysis.
LA-ICP-MS Setup

LA-ICP-MS analysis of magnesium-to-calcium (Mg/Ca) and barium-to-calcium (Ba/Ca) trace element ratios were conducted at the Department of Earth Sciences at the University of Gothenburg, using an Agilent 7500a Quadrupole ICP MS attached to a ESI NWR213 laser ablation system equipped with a large format cell. Laser measurements were performed using laser energy densities of ca 7 J/cm², and 0.9 l He/min as a carrier gas. 2 ml N₂/min were added downstream to the He carrier gas before mixed with 0.7 l/min Ar. The addition of N₂ along with the use of a second rotary vacuum pump lead to an improvement in sensitivity by ca a factor 2 compared to previous measurements (e.g., Chan et al. [2011]). Transect lengths were limited to 10000 µm in order to minimize the effect of ICP-MS drift and were analyzed at a scan speed of 10 µm/second, with a 60 µm spot size and frequency of 10Hz. NIST SRM 610 (U.S. National Institute of Standard and Technology Standard Reference Material) glass reference material was used as an external standard. NIST SRM 610 concentration used are 81595 ppm for Ca, 435 ppm
for Mg and 452 ppm for Ba, extracted from the GeoReM database available from: http://georem.mpch-mainz.gwdg.de/, version 04/01/2012). Detection limits were: Mg = 0.01 ppm, Ca = 4 ppm, Ba = 0.005 ppm. All data are reported as Element/Ca mass ratios [(mg/g)/(mg/g)] which can be converted to molar ratios (mol/mol) by dividing ratios by a conversion divisor (0.60644 for Mg/Ca, 3.42649 for Ba/Ca) [Hetzinger et al., 2013].

5.2.3 Development of Age Model

Subannually-resolved age models were established based on seasonal cyclic variations in Mg/Ca element ratios. According to monthly averages of Extended Reconstructed Sea Surface Temperatures (ERSST v4; 1854 – 2010; [Huang et al., 2014], the maximum (minimum) Mg/Ca values of seasonal cycles were interpreted to correspond to months exhibiting the warmest – August (coolest – March) temperatures off Kingitok Island, Labrador. Maximum and minimum Mg/Ca values were determined by graphically superimposing seasonal Mg/Ca cycles on a photomosaic of the sample surface depicting laser transects of the LA-ICP-MS, with the maxima of Mg/Ca aligned to the central portion of each growth increment, and the minima aligned to growth increment transitions (Figure 28B).

This cross-checking method ensured that each individual Mg/Ca cycle was matched with a corresponding annual growth increment in order to avoid possible errors in the age model. As previously mentioned, the coralline alga specimen was collected in July 2011, therefore anchor points were assigned beginning with the first Mg/Ca minimum (ex. March 2011), followed by the subsequent Mg/Ca maximum (ex. August 2010) and continuing back in time. The algal Mg/Ca time series was then linearly interpolated between these anchor points using AnalySeries software [Paillard et al., 1996] to obtain an equidistant proxy time series at a resolution of 12 samples per year. As the sample collection took place in summer, the final year of algal growth prior to collection (ex. 2011) was incomplete and therefore excluded from the analyses. Lastly, since algal Ba/Ca ratios do not exhibit annual cyclicity, Mg/Ca anchor points were transferred to barium laser measurements in order to create the Ba/Ca age model.
5.3 Data Collection

5.3.1 CIS Historical Ice Coverage Data

Historical ice coverage data from northern Canada was obtained from the Canadian Ice Service (CIS), Environment Canada (https://www.ec.gc.ca/glaces-ice/). Percentage ice cover data in the Northern Canadian Waters region was obtained from IceGraph Tool 2.0 for the month exhibiting the minimum sea-ice cover (September) from 1971 to 2010 [Tivy et al., 2011]. The data from the IceGraph Tool 2.0 is derived from weekly regional ice charts from predefined regions (based on the compilation analysis of satellite imagery, weather and oceanographic information, and observations from ships and aircrafts), and is calculated by multiplying the percentage of sea area in a specified region that is covered by ice, by the concentration of ice.

5.3.2 Newfoundland Historical Sea-Ice Extent Observations

Newfoundland sea-ice extent observations were reconstructed from seasonal ice charts (January to April; 1810 to present (http://archive.nrc-cnrc.gc.ca/eng/ibp/iot/research/ice-databases.html) depicting ice conditions off the east coast of Newfoundland and the Grand Banks [Hill and Jones, 1990].

5.3.3 Fram Strait Data

Fram Strait sea-ice export was reconstructed based on historical observations of multiyear ice called “Storis” from ship logbooks and ice charts off the southwestern coast of Greenland. Since summer months contain the most data coverage (with the least missing data), summer (May – July) storis observations extracted from historical records were used to create the storis extent index [Schmith and Hansen, 2003].
5.4 Results and Discussion

5.4.1 Ba/Ca & Sea-Ice Relationships

5.4.1.1 Northern Canada Sea-Ice Cover

Annual coralline algal Ba/Ca ratios are significantly correlated to CIS historical ice coverage for the month of September in northern Canadian waters from 1971 – 2010 (Figure 29; n = 40, \( r = 0.54, p < 0.001 \)), such that increases (decreases) in sea-ice cover are associated with higher (lower) Ba/Ca values. The positive relationship reflects the biological scavenging of barium from the surface seawater as a result of enhanced phytoplankton productivity associated with the melting of sea-ice. This is in agreement with a number of Arctic-wide investigations of ocean primary productivity, which have indicated an overall increase in net primary productivity (NPP) as a result of declining sea-ice cover [Arrigo and van Dijken, 2011; Arrigo and van Dijken, 2015; Brown and Arrigo, 2012; Pabi et al., 2008]. Increasing NPP has been associated with larger open water areas (providing suitable ice-free habitats for phytoplankton growth) and longer open water seasons (the timing between spring melt and fall freeze-up which determines the length of the phytoplankton growing season) [Arrigo et al., 2008]. Although no records of barium depletions associated with the recently observed increases in primary productivity currently exist; intensified biological productivity during seasonal phytoplankton blooms in the Chukchi Sea (associated with increases in solar radiation and melting of sea-ice) have been shown to be capable of severely depleting and essentially “stripping” barium from the surface seawater [Falkner et al., 1994; Guay and Falkner, 1997].
A comparison of annual Ba/Ca ratios to monthly averaged CIS sea-ice cover data (1971 – 2010) delimits the months of significant Ba/Ca – sea-ice cover relationships (Figure 30). Results indicate that the months July – October of the ice-melt season show a significantly positive association to algal Ba/Ca at the 95% level (grey bars), with the strongest correlations between August – October significant at the 99% level (dark grey bars). Algal Ba/Ca demonstrates the highest correlations to monthly historical CIS sea-ice data during months of minimum sea-ice cover (Black line, August – October), which suggests that the positive relationship between algal Ba/Ca and CIS sea-ice cover is most strongly coupled during the months where ice-melt is most significant. This finding is in support of the above interpretation, indicating that months with the lowest sea-ice cover (with more open water areas) have the largest influence on primary productivity, thus effectively removing Ba from the surface seawater, and resulting in lower levels of Ba/Ca recorded in the algae living on the shallow seafloor below. While January and
May also demonstrate a significant correlation to algal Ba/Ca, the number of in-situ observations during those months are noticeably sparser than the following months from late summer to fall. This can be attributed to the strong presence of sea-ice obstructing shipping navigation for in-situ observations, and is supported by NSIDC monthly sea-ice extent satellite images (1979 – 2011; http://nsidc.org/data/seaice_index/) indicating that the northern coastal Labrador region remains ice-covered well into the month of June. In addition, growth rates of coralline algae are significantly reduced or halted by the month of May as overwintering stores of photosynthates are used up with time under the sea-ice [Adey et al., 2013; Halfar et al., 2013]. Therefore, algal Ba/Ca is expected to show the strongest relationship to CIS historical sea-ice cover during months where a sufficient number of in-situ observations have been made, and when algal growth rates have returned to optimal levels.
Figure 30 | Correlation of annually averaged Ba/Ca ratios to monthly averaged CIS sea-ice cover. Data plotted during months of significant algal growth (May-Dec; 1971 – 2010) [Halfar et al., 2013, Adey et al., 2013]. Black line indicates monthly CIS sea-ice cover monthly average for the same time period. July – October of ice-melt season shows significant correlations to algal Ba/Ca at 95% level (grey bars), with strongest correlations during August – October significant at 99% level (dark grey bars). White bars indicate insignificant correlations. Bottom table shows total number of in-situ observations made during each month from 1971-2010.

<table>
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<tr>
<th>Month</th>
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5.4.1.2 Comparison to Centennial-scale Proxy and Observational Sea-ice Records

To examine whether the algal barium relationship to ice-melt induced primary productivity is sustained over longer timescales, coralline algal Ba/Ca was compared to a marine sea-ice proxy reconstruction [Halfar et al., 2013]; and a century-long observation of winter sea-ice extent off the coast of Newfoundland [Hill and Jones, 1990] (Figure 31A – C). Arctic sea-ice variability reconstructed from combined records of Mg/Ca element ratios and growth increment widths from crustose coralline algae has been used as a proxy for the duration of the open water (ice-free) season associated with increased SST and sunlight reaching the surface seawater [Halfar et al., 2013]. The coralline algal Ba/Ca record shows a clear negative association to the combined proxy sea-ice record (Figure 31B; 1646 – 2010; 10 year mean; n = 318, \( r = 0.75, p_{adj} < 0.0001 \) adjusted for loss of degrees of freedom). This result indicates that increases in sea-ice proxy values (related to warming, less sea-ice, and increased light levels) are associated with decreases in algal Ba/Ca (resulting from increased primary productivity), and vice versa.

Similarly, Newfoundland sea-ice extent observations (km²) demonstrate a strong relationship to algal barium (Figure 31C; 1870 – 2010; 10-year mean April: n = 132, \( r = 0.72, p_{adj} < 0.01 \)). The Newfoundland sea-ice extent record represents the maximum winter extent of sea-ice in the Newfoundland region [Hill and Jones, 1990]. Winter sea-ice extent is likely related to the timing of sea-ice retreat during the following spring melt season; such that years exhibiting lower (higher) than normal winter sea-ice extent are associated with earlier (later) sea-ice retreat in spring. The timing of sea-ice retreat and advancement dictates the length of the phytoplankton growing season, with longer and more productive seasons coinciding with years of earlier spring melt and later freeze-up in the fall of the same year [Arrigo and van Dijken, 2015]. Therefore, the findings from this paper suggests that periods of reduced winter sea-ice extent results in a longer phytoplankton growing season, increasing primary productivity and reducing available barium for uptake into the coralline algal skeleton.
Figure 31 | Coralline algal Ba/Ca (A; red) compared to observational (C, D, E) and proxy (B, F) data. Coloured lines represent 10-year means. Light grey bars show periods of increased Ba/Ca, associated with: 1) increased sea-ice: (B) sea-ice proxy [Halfar et al., 2013]; (C) Newfoundland sea-ice extent [Hill and Jones, 1990]; (D) Fram Strait sea-ice export [Schmith and Hansen, 2003]; and 2) cool phases of the Atlantic Multidecadal Oscillation: (E) Instrumental AMO index [Enfield et al., 2001]; (F) Tree-ring based proxy AMO index [Gray et al., 2004]. The long-term increase in cod fish landings indicate increasing productivity up until late 1960s, and is associated with a long-term decline in algal Ba/Ca. Note that proxy sea-ice data (B), instrumental AMO index (E), and proxy AMO Index (F) are plotted inversely to show increasing sea-ice and cooling.
5.4.1.3 Fram Strait Sea-Ice Export

The Labrador Sea in the Subarctic North Atlantic receives outflows of Arctic sea-ice and freshwater through the Canadian Arctic Archipelago and the Fram Strait, with the latter serving as the major gateway for polar outflows [Serreze et al., 2007]. The Fram Strait represents the single largest export of sea-ice out of the Arctic [Aagaard and Carmack, 1989; Koenigk et al., 2006], accounting for approximately 26% of the total freshwater [Serreze et al., 2006] and 10-25% of the total sea-ice discharge from the Arctic Ocean respectively [Kwok et al., 2004; Serreze et al., 2006]. Global coupled atmosphere – sea-ice – ocean models indicate that during periods of anomalously large ice exports through the Fram Strait, additional ice is delivered southwards into the East Greenland Current, which subsequently melts and flows into the Labrador Sea as a freshwater/local sea-ice anomaly 1 – 2 years later [Haak et al., 2003; Karcher et al., 2005; Koenigk et al., 2006]. These model simulations indicate that following large Fram Strait ice export events, anomalously cold and fresh waters enter into the Labrador Sea, with the ability to reduce salinity, deep convection, and ocean heat release - thus promoting sea-ice formation [Hakkinen, 1999; Koenigk et al., 2006].

In order to test whether freshwater and sea-ice export through the Fram Strait influences local sea-ice formation (and hence productivity) in the Labrador Sea, coralline algal Ba/Ca was correlated to an annually reconstructed record of Fram Strait sea-ice export (km³/yr) from 1870 to 2000 [Schmith and Hansen, 2003]. Results indicate a positive relationship between coralline algal Ba/Ca ratios and sea-ice export from the Fram Strait with a two year lag (Figure 31D; 1870 – 2000; 10 year mean; n = 122, r = 0.65, p_{adj} = 0.02). The latter was applied to the algal Ba/Ca time series in order to account for the transit time required for the Fram Strait ice/freshwater signal to propagate into the Labrador Sea [Koenigk et al., 2006].

5.4.2 Atlantic Multidecadal Oscillation

Historical records of North Atlantic sea-ice variability have recently been shown to covary with phase changes of the Atlantic Multidecadal Oscillation (AMO) index [Miles et al., 2014]. The AMO is manifested as basin-wide sea surface temperature (SST) anomalies in low to mid-latitudes (0 – 60°N), oscillating between positive (negative) phases associated with warm (cool)
North Atlantic SSTs with a periodicity of approximately 60 – 90 years. This multidecadal pattern of sea-surface temperature variability is thought to be driven by the varying intensity of the Atlantic Meridional Overturning Circulation (AMOC) [Delworth and Mann, 2000]. Modelling simulations suggest that North Atlantic sea-ice extent may be driven by changes in the AMO and AMOC indices; such that during periods of high index values (warm phases), intensification of deep convection leads to a subsequent release of heat that warms surface air and seawater temperatures in the Labrador and Nordic Seas, thus inhibiting the formation of sea-ice [Day et al., 2012; Mahajan et al., 2011]. In contrast, periods of low AMO/AMOC index values (cool phases) have been linked to weakening of deep convection, enhanced sea-ice export through the Fram Strait, and an increased presence of sea-ice in the subarctic North Atlantic [Dima and Lohmann, 2007; Miles et al., 2014; Schmith and Hansen, 2003].

In order to assess whether long-term AMO climate driven sea-ice variability influences marine primary productivity, coralline algal Ba/Ca was compared to the AMO index. Closer examination of the algal Ba/Ca time series also reveals long-term multidecadal variability with a characteristic AMO-like frequency as demonstrated using a multi-taper power spectrum (Figure 32; 1646 – 2010; ~55 – 60 years significant at the 99% level). Multidecadal Ba/Ca variability demonstrates strong covariability with the instrumental AMO index [Enfield et al., 2001] (Figure 31E; 1900 – 2010; detrended 10-year mean: n = 102, r = 0.82, p_{adj} = 0.003), such that AMO cool phases (1900 – 1927 and 1963 –1995) are associated with higher Ba/Ca; and AMO warm phases (1928 – 1962 and mid-1990s to present) are associated with lower Ba/Ca levels.
Figure 32 | Multi-taper power spectrum of annually averaged gap-filled Ba/Ca time series 1646 - 2010. Significance estimates based on a red-noise AR (1) model are shown in Supplementary Figure 33. Periods (years) of peaks significant at 95/99% level are indicated.

On longer timescales, coralline algal Ba/Ca also demonstrates an inverse relationship to a multi-centennial tree-ring proxy based reconstruction of the AMO index [Gray et al., 2004] (Figure 31F; 1646 – 1990; detrended 10-year mean: n = 298, $r = 0.58$, $p = 0.00076$). The extended comparison between coralline algal Ba and the proxy-based AMO index reveals additional cycles of depressed algal Ba/Ca values that roughly correspond to the AMO warm phases (1670 – 1710 and 1778 – 1800), and increases in algal Ba/Ca during AMO cool phases (1710 – 1735, 1740 – 1755, and 1765 – 1780, and 1800 – 1820). Covariability between the two proxy records indicate more frequent periods of enhanced algal Ba/Ca associated with AMO cool phases during the Little Ice Age (LIA: 1550 – 1850).

Between 1870 and 1910 the AMO proxy index indicates a shift from a warming to cooling phase, associated with a dip and subsequent rise in algal Ba/Ca. This shift is also evident in the algal sea-ice proxy time series (indicating a shift to reduced SST and sunlight levels); and also
coincides with increases in Newfoundland sea-ice extent and Fram Strait sea-ice cover. Prior to 1910 higher algal Ba/Ca ratios (1646 – 1840) coincide with the Little Ice Age, which indicates lower levels of productivity coinciding with cooling climates and the expansion of sea-ice (Figure 31A).

5.4.3 Recent Decline in Coralline Algal Ba/Ca

Similarly, a 500-year long historical record of Atlantic cod landings off Newfoundland indicates that stocks remained low throughout the Little Ice Age (16th – late 19th century) [Rose, 2004]. This may be related to suppressed marine productivity in the North Atlantic during the LIA, as evidenced by an extreme minima in phytoplankton carbonate content of marine sediments (a proxy for productivity) [Andrews et al., 2003; Keigwin, 1996]. Following the LIA, increases in cod catches indicate a period of high productivity from 1900 – 1960 [Rose, 2004]. This pattern is mirrored by a steeply declining trend in algal Ba/Ca since 1910, suggesting that the termination of the LIA and the subsequent reduction in sea ice led to significant increases in phytoplankton productivity in the Subarctic North Atlantic. Since the 1960s, the correspondence between algal Ba/Ca and Atlantic cod landings breaks down due to the decline and eventual collapse of the Newfoundland cod fisheries due to overfishing in the early 1990s [Lilly et al., 2013; Rose, 2004].

5.5 Conclusion

This multicentennial record of coralline algal Ba/Ca ratios indicates that the recently observed productivity increase in the Subarctic North Atlantic is unprecedented in the last 360 years. Interactions between large scale climate oscillation patterns, and the ongoing melting of sea-ice are resulting in higher levels of primary productivity. The trend in sea-ice melt leading to increasing phytoplankton productivity is predicted to influence nutrient cycling patterns, carbon export to the deep sea, as well as impacts on local marine ecosystems [Arrigo et al., 2008]. This is particularly true for regions such as the Labrador Sea where there are a low number of trophic links, thus increases in primary productivity are likely to result in wholesale shifts in local species abundance and diversity [Frajka-Williams and Rhines, 2010].
This investigation demonstrates the utility of coralline algal Ba/Ca as a proxy for local productivity in association with warming and sea-ice melt in the Labrador Sea. Algal Ba/Ca ratios consistently demonstrate significant correlations to both observational and satellite records of sea-ice variability, and follow general trends in productivity as indicated by historical catch records of Newfoundland fisheries. However, intensified fishing pressure since the 1960s lead to the eventual collapse of Atlantic cod off Newfoundland and Labrador, resulting in a breakdown in the productivity relationship to algal Ba/Ca. During this time, the availability of satellite-derived net primary productivity estimates demonstrate a continual increases ocean productivity, mirrored by a general decline in algal Ba/Ca. Therefore, the here established sea-ice – phytoplankton productivity – algal Ba/Ca relationship is likely to be a persistent feature of the subarctic northwest Atlantic.

5.6 Supplementary Material

5.6.1 Crustose Coralline Algae

Crustose coralline algae are calcareous photoautotrophic marine algae that are abundant and geographically widespread, forming hard rock-like encrustments in shallow rocky sublittoral zones worldwide [Steneck, 1986]. Annual growth increment forming coralline algae of the genus Clathromorphum are exceptionally abundant in subarctic regions of the North Atlantic, North Pacific, and Arctic oceans, where they can form accretions of up to 50 cm in thickness (at an average vertical extension rate of 380 µm/year), resulting in age spans of several hundred years [Frantz et al., 2005; Lebednik, 1976]. In fact, a living specimen of Clathromorphum nereostratum collected from the Aleutian Islands, Alaska has been U/Th dated to 850 ± 28 years, making it one of the oldest known marine organisms [; Halfar et al., 2007]. Thus, as a result of their abundance, distribution, longevity, and formation of clear and distinct annual growth increments; coralline algae have become excellent high-resolution paleoclimate archives for reconstructing past changes in the ocean environment.

Over the past decade, a number of different geochemical methods have been utilized to examine stable oxygen isotopes (δ¹⁸O), trace element compositions (ex. Mg/Ca, Ba/Ca, Sr/Ca, U/Ca), growth increment widths, and calcification within the coralline algal skeleton. Proxy data
obtained from *Clathromorphum* have been calibrated in a year-long in-situ field study [Halfar et al., 2008], and inter and intra-specimen comparisons have demonstrated high geochemical data reproducibility [Hetzinger et al., 2011; Hetzinger et al., 2013]. This wealth of proxy information spanning multidecadal to multicentennial timescales has provided us with a better understanding of variations in sea surface temperature [Gamboa et al., 2010; Hetzinger et al., 2009; Kamenos et al., 2008], cloud cover and shallow marine light dynamics [Burdett et al., 2011; Halfar et al., 2011b], Arctic sea-ice cover [Halfar et al., 2013]; and their associations with large-scale multidecadal climate oscillation patterns (ex. Pacific Decadal Oscillation, North Atlantic Oscillation, El Niño Southern Oscillation, Aleutian Low) that were previously unresolvable due to the short record of oceanographic observations.

5.6.2 LA-ICP-MS Analyses

A 30 year hiatus in the algal Ba/Ca time series is evident from 1840 to 1870 as a result of a gap in LA-ICP-MS measurements. LA-ICP-MS transects were taken parallel to previously measured electron microprobe lines extending across the entire lifespan of the algal specimen [Halfar et al., 2013]. This was done in order to obtain Ba/Ca records from the algal specimen as microprobe analyses were only capable of measuring magnesium-to-calcium (Mg/Ca). Through electron microprobe analyses, a geochemically altered region of the specimen was discovered (as indicated by significantly depressed amplitudes of Mg/Ca cycles), and was therefore avoided for subsequent laser analyses. However, detailed microscopic inspection of the sample surface within the altered region displayed clear growth banding, which enabled precise calendar dating of the hiatus period. The age model for algal Ba/Ca was then developed accordingly to account for the years of missing data in the hiatus region. A detrended Ba/Ca record was used on correlations exhibiting a trend. Prior to detrending, a singular spectral analysis was performed in order to fill the gap in laser data collection. This method ensured that the linear trend in the gap-filled time series was not significantly different than the unfilled time series (significant at the 99% level; Supplementary Figure 33).
Figure 33 | Annual and Gap-filled Ba/Ca time series. Annually averaged Ba/Ca time series (solid) and the gap-filled Ba/Ca time series (dashed) from 1646 to 2010. Linear least squares fits to both time series shown in blue.

It is important to note here that although the Newfoundland and Fram Strait sea-ice records date back to the early 1800s, decadal-scale relationships to algal Ba/Ca (based on 10-year running means) were only examined for the period from 1870 – 2010 due to the gap in the coralline algal record.
Conclusions, Summary, and Future Research

6 Conclusions

The research presented in this dissertation demonstrates the utility of *Clathromorphum* spp. crustose coralline algae as paleoclimate archives in the reconstruction of past climate and environmental changes in the Subarctic North Pacific and North Atlantic Oceans. Within the realm sclerochronology, coralline algae are emerging as a powerful tool for high-resolution paleoclimate research, due to their: 1) Abundance and ubiquitous distribution, particularly in mid-to-high latitude oceans where instrumental oceanographic observations are sparse; 2) Longevity on multi-decadal to multi-centennial timescales; and 3) Formation of distinctive annual growth increments that do not suffer from ontogenetic-related declines, and allows for precise calendar dating of calcified tissue. The work presented here aims to demonstrate new techniques and applications in coralline algal sclerochronology, in order to better understand the response of mid-to-high latitude algal-dominated ecosystems to anticipated consequences of climate and environmental change.

The micro-CT method (originally used in medical sciences) is adapted for the first time to sclerochronology, for the 3D visualization and quantification of structural changes in the coralline algal skeleton (Chapter 2). High resolution seasonal-scale records of coralline algal growth, skeletal density, and calcification rates were obtained using this technique to demonstrate the physiological stresses of warming and locally acidifying waters on algal calcification in the Subarctic North Pacific (Chapter 3). This thesis also explores new applications for Ba/Ca trace element ratios in different environmental settings: (1) as nutrient-type proxies for North Atlantic phytoplankton productivity in relation to sea-ice variability (Chapter 4); and (2) as indicators of freshwater runoff from mountainous coastal regions with high sediment loads contained in seasonal runoff (Chapter 5).

In the following sections, the major conclusions from the previous chapters are summarized, followed by a brief statement on future research needs.
6.1 Summary

The application of high-resolution micro-computed tomography for the sclerochronological analysis of slow-growing crustose coralline alga *Clathromorphum nereostratum* reveals seasonal-to-annual scale changes in algal calcification. Micro-CT is a non-destructive x-ray technique that allows for direct 2D imaging and 3D quantification of internal skeletal features and volumes, meanwhile preserving pristine specimens for further geochemical analyses. 20 µm resolution scans generate 2D x-ray slices at 20 µm intervals along the X, Y, and Z-axis plane, and is then compiled into a 3D reconstruction of specimen using MicroView software. This setup enables users to visualize and freely manipulate digital images (ex. rotate/tilt specimen, scroll through individual slices) for selection of the most pristine growth regions and longest records for more accurate quantification of structural and/or growth parameters. Additionally, MicroView allows for empty spaces such as the reproductive conceptacle cavities to be removed from the density evaluation to ensure that sampling focused on skeletal calcite only. High-resolution skeletal density data generated using micro-CT displays distinct seasonal cyclicity that is associated with summer vs. winter calcification changes, and agrees well with previous investigations of the algal skeleton using scanning electron microscopy (SEM) [Adey et al., 2013]. Precise determination of subannual-scale changes in skeletal densities allow for more accurate differentiation between seasonal and long-term variability, which is key for interpreting covarying climatic and environmental influences on growth and calcification of carbonate organisms. The purpose of this study is to broaden the applications of micro-CT for non-destructive viewing and quantification of structural and morphometric changes in other sclerochronological archives, especially those that are small in size or exhibit ontogenetic declines or compressed growth.

Utilizing the micro-CT techniques described in Chapter 2, annually-resolved multidecadal records of algal growth, skeletal density, and calcification rates were generated to investigate the long-term in-situ response of *Clathromorphum nereostratum* to declining seawater pH in the subarctic North Pacific. Algal specimens were live-collected off of Amchitka Island and Attu Island of the Aleutian archipelago in Alaska, in a region that has recently been shown to have undergone a long-term decline of 0.08 ± 0.01 pH units since the late 19th century [Fietzke et al., 2015]. High-Mg calcite skeletons (such as those found in *Clathromorphum* spp. coralline algae) have been shown to exceed the solubility of both calcite and aragonite in controlled dissolution.
experiments [Andersson et al., 2008; Andersson et al., 2003; Ries, 2011]. Therefore, highly soluble coralline algae residing in cold-water regions where seawater carbonate saturation states are exceptionally low, are expected to be among the first to respond to ocean acidification.

Multi-decadal variability in coralline algal calcification rates demonstrate an inverse relationship to the Arctic Oscillation Index (AO). Positive (negative) AO phases are associated with strengthening (weakening) of the Northern Hemisphere polar vortex such that: During the positive AO phase – low pressure systems induce wind mixing and upwelling of nutrients for phytoplankton production, which reduces solar insolation available for coralline algal photosynthesis and hence algal calcification rates. Similarly, algal skeletal density demonstrates a negative correlation to sea surface temperatures such that increases (decreases) in coralline algal skeletal density are associated with cooler (warmer) SST. Taken together, these findings indicate that in addition to acidification, sunlight availability, and temperature stress may also be important factors that influence the ability of corallines to calcify.

Additionally, the 70 year algal record indicates a modest decline in algal growth and calcification rates, with skeletal density exhibiting the steepest reduction, particularly since 1980. While continued vertical growth can calcification may allow coralline algae to quickly heal and regenerate skeletal/thallus material following wave action and herbivore grazing [Steneck, 1986], the reallocation of metabolic energy may have translated into the production of weaker (less dense) skeletons. Ultrastructural weakening of the algal skeleton may increase its susceptibility to breakage and bioerosion, which can have adverse effects on the diverse reef-like invertebrate communities associated with the coralline algal-dominated ecosystems of the subarctic North Pacific.

I also examined *C. nereostratum* from the Aleutian Islands, in the pathway of the Alaska Coastal Current (ACC) off of Akun Bay, Alaska. In this investigation, trace element ratios of Mg/Ca and Ba/Ca were obtained using LA-ICP-MS and utilized as proxies for the reconstruction of temperature and freshwater variability respectively; in a region where instrumental observations are nonexistent prior to 1970. Annual algal Mg/Ca ratios show a weakly positive lagged relationship to the remotely located (but only station available) GAK 1 oceanographic station in the Gulf of Alaska. However, on multidecadal timescales, algal Mg/Ca closely tracks temperature variability associated with the Pacific Decadal Oscillation (PDO). Conversely, algal
Ba/Ca ratios demonstrate a lagged negative relationship to GAK 1 measured salinity. Seasonal variations in Gulf of Alaska surface salinity are attributed to the fall maximum discharge due to increased precipitation/storm activity and enhanced glacial melt off mainland Alaska [Royer, 1982]. In the mountainous coastal regions of the Gulf of Alaska, intense seasonal runoff is capable of delivering high suspended sediment loads and barium-enriched freshwater into coastal regions [Coffey et al., 1997; Guay and Falkner, 1997].

A freshening episode of the Alaska Coastal Current was recorded by both a decrease in GAK 1 salinities and an increase in algal Ba/Ca from 2001 to 2006. Freshening of the ACC is likely the result of increasing annual mean air temperatures [Stroeve et al., 2005; Woodgate et al., 2006], leading to enhanced moisture transport and coastal precipitation [Weingartner et al., 2005; Janout et al., 2009], and increased rate of glacial thinning [Arendt et al., 2002, 2009] along the Gulf of Alaska. Based on the previously established Ba/Ca – salinity relationship, the multidecadal algal record indicates a long-term decline in ACC salinity since the 1970s, culminating in a sharp decline during the recent freshening event that is unprecedented in the last 60 years. Freshening of the Alaska Coastal Current can have a significant impacts on downstream regions, with the potential to influence ocean stratification, circulation, and sea-ice formation in the Bering Sea and Arctic Ocean [Serreze et al., 2006].

The versatility of coralline algal Ba/Ca trace element ratios was demonstrated using a different species of coralline algae *Clathromorphum compactum*, collected from northern Labrador, Canada. In this study, algal Ba/Ca is utilized for the first time as a proxy for North Atlantic primary productivity associated with climate driven sea-ice variability that extends well into the Little Ice Age (LIA) (1646 AD). The northwestern Atlantic region is characterized by intense seasonal phytoplankton blooms that can effectively scavenge trace elements and nutrients from the surface water column. Therefore in this setting, Ba/Ca recorded by shallow benthic coralline algae may be used as a proxy for surface ocean productivity.

Coralline algal Ba/Ca demonstrates significant positive correlations to observational records of sea-ice cover, sea-ice extent, and sea-ice export for different regions within the Subarctic North Atlantic; such that increases (decreases) in sea-ice cover are associated with higher (lower) Ba/Ca values. This positive relationship reflects the enhanced biological scavenging of barium from surface seawater during periods of ice melt and vice versa. On multi-centennial timescales,
algal Ba/Ca was compared to a marine sea-ice proxy reconstruction from crustose coralline algae [Halfar et al., 2013], which showed decreased Ba levels associated with longer open water (ice-free) seasons. A multi-taper power spectrum of the algal Ba/Ca record indicates periodicities of ~55 – 60 years, which is associated with the Atlantic Multidecadal Oscillation (AMO), a basin-wide pattern of SST anomalies in low to mid-latitudes (0 – 60°N) with a periodicity of ~60 – 90 years. In fact, historical records of North Atlantic sea-ice variability have recently been shown to covary with phase changes of the AMO [Miles et al., 2014]. Coralline algal Ba/Ca demonstrates a persistent pattern of covariability that is broadly consistent with the timing and phasing of both the instrumental AMO index (1900 – 2010) [Enfield et al., 2001], and a multi-centennial tree-ring proxy based reconstruction of the AMO index (1646 – 1990) [Gray et al., 2004]; such that AMO cool (warm) phases are associated with expansion (melting) of sea-ice resulting in decreased (increased) primary productivity, and hence higher (lower) Ba/Ca levels. More importantly, correlations to the AMO proxy record indicate more frequent periods of enhanced algal Ba/Ca (indicating reduced productivity) associated with AMO cool phases during the Little Ice Age from 1646 to 1840.

Ba/Ca values enter a decline beginning in 1910 to present which coincides with an increasing trend in cod catches off Newfoundland up until 1960, indicative of rising marine productivity levels. Closer examination of the multicentennial record of coralline algal Ba/Ca indicates that the recently observed productivity increase in the Subarctic North Atlantic is unprecedented in the last 360 years. Availability of satellite-derived net primary productivity over the last decade and a half [Arrigo and van Dijken, 2015], mirrored by an ongoing decline in algal Ba/Ca. Therefore, the here established climate-driven sea-ice variability → phytoplankton productivity → algal Ba/Ca relationship is likely to be a persistent feature of the subarctic northwest Atlantic. Increasing phytoplankton productivity is expected to influence nutrient cycling patterns and carbon export to the deep sea [Arrigo et al., 2008], and fundamentally alter marine biodiversity and ecosystem functioning [Frajka-Williams and Rhines, 2010] as warming and freshening of the surface layer is projected to intensify over the coming century.
6.2 Recommendations for Future Research

Proxy records obtained from sclerochronological archives may be the key to understanding long-term, natural climate variability - previously unresolvable through spatiotemporally limited instrumental observation. The results gathered from this dissertation contribute to the growing body of work on crustose coralline algal sclerochronology, adding novel analytical methods, and applications for trace element geochemistry in the reconstruction of past climate and environmental variability.

Based on the findings of the research presented, future research should focus on the following:

- Applications of the here described micro-CT technique for high-resolution non-destructive viewing and quantification of structural and morphometric changes in other sclerochronological archives such as corals, fish otoliths, and bivalve molluscs. Continued micro-CT based research will help establish records that may be able to quantify the effects of ocean acidification on weakening/dissolution of skeletal structures, and/or frequency and magnitudes of biological erosion.

- Based on the findings from Chapter 3, algal skeletal density appears to be the most sensitive in comparison to the other growth parameters measured, and may possibly be the best metric for monitoring future impacts of changing climate and ocean chemistry.

- Additional collections of coralline algae from the Aleutians can provide longer records of calcification changes in response to low frequency climate variability. This will allow researchers to better assess the role of atmosphere-ocean feedback mechanisms, and potentially identify underlying mechanisms for the observed changes in algal calcification.

- More research is needed to understand Ba/Ca as promising new proxy for the reconstruction of phytoplankton productivity in other regions of known phytoplankton blooms (ex. Aleutian Islands, Bering Sea, Chukchi Sea, etc.)
• Additional laboratory experiments and in-situ specimen collections combined with water sampling can enhance our mechanistic knowledge of coralline algal biomineralization and proxy element incorporation in controlled and natural settings.

• The development of additional proxies (ex. metal-to-calcium trace element ratios as a biomonitoring tool, Δ47 clumped isotope thermometry, etc.) and combined proxies such as in Halfar et al., [2013] and Kamenos et al., [2012] to reveal information about new environmental parameters.
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