OTOLITH DERIVED HATCH DATES, GROWTH RATES, AND MICROCHEMISTRY OF

ARCTIC COD (*BOREOGADUS SAIDA*) SUPPORT THE EXISTENCE OF SEVERAL

SPAWNING POPULATIONS IN ALASKAN WATERS

By

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Abstract

The changing climate in the Arctic is resulting in increased air and water temperatures as well as a reduction in sea ice, affecting native species that evolved to live in the unique habitats of the Arctic Ocean. One species of significant importance to Arctic marine ecosystems is the Arctic Cod (*Boreogadus saida*), a keystone species that serves as vital prey for many marine mammals, seabirds, and fishes. Arctic Cod have a strong association with sea ice for spawning and for much of their early life history. In the Pacific Arctic, little is known about their early life history, especially with regards to hatch timing and locations. To address these gaps, I estimated the hatch timing and growth rates of Arctic Cod during their first year by examining incremental growth patterns in their otoliths. Specifically, I counted daily growth increments to estimate age, and used those estimates to describe the relationship between length and age. Using this relationship, length measurements of age-0 Arctic Cod were converted to estimated daily ages and subtracted from the day of capture to estimate hatch date distributions for multiple sampling regions. Results suggest that fish caught during spring in the northern Bering Sea and southern Chukchi Sea hatched near their capture location over a relatively short period that coincided with the timing of local sea ice recession. Hatch dates from summer samples over multiple sampling regions indicated a prolonged hatching event that lasted from early winter (December) through early summer (July). Summer aggregations in the northeast Chukchi Sea likely represented a mix of different hatching populations that had been transported from the south and retained in the northern regions. Within each sampling region, mean hatch dates differed between pelagic and demersal caught Arctic Cod, which supports the existence of multiple hatching populations mixing within each region during the summer. In general, hatching occurred earlier the further south they were captured for summer-captured fish, whereas their growth rate declined as one moved northward, possibly due to the higher average temperatures during the larval stage in southern hatching locations. By analyzing the elemental composition of otoliths, I was able to infer environmental conditions such as salinity near the time of hatching of age-0 Arctic Cod. Regional differences in elemental concentrations at the time of hatching suggest a stronger freshwater influence in the eastern Beaufort Sea compared to the Chukchi Sea and western Beaufort Sea and support the existence of separate hatching populations. This study expands the understanding of the early life history of Arctic Cod and informs managers and policy makers to better protect critical life stages of this key species in a changing environment.

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General introduction

The Arctic is undergoing rapid change, resulting in a reduction in sea ice and increased air and water temperatures (Onarheim et al., 2018; Thoman et al., 2020). Understanding how these changes impact the Arctic ecosystem is critical for preparing resource managers for an uncertain future. One species of significant importance to the Arctic ecosystem is the Arctic Cod (*Boreogadus saida*), which is distributed throughout the circumpolar Arctic (Mecklenburg et al. 2002). In Alaskan waters, Arctic Cod have been captured as far south as the middle shelf of the southeastern Bering Sea (Marsh and Mueter, 2020) and they occur in large numbers throughout the Chukchi and Beaufort seas (Parker-Stetter et al., 2011; De Robertis et al., 2017; Forster et al., 2020). Arctic Cod can be found near the seafloor, in large midwater aggregations, and often occur in association with sea ice (Craig et al., 1982; Hop and Gjøsæter, 2013; David et al., 2016). The life history of Arctic Cod and their critical role in the Arctic ecosystem make them vulnerable to environmental change. They are relatively small, with adults averaging 300 mm in length (Scott and Scott 1988) and have a life span of about 7 to 8 years (Hop and Gjosaeter, 2013). Males reach sexual maturity at 1 to 3 years of age and females at 3 years of age (Craig et al., 1982; Hop and Gjosaeter, 2013; Nahrgang et al., 2015). Spawning typically occurs November through March under sea ice (Bouchard and Fortier, 2011; Hop and Gjøsæter, 2013).

Although much is known about the life history of adult Arctic Cod (Mueter et al., 2016), less is known about their early life history, especially with regards to hatch timing and hatching location (Craig et al., 1982), an important gap that is addressed in this thesis. Because knowledge about early life history is needed to protect this vulnerable life stage, I estimated hatch timing and growth rates of Arctic Cod in Alaskan waters using otolith increment analysis. In addition, I tried to differentiate hatching events and infer water characteristics at the time of hatching through otolith microchemistry. The use of otoliths, calcified structures located in the inner ear used for hearing and balance, has a long history in fisheries studies (Campana 1999). Because they are biologically inert, otoliths can be used to reconstruct many aspects of a fish's life history (Campana, 2005). As a fish ages, calcium carbonate is deposited incrementally to form rings on the otolith which can be used for aging. Each of these growth increments contain elemental signatures that reflect the surrounding environment, which can be used to determine water characteristics such as temperature and salinity at a specific time in their life (Campana 1999; Bouchard et al., 2015).

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To survive extremely cold temperatures, the cells of Arctic Cod contain a glycoprotein that acts as an antifreeze which prevents the formation of ice crystals (Zhuang et al., 2018). This is an important adaptation because Arctic Cod eggs are highly buoyant and lie just under the sea ice; this allows the eggs to freeze and, once thawed, to remain viable (Laurel et al., 2015). Arctic Cod are generalists and feed on a variety of zooplankton prey during their adult stage, but prefer large energy-rich *Calanus* spp. during larval and juvenile stages (Bouchard and Fortier, 2020). Due to their high assimilation efficiency (~80%) Arctic Cod are lipid dense, making them a primary food source for many predators such as seals, whales, sea birds, and some fishes (Hop et al., 1997; Bluhm and Gradinger, 2008, Crawford et al., 2015). Large predation events on Arctic Cod have been documented with hundreds of belugas (*Delphinapterus leucas*), harp seals (*Pagophilus groenlandicus*), and ringed seals (*Pusa hispida*), as well as thousands of fulmars (*Fulmarus* spp.) and kittiwakes (*Rissa* spp.) feeding on large aggregations of Arctic Cod (Welch et al., 1993). Artic Cod are also harvested by Indigenous peoples for subsistence and are harvested commercially in the Barents Sea (Gjøsæter 1995; Magdanz et al., 2010). Because of their importance to the Arctic ecosystem, Arctic Cod are considered to be a keystone species in the region (Welch et al., 1993; Hop and Gjosaeter, 2013; Majewski et al., 2017).

Studies have identified the thermal thresholds for Arctic Cod at different life stages and indicate what may happen as ocean temperatures increase. In laboratory studies, the eggs of Arctic Cod survive freezing temperatures but do not remain viable when temperatures exceed 3.8°C (Drost et al., 2016; Kent et al., 2016). The ability to thrive in freezing Arctic waters has allowed Arctic Cod to maintain high abundances and provide energy for higher trophic level species; however, their physiological preference for cold conditions makes them vulnerable to warming temperatures. In laboratory studies, juvenile Arctic Cod reached their maximum growth rate at 7.3°C and did not survive temperatures exceeding 16°C (Laurel et al., 2015).

As species that are better adapted to warmer waters begin to move north, they may prey on Arctic Cod or outcompete them for resources (Marsh and Mueter, 2020). This displacement has already been documented in the northern Bering Sea, where the biomass of benthic fish and invertebrates increased from 3.0 million tons in 2010 to 4.5 million tons in 2017, primarily due to increases in Walleye Pollock (*Gadus chalcogrammus*) and Pacific Cod (*Gadus microcephalus*), whereas Arctic Cod were largely absent in 2017 and other recent warm years (Lauth et al., 2019, Stevenson and Lauth, 2019; Marsh et al. 2020b; Baker, 2021).

Increasing water temperatures will create a more favorable environment for warmer water species and may increase prey biomass at the cost of prey quality. Other gadids such as Saffron Cod (*Eleginus gracilis*), Pacific Cod, and Walleye Pollock have higher growth rates than Arctic Cod at temperatures above 5°C (Laurel et al., 2015), possibly allowing them to outcompete Arctic Cod. These species, however, have a lower lipid richness than Arctic Cod, requiring predators to consume larger amounts of less calorie-rich prey to meet their metabolic requirements. Moreover, the ability of Arctic Cod to store lipids decreases with temperature, impacting their body condition and ultimately the predators that rely on them (Copeman et al., 2020). To better understand how Arctic Cod populations and their predators will be affected as the climate warms, more information about their life history is required, especially the most abundant and vulnerable early life history stages.

In addition to the direct effects of warming on the Arctic marine ecosystem, Arctic Cod and other species may be impacted by increased anthropogenic activity as Arctic sea ice continues to retreat (Thoman et al., 2020). Reductions in ice cover and a longer ice-free season make the Arctic more accessible, allowing for increased vessel traffic and easier access for potential oil and gas exploration. Existing and projected shipping routes through Bering Strait and along the Canadian and Russian coastlines may overlap with essential Arctic Cod spawning areas during the ice-free season (Stephenson et al., 2013). Arctic Cod are most vulnerable during the egg and larval stages when they are associated with the top layer of the water (Graham and Hop, 1995). Arctic Cod eggs are particularly susceptible to damage from turbulence during open water conditions (Eriksen et al., 2020), including turbulence generated from vessel traffic, which may render them inviable. Sounds generated by vessel traffic have also been observed to cause Arctic Cod to leave feeding areas and change their movement and behavior (Ivanova et al., 2020). These changes can cause them to spend more energy, which reduces their ability to feed optimally.

The potential for increased oil exploration also poses significant risks to Arctic Cod. The eggs and larvae of Arctic Cod are highly susceptible to oil spills due to their buoyancy and association with the top portion of the water column (Laurel et al., 2019). Sensitivity to oil is also dependent on the life stage, with the late yolk-sac stage being much more sensitive than the juvenile stage (Gardiner et al., 2013). Both physical and chemical dispersants have been shown to negatively impact Arctic Cod (Word et al., 2011). Exposure to crude oil during their early developmental stage can cause jaw and heart deformation as well as change lipid metabolism and growth (Laurel et al., 2019; Bender et al., 2021). When crude oil exposure is combined with a 2.3°C increase in water temperature, deformities and

mortality was observed in 64% of sampled fish, this provides a potential scenario if an oil spill occurs as water temperatures increase (Bender et al., 2021). Bioaccumulation of crude oil in Arctic Cod can also occur indirectly through the consumption of contaminated *Calanus* spp., which in turn will increase accumulation in important subsistence species that feed on Arctic Cod (Agersted et al., 2018). Models of oil spills and their impacts on Arctic Cod indicate that even a small exposure of a large aggregation would greatly reduce their chance of survival to age-1. A 100,000-ton spill of crude oil in the Beaufort Sea treated with dispersants has been estimated to cause the death of 1.9 million larval and juvenile Arctic Cod, whereas an untreated spill of similar size would result in the loss of approximately 0.5 million larvae (Gallaway et al., 2017). Although these numbers may seem high, the loss only reduces the adult population by roughly 0.7%. However, this scenario depicts the negative impact spills can have if they occur at a hatching location, which demonstrates the need for a better understanding of where Arctic Cod are hatching.

The important ecological role of Arctic Cod and their vulnerability to environmental change highlight the need for more information about their early life history. Therefore, the main goal of this thesis was to provide a better understanding of hatch timing and locations of Arctic Cod in the Pacific Arctic. To achieve this goal, I examined incremental growth in otoliths to estimate hatch dates and growth rates. In addition, I determined the elemental composition of otoliths at the time of hatching to infer environmental characteristics near the time of hatching. For Chapter 1, I first estimated hatch dates of Arctic Cod from several regions in the Pacific Arctic by counting daily growth increments on their otoliths and subtracting the estimated ages of the fish from the corresponding capture dates. Second, I used the growth increments from a sample of fish of different sizes and ages to estimate and compare the average growth rates for several regions of the Pacific Arctic based on the slope of the length-at-age relationship. In Chapter 2, I assessed the elemental composition around the hatch mark in the otolith to infer environmental characteristics at the time of hatching based on previously established relationships between salinity and otolith elemental compositions (Bouchard et al., 2015). Results from both chapters provided new information on the early life history of Arctic Cod, which otherwise would be difficult to obtain due to the challenges associated with sampling during the ice-covered winter season.

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Chapter 1: Otolith-derived hatch dates and growth rates of Arctic Cod (*Boreogadus saida*) support existence of several spawning populations in Alaskan waters

## 1.1     Abstract

Arctic cod is an important prey species in Arctic marine ecosystems that provides efficient energy transfer from producers to consumers in the food web. Arctic cod are locally abundant throughout the Arctic, though little is known about their early life stages in the Pacific Arctic, especially when and where they spawn and hatch. This study estimated hatch dates and growth rates of first year Arctic Cod through analysis of otolith growth increments. First-year Arctic Cod were captured in the northern Bering, Chukchi, and Beaufort seas during the spring or summer between 2012 and 2017. Estimated hatch dates ranged widely from November to July with peak hatching occurring from February through May, depending on the region of capture. Combined with large individual and regional variability in growth rates, this suggests a bet-hedging strategy to ensure some larvae encounter favorable growth conditions. In addition to regional differences, we identified a clear separation of hatch dates between spring- and summer-caught Arctic Cod, suggesting different origins or strong size-dependent mortality. Finally, differences in hatch dates between pelagic and demersal juveniles support the settlement of older, larger juveniles to the seafloor on deeper portions of the shelf in late summer. Differences in hatch timing and growth in the context of variability in sea ice retreat, river discharge and other environmental conditions can provide new insights into the future of Arctic Cod as the Arctic climate continues to change.

## 1.2     Introduction

Arctic Cod (*Boreogadus saida*) was identified as a keystone species within Alaska's Arctic waters by the Fisheries Management Plan for Marine Resources in the Arctic (NPFMC, 2009) because it provides an important pathway for energy transfer from planktonic prey to larger animals. With large abundances, high energy content, and an assimilation efficiency around 80%, Arctic Cod are ideal prey for many Arctic predators (Hop et al., 1997; Bluhm and Gradinger, 2008, Crawford et al., 2015). Many larger predators such as seals, whales, and seabirds depend on Arctic Cod as a source of energy; in turn, polar bears and Alaska Native communities rely on some of these marine mammals for their caloric intake (Welch et al., 1993). Arctic Cod are also targeted by commercial fisheries in the Barents Sea (Gjosaeter 1995) and by subsistence fisheries in the Pacific Arctic (Magdanz et al., 2010).

Previous research on Arctic Cod has identified characteristics of their life history as adults; however, less is known about their early life history due to the difficulties associated with sampling early stages. Male Arctic Cod reach sexual maturity between 1 and 3 years of age whereas females mature at 2 to 3 years of age. They have a life span of 7 to 8 years (Hop and Gjosaeter, 2013), and reportedly spawn mostly between January and March in large groups underneath the ice (Craig et al., 1982; Bouchard and Fortier, 2011; Gallaway et al., 2017). Based on laboratory studies, Arctic Cod eggs remain suspended at the surface of the water just under the sea ice and can tolerate sub-zero temperatures without affecting survival (Laurel et al., 2015). Artic cod spawning locations and dates, as well as hatch times, are currently unknown, although such information is needed to protect these vulnerable life stages. Early larval stages have been found throughout the spring (Deary et al., 2021) and summer sampling seasons (Vestfals et al. 2019), suggesting a broad distribution of spawning and hatching in space and time. The bongo nets used in these studies, however, under-sample larger larvae and juveniles in the summer. Large abundances of larvae and juveniles have been observed over multiple years over the northeast Chukchi Sea shelf (De Robertis et al., 2017; Levine et al. 2021), but their origins remain unknown. Large numbers of larval and juvenile Arctic Cod have also been documented in the western Beaufort Sea (Parker-Stetter et al., 2011; Forster et al., 2020; Vestfals et al., 2019), but it is unclear if this population is connected to that found in the northern Chukchi Sea. Despite these recent observations, large gaps remain in the understanding of the reproductive biology and early life history of Arctic Cod in the Pacific Arctic (Mueter et al., 2016; 2020).

The Arctic marine environment is changing rapidly; how this may affect the distribution, abundance, condition, and phenology of Arctic Cod is of great interest to researchers and resource-dependent communities. The Arctic has seen a significant reduction in sea ice, an increase in water temperatures, and a rate of air temperature change that is double the global average (Thoman et al., 2020). These changes are reshaping the ecosystem, allowing southern, warmer-water species such as Capelin (*Mallotus villosus*), Saffron Cod (*Eleginus gracilis*), and other gadids to move north and compete with Arctic Cod for habitat and food resources (Hop and Gjøsæter, 2013, Marsh and Mueter, 2020). Arctic Cod have a lower thermal tolerance when compared to other southern gadid species and experience a high mortality occurring at 16°C and a peak growth rate at 7.3°C as juveniles (Laurel et al., 2015). The eggs of Arctic Cod have a much narrower temperature tolerance and will not survive in temperatures exceeding 3.8°C (Drost et al., 2016). As the Arctic environment continues to change, the impact of these changes on Arctic Cod will have ripple effects across the Arctic ecosystem.

Arctic Cod growth and hatch timing have been examined in Canadian and European Arctic waters using daily otolith increment deposits. These analyses revealed two hatching patterns: a short hatch event that occurs concurrently with ice break-up and the beginning of increased biological production (May to June) as well as a protracted hatching event that can occur under the sea ice beginning in the winter (January) and extending into the summer (July) (Bouchard and Fortier, 2011). Hatch timing may be an important determinant of subsequent growth and survival. Early hatching Arctic Cod are able to attain a greater pre-winter size but experience increased mortality during the long larval phase. Later hatching Arctic Cod have reduced pre-winter size, but experience less larval morality (Fortier et al., 2006). Therefore, there is greater potential for a higher abundance of later hatching fish at the end of the summer season, but these fish are on average smaller than the early hatching Arctic Cod (Fortier et al., 2006; Bouchard and Fortier, 2008) and may experience higher overwinter mortality as a result. If hatch timing is linked to sea-ice retreat, reductions in sea ice and early ice melt may contribute to observed changes in the abundance and distribution of Arctic Cod at the southern end of their range such as the Bering Sea (Marsh and Mueter, 2020). However, the links between changing ice conditions, hatching, and the survival of larval Arctic Cod in the Pacific Arctic are not currently understood.

Understanding the timing and location of spawning and hatching, as well as the subsequent growth, movements and survival of eggs and larvae, is critical to identifying habitat requirements for early life history stages of Arctic Cod. This is especially true in areas of potential oil exploration, as Arctic Cod are highly vulnerable to crude oil during their early life history (Gallaway et al., 2017; Laurel et al., 2019). Limited information is available on the distribution of eggs and early larval stages due to the challenges of sampling these life stages. To better understand the early life history and provide information to inform management, analysis of otolith microstructure could be used to provide insight into growth rates and hatch timing. These inferences, in turn, can be used to inform our understanding of earlier life stages in several ways. First, information on hatch timing is needed to parameterize biophysical transport models for Arctic Cod (Deary et al., 2021; Vestfals et al., 2021). Such models, in turn, can help identify and protect potential spawning aggregations and can be used to simulate how sea ice reduction and changes in water temperature may affect early life survival. Second, inferred hatch dates can be compared to the known hatch dates for other Arctic Cod stocks to help identify environmental drivers that control hatch timing and to compare the time of hatching across different stocks (Bouchard and Fortier, 2011). Finally, age-length relationships based on otolith-derived ages can

be used to estimate growth rates of larval and juvenile Arctic Cod in the field during their first few months and to compare growth rates among regions.

To address the gaps in understanding of Arctic Cod early life history, this study estimated and compared the distribution of hatch dates of larval and early juvenile Arctic Cod sampled during the spring and summer in the Chukchi and Beaufort seas. Specifically, we estimated age in days of Arctic Cod larvae based on daily otolith growth increments and used age-at-length relationships to infer the distribution of hatch dates from observed length-frequencies. A second objective was to derive field-based estimates of average daily growth rates and to compare growth rates among stocks from different regions to laboratory-derived and other field-based estimates from the literature.

## 1.3    Methods

### 1.3.1    Study region

Arctic Cod samples were obtained from the Bering, Chukchi and Beaufort seas and were assigned to five distinct regions based on differences in bathymetric and oceanographic characteristics within each of the seas (Figure 1.1). From south to north, these regions are northern Bering Sea (NBS), southern Chukchi Sea (SCS), northern Chukchi Sea (NCS), western Beaufort Sea (WBS), and eastern Beaufort Sea (EBS). The NBS has a broad shelf that encompasses Norton Sound and the Chirikov Basin between St. Lawrence Island, AK and Bering Strait, with depths generally less than 50 m. It connects to the Chukchi Sea via the Bering Strait at 56.9°N, which has a depth of less than 50 m. The majority of the Chukchi Sea has a shallow (40—60 m) continental shelf, which was split into a southern and northern region for our analyses due to differences in water masses. Alaska coastal water, Bering shelf water and Anadyr water from the Bering Sea converge in Bering Strait before entering the Chukchi Sea (Eisner et al., 2012; Danielson et al., 2017a) and continuing to flow north. These water masses of recent Pacific origin cover a variable portion of the SCS shelf but are typically separated from distinct water masses in the NCS, referred to as winter water and recent melt water, by a semi-permanent front that extends from the surface to the sea floor (Weingartner 1997). The colder and more saline winter water extends as far south as 70°N, therefore 70°N was used as the dividing line between the NCS and SCS for our analyses (Pickart et al., 2010). In contrast to the Chukchi Sea, the Beaufort Sea has a narrow shelf that quickly drops into the Arctic Basin to depths exceeding 2,000 m. The Beaufort Sea shelf receives relatively nutrient-poor water via the Alaska Coastal Current entering from the west and is influenced by fresher waters from the Mackenzie River, as well as by deeper Atlantic waters from the basin (Carmack and Macdonald 2002, Pickart 2004). For this analysis, the Beaufort Sea was separated into an eastern

(samples east of 147°W; EBS) and western region (153 °W to 147 °W; WBS) (Figure 1.1), with the EBS experiencing a stronger influence from the Mackenzie River than the WBS (MacDonald et al., 1987). Because of the strong connectivity between the NCS and the westernmost portion of the Beaufort Sea (west of 153° W), we pooled samples from these regions and refer to them collectively as the NCS.

### 1.3.2    Sample processing and collection

Larval and juvenile Arctic Cod were collected during five Arctic surveys conducted during the spring (June) and summer (August/September) seasons: the Arctic Shelf Growth, Advection, Respiration and Deposition (ASGARD) rate measurement survey in the NBS and SCS in the spring of 2017 (Danielson et al., 2017b), the Arctic Marine Biodiversity Observation Network (AMBON) survey in the Chukchi Sea in the summer of 2017 (Iken et al., 2018), the Arctic Integrated Ecosystem Survey (Arctic IES II) in the Chukchi Sea and WBS in the summer of 2017 (Farley et al., 2017), and the summer Transboundary Surveys conducted in the WBS in 2012 (TB12) and in the EBS in 2013 (TB13) and 2014 (TB14) (Norcross et al., 2017; Table 1.1). Four types of sampling methods were used: (1) an Isaacs-Kidd Midwater Trawl (IKMT, Methot 1986) with a 3-mm-mesh body and 1-mm-mesh cod-end liner was deployed obliquely to collect larval and juvenile pelagic fishes from near bottom to the surface during the AMBON, TB12, and TB13 surveys; (2) a modified Marinovich trawl with a 64-mm-mesh body which tapered to a 3-mm-mesh codend was used to target aggregations identified by acoustic backscatter as part of an acoustic-trawl survey during Arctic IES II (De Robertis et al., 2017); (3) two types of bottom trawls were used to sample demersal fishes, including a 3-m modified Plumb Staff Beam Trawl (PSBTA, Abookire and Rose, 2005) with a 7-mm-mesh body and a 4-mm liner during the AMBON, Arctic IES II and Transboundary Surveys, and a Canadian Beam Trawl (CBT, Majewski et al., 2017) with a 10-mm-mesh body and a 6-mm-liner during the Transboundary Surveys; and (4) a 60-cm-diameter Bongo net with a 505-μm-mesh body was used to sample zooplankton and ichthyoplankton during ASGARD and Arctic IES II surveys.

Two sets of Arctic Cod samples were used for analyses. The first sample consisted of all Arctic Cod sampled in the field and was used to characterize the length-frequency distribution of the population and the second sample consisted of a length-stratified subset to remove otoliths for aging. Standard lengths of all larval and juvenile Arctic Cod sampled in a given season, cruise, region, and vertical location (demersal or pelagic) were measured in the field or laboratory to the nearest mm (Table 1.1). Length-stratified subsamples for otolith aging were obtained from each region and season, except the WBS and 2012 EBS, to estimate region-specific relationships between length and age (Table 1.2) and to convert observed length-frequencies to estimated hatch date distributions using the

approach described below. To summarize length-frequencies and hatch date distributions, samples from different seasons, regions, and vertical locations were organized into groups. We defined a total of 10 groups consisting of pelagic spring samples from the NBS (1 group), pelagic spring and pelagic and demersal summer samples from the SCS (3 groups), and pelagic and demersal summer samples from the NCS, WBS, and EBS (2 groups each).

To obtain representative length-frequency distributions for larval and juvenile Arctic Cod for each group, the length of fish from different gear types and cruises were used (Table 1.1). Length-frequency distributions during spring were quantified using Bongo samples because larval fish are generally small (< 20 mm) at that time. Although the Bongo may select against some of the larger larvae in the water column, because of its small mesh size Bongo samples were considered to be most representative of the size distribution of larval Arctic Cod in the sampling area during spring. This was supported by opportunistic IKMT samples that supported our assumption that larvae were generally less than 20 mm in June. During the summer sampling period the bongo trawl was not used for Arctic Cod collection because age-0 fish were generally larger than 20 mm and were distributed throughout the water column or had settled to the bottom. We used fish collected by either the Marinovich trawl or the IKMT (when Marinovich was unavailable) to quantify length-frequencies of pelagic juveniles, whereas samples from the bottom trawls (PSBTA or CBT) were used to characterize lengths of demersal fish. The Marinovich has little size selectivity over the size range of interest (De Robertis et al., 2017; A. De Robertis, NOAA, Seattle, pers. comm.). Similarly, the PSBTA has been estimated to retain all or most age-0 Arctic Cod (Marsh et al., 2020a). We focused primarily on the pelagic fish for comparisons among regions because they are assumed to be age-0 fish, whereas the bottom trawl catches may include some small Arctic Cod (< 75 mm) that could be age-1 or older as there is considerable overlap in sizes among ages (Helser et al., 2017). Length data for the Transboundary Surveys from both the PSBTA and CBT were combined to characterize the length-frequency distribution of Arctic Cod in the eastern Beaufort Sea because there was no evidence that the size composition of the catches differed significantly between these gear types (Norcross et al., 2017). The combined gear type will be referred to hereafter as bottom trawl. In summary, pelagic fish in spring were represented by Bongo samples, whereas in the summer Marinovich and IKMT samples were used to represent pelagic samples, and bottom trawls were used to represent demersal fish.

To assess length frequency distributions of age-0 fish, as well as for aging age-0 fish, we included only individuals equal to or less than 75 mm in the Bering Sea, Chukchi Sea and WBS and up to 60 mm in

the EBS. The upper limits were estimated to be the maximum size of age-0 Arctic Cod in late summer based on the length-frequency distribution of all Arctic Cod sampled in a given region (Appendix A.1). The estimated cutoff of 75 mm for the southern regions was consistent with that used for age-0 fish sampled in the Chukchi Sea in late summer 2012 and 2013 (Marsh et al., 2020a). In the EBS, a cutoff of 60 mm was used as larger fish were clearly separated from a dominant mode of smaller, age-0 fish and were continuous with a mode of larger, presumably age-1 or older fish (Appendix A.1). This cutoff is consistent with previous studies in the Beaufort Sea (Norcross et al. 2017).

To sample fish for otolith aging, age-0 Arctic Cod were sampled over the full size range of approximately 6 mm to 75 mm to obtain samples that were representative of the full range of sizes of larval Arctic Cod in the spring and of young-of-year Arctic Cod in late summer within each sampling region. We collected size-stratified random subsamples of specimens collected across much of the study region (Figure 1.1), subject to other sampling priorities. Lengths were stratified into thirds to form small, medium, and large groups for each region to ensure that a broad range of lengths was represented for aging. Subsamples of larval and juvenile gadids for otolith analyses were frozen or stored in 95% ethanol and shipped to the University of Alaska Fairbanks in Juneau, Alaska, where they were identified and processed for further analysis. Samples of archived otoliths from the Transboundary Surveys in the Beaufort Sea and the corresponding standard lengths were obtained from the Fisheries Oceanography Lab at the University of Alaska Fairbanks (UAF). Of the smaller specimens (< 15 mm) shipped to Juneau, about 60% were examined by a larval taxonomist to confirm species identification. Because larger fish are difficult to identify in the field, IDs of fish greater than 20 mm captured during the Arctic IES II survey were verified by sequencing the mitochondrial cytochrome oxidase c, subunit 1, and aligning them with known gadid sequences at the National Oceanic and Atmosphere Administration (NOAA) Ted Stevens Marine Research Institute in Juneau, Alaska (Sharon Wildes, NOAA, Seattle, pers. comm.). All samples were processed and analyzed at UAF's Lena Point Fisheries Facility and at the Alaska Department of Fish and Game Mark, Age and Tagging laboratory in Juneau.

### 1.3.3 Otolith aging

To estimate age of juvenile Arctic Cod, sagittal otoliths were examined for daily growth increments. After measuring standard lengths, the otoliths were removed under a dissecting microscope with fine-tipped forceps. The left otolith was extracted, rinsed with 95% ethanol to remove organic matter, and mounted to a glass slide using clear, thermal plastic cement. The right otolith was removed, cleaned, and stored dry to be used if the left otolith was damaged or unusable. The mounted otoliths

were polished with various grades of lapping film to expose the daily growth increments within the otolith. Due to their uneven shape, some otoliths required polishing on both sides.

To estimate hatch dates, daily growth increments were counted on the otoliths. The presence of daily growth increments was previously confirmed by Bouchard and Fortier (2011). Otoliths images were captured using Image Pro Plus© (Media Cybernetics), and these images were used to enumerate daily rings. Each visible ring, a light band followed by a dark band in the otolith matrix was assumed to represent one day of growth (Figure 1.2 A). These daily growth increments were counted from the hatch mark to the edge of the otoliths (Figure 1.2 B). Hatch marks were identified and validated using otoliths of lab-reared, known-age Arctic Cod from the Hatfield Marine Science Center in Newport, Oregon, as reference specimens (Benjamin Laurel, NOAA Alaska Fisheries Science Center, pers. comm.; Figure 1.2 C). The otoliths of lab-reared Arctic Cod were aged to confirm the reader's ability to identify and enumerate daily rings. Using the known age and hatch date of the fish, the location of the hatch mark was then identified and confirmed to further improve age determinations. This method of hatch mark identification is similar to other studies that determined the hatch mark by examining otoliths shortly after the fish hatched (Eckmann and Rey 1987).

To ensure accuracy of otolith aging, all otoliths were aged at least twice and a third time if the first two ages were not within a 5% coefficient of variation (CV). The second and third ages were done on different dates from the previous read to minimize potential bias. If the first two ages fell within a 5% CV of each other, the second age was used. In the event the first two ages had a greater than 5% CV the otoliths was aged a third time and the final age was used if it was within 5% of either the first or second age. If the third read had a CV greater than 5% the ages were not used for analysis. This occurred on otoliths that became damaged from over-polishing. Although there are other methods for validating age determinations, the CV is statistically more rigorous and flexible (Chang, 1982; Campana 2001). A subsample of the aged otoliths (n = 15) was examined by a second otolith aging expert to confirm that the images, measurements and ages had no errors.

Growth increments in the center of some otoliths were unreadable because too much material had been removed during polishing. Therefore, the saved otolith was used for aging, but for some of the larger fish (20—54mm), the center again became washed out. In these cases, daily ages were counted using the otolith with the most amount of visible increments starting at the first readable growth increment. The number of increments that were unreadable was estimated based on a regression approach using completely aged otoliths from the same region (Appendix B).

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### 1.3.4    Length-frequency distributions

Length-frequency distributions for the entire sample of captured larval or juvenile Arctic Cod were visually examined by season and region, and separately for pelagic and demersal sampling gear. In addition, the mean lengths of demersal and pelagic larvae and juveniles were plotted for each station to visualize spatial patterns in mean size. These distributions, combined with age-at-length regressions for a given region, provided the basis for determining the hatch date distribution.

### 1.3.5    Hatch date estimation

To estimate the distribution of hatch dates for Arctic Cod in each group we first estimated age-at-length relationships and their uncertainty for a subsample of fish using linear regressions. The resulting relationships were then used to convert all observed lengths in a group to estimated ages. Finally, the estimated ages were subtracted from the dates of capture to obtain an estimated hatch-date distribution. We assumed a linear relationship between age and length based on a previous study of larval Arctic Cod (Bouchard and Fortier, 2011) and visual examinations of age-at-length. Therefore, counts of daily growth increments (hereafter 'age') within each season were modeled as a linear function of length and region with an interaction term to allow for possible differences in age-at-length by region($r$):

Eq. 1 $$age_{r,i} = \alpha_r + \beta_r \cdot length_{r,i} + \varepsilon_{r,i} \quad \varepsilon_{r,i} \sim N(0, \sigma_\varepsilon^2)$$

where $age_{r,i}$ and $length_{r,i}$ are the estimated age and length of the $i$[th] specimen in region $r$, $\alpha_r$ and $\beta_r$ are the intercept and slope for region r, and the $\varepsilon_{r,i}$ are residuals that are assumed to be normally distributed with mean 0 and variance $\sigma_\varepsilon^2$. Region-specific coefficients ($\alpha_r, \beta_r$) were only estimated if the interaction term was significant ($p < 0.05$), otherwise a single regression line was estimated across the sampled regions ($\alpha, \beta$). Preliminary analyses indicated that the standard deviation in estimated ages increased linearly with the predicted mean ages, therefore a variance structure accounting for this mean-variance relationship was incorporated into the models. All models were fit using a weighted least squares approach as implemented in the 'nlme' package in R (Pinheiro et al., 2020). Residual diagnostics did not suggest any violations on the linearity or normality assumptions.

To estimate age distributions within each region, we used the best age-at-length model for each region to predict ages from observed lengths. Age data were not available for the WBS so the age-at-length model for the NCS was applied to this region because it is contiguous with and immediately downstream of the NCS. Juveniles from the NCS are likely advected into the western Beaufort Sea

(Levine et al. 2021). To appropriately reflect variability in age-at-length arising from individual variations in growth, we randomly simulated up to 10 ages for each observed length based on the estimated mean age and its standard deviation at a given length. Occasionally, the age of a simulated fish exceeded one year due to the large estimated variance in the age of larger fish; those fish were removed from the simulated age distribution. The number of simulated ages per measured individual was chosen to generate at least 10,000 ages for obtaining a smooth age distribution for plotting and this choice did not affect results. Simulated ages were subtracted from the corresponding capture dates to obtain estimated hatch dates for further analyses of hatch date distributions. Regression models to predict ages from lengths for the SCS were fit separately to data from spring and summer surveys due to large differences in the observed length ranges of fish between spring and summer. The SCS was the only region where data from both seasons were available.

### 1.3.6  Hatch date comparisons

The estimated hatch date distributions were compared among groups using density plots. To statistically compare these distributions among groups, we calculated the mean hatch dates for each group of Arctic Cod and used a bootstrap approach to construct confidence intervals for the means. Bootstrap samples were generated for each group by randomly re-sampling with replacement both the observed length-frequencies for a given group and the age-length samples used for estimating age-at-length for that group. For each set of bootstrap samples, a hatch date distribution was simulated following the same series of steps used in estimating the hatch date distribution from the original samples and the mean hatch date of the simulated distribution was calculated. This was repeated for each of 10,000 sets of bootstrap samples to construct 95% confidence intervals for the mean hatch date of each group, as well as for pairwise differences between groups. If the confidence interval for a pairwise difference did not include zero, mean hatch dates between groups were considered statistically different. In addition, p-values for all pairwise comparisons were computed based on the proportions of simulated differences that were less than and larger than zero. The smaller of these proportions was multiplied by 2 for a two-sided test of the null hypothesis that the difference is zero. The proportion was multiplied by two for a two-sided test because we did not specify *a priori* hypotheses about which groups had earlier or later hatch dates.

Initial comparisons among regions showed no difference in mean hatch dates between Arctic Cod captured in the most western portion of the Beaufort Sea and the Northern Chukchi Sea from the 2017 Arctic IES survey. This was true for both the pelagic and demersal captured fish with p-values of

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0.647 and 0.952 respectively. Along with the lack of significant difference, there were relatively few samples from this survey collected from the western Beaufort Sea (n = 68 pelagic and 77 demersal Arctic Cod), therefore these two regions were pooled and will be referred to as NCS hereafter. The similarity between the two regions was not surprising given their close proximity and oceanographic connectivity, and the assumption that most northern Chukchi and western Beaufort Sea Arctic Cod are advected into those regions from southern hatching locations (Levine et al., 2021).

In the Chukchi Sea during summer, data for both pelagic and demersal Arctic Cod were available from two overlapping surveys and were analyzed separately. Mean hatch dates for pelagic Arctic Cod collected during the transect-based AMBON survey (IKMT) and those collected during the grid-based Arctic IES II survey (Marinovich) (Table 1.1) were quantified separately as the surveys covered different areas. For comparing hatch dates of fish among regions, we present results for Arctic IES II samples collected in the SCS and NCS using the Marinovich trawl (pelagic fish) or bottom trawl (demersal fish) because the Arctic IES II survey sampled a systematic grid and covered a larger geographical area within each region, providing more representative length-frequency distributions.

### 1.3.7    Growth rates

To obtain in situ estimates of age-0 growth rates for Arctic Cod we fit linear regressions of length on age by region and season. A simple linear regression of length as a function of age was used, where the slope (mm $d^{-1}$) represents the estimated average growth rate of the sampled population in a given region and season. Growth rates were estimated separately by region to account for differences in temperature, prey availability among regions, and genetic differences among stocks, all of which can affect the rate of growth (Laurel et al., 2015; Helser et al., 2017; Laurel et al., 2018). Growth rates were also estimated by season to account for potential differences between the growth of early larval and juvenile stages and the apparent growth of the surviving age-0 fish sampled later in the summer, whose average growth may differ due to size selective mortality or seasonal changes in temperature.

### 1.4    Results

### 1.4.1    Otolith-based ages

A total of 181 Arctic Cod otoliths were examined, with ages ranging from 10 to 161 days for Arctic Cod sampled in the spring, and from 55 to 308 days for those collected during summer (Table 1.2). The age range was greater for samples from the Chukchi Sea (55—308 days) than the Beaufort Sea (76—241 days), which may in part be due to smaller sample sizes in the Beaufort Sea (Table 1.2). The mean

CV between first and second age assessments was 0.02 (range from 0.00 to 0.05), indicating age estimates were accurate.

### 1.4.2 Length-frequency distributions

Length-frequency distributions of age-0 Arctic Cod differed by region, season, and depth of capture. Arctic Cod captured in the spring were on average 80.5% smaller than those captured in the summer (Table 1.1). In the SCS, where Arctic Cod were sampled in both seasons, pelagic spring-caught fish had a mean length of 8 mm (range: 5 — 17 mm) whereas those caught in summer had a mean length of 52 mm (range: 28 — 74 mm).

Within seasons, differences in length frequency distribution were observed among regions (Figure 1.3). The WBS had the largest mean length of age-0 Arctic Cod followed closely by the SCS (Table 1.1). Within regions, demersal-caught fish were 0% to 36% larger than fish caught in pelagic nets (Table 1.1) We also observed differences in mean length among surveys within the same regions sampled during the summer of 2017. Specifically, pelagic fish were 29% to 31% larger in the Arctic IES II survey than the AMBON survey. The surveys overlapped spatially, but the Arctic IES II survey took place about one month later (Table 1.1), which likely accounts for the larger mean sizes. Spatial patterns in mean length across the study region suggest a gradient from larger fish in the south to smaller fish in the north during both spring and summer in the Chukchi Sea (Figure 1.4). During summer, the smallest average length of fish was observed in the EBS, whereas WBS fish had an average length similar to the NCS fish. These spatial differences are confounded with differences in the timing of sampling as the SCS and WBS were sampled later in the year than the NCS and EBS (Table 1.1). The number of Arctic Cod caught varied across regions and seasons from 19 cod in the NBS during spring to >4,000 in the NCS during summer, reflecting differences in average catch-per-unit-effort and differences in the number of stations sampled (Table 1.1).

### 1.4.3 Age-at-length regressions

The best age-at-length model included a significant interaction between length and region for spring-caught samples (F = 12.76, p = 0.001, $R^2$ = 0.909), indicating that slopes differed between the NBS ($\beta_{NBS}$ = 8.210, se = 0.561) and SCS ($\beta_{SCS}$ = 5.485, se = 0.538; Table 1.3, Figure 1.5A). In contrast, there was no interaction between length and region in the summer (F=0.45, p = 0.715), indicating that age increased at the same rate with length across sampling regions ($\beta$ = 4.09, se = 0.24, $R^2$ = 0.696; Table 1.3, Figure 1.5B). However, intercepts differed among regions (F = 4.837, p = 0.009) and fish at a given length

were on average 19 days older in the NCS compared to the EBS and 20.5 days older in the SCS than in the NCS (Table 1.3).

### 1.4.4    Hatch timing

The estimated distribution of hatch dates differed between Arctic Cod sampled in the spring and in the summer, between Arctic Cod from different regions and between pelagic and demersal age-0 Arctic Cod. Within the SCS, spring-captured pelagic fish had a mean hatch date that was 131 days later than summer-captured pelagic fish (Table 1.4; Figure 1.6). Arctic Cod hatch dates of summer-caught fish differed significantly among regions (p<0.05) except for pelagic fish caught in the NCS and WBS (p=0.957, ~1-day difference) and demersal fish from the WBS and EBS (p=0.059, 12-day difference; Table 1.5; Figure 1.6). Demersal SCS fish sampled during the summer had the earliest mean hatch date (December 27) and pelagic EBS fish had the latest mean hatch date (March 10, Table 1.4). Summer-caught pelagic fish in the SCS hatched on average 14 days earlier than those in the NCS, 26 days earlier than those in the WBS, and 51 days earlier than EBS pelagic fish (Table 1.5). Within each region, pelagic Arctic Cod had mean hatch dates significantly later (p<0.05) in the year than demersal Arctic Cod, except in the Chukchi Sea where SCS and NCS demersal and pelagic fish did not differ significantly in mean hatch dates. The NCS was the only region where demersal fish hatched later than pelagic fish with a mean hatch date of nine days later (Figure 1.6).

### 1.4.5    Growth rates

Estimated growth rates differed among regions (Table 1.2). Growth rates of spring-caught Arctic Cod larvae were 37% slower in the NBS (0.115 mm d$^{-1}$) than in the SCS (0.183 mm d$^{-1}$) (Table 1.2; Figure 1.7A). Growth rates of Arctic Cod captured in the summer season similarly differed significantly among regions (F=3.14, p=0.047), ranging from 0.097 mm d$^{-1}$ in the EBS to 0.200 mm d$^{-1}$ in the SCS (Table 1.2; Figure 1.7B).

### 1.5    Discussion

Arctic Cod from all regions hatched over a protracted period between November and June, with peak hatch dates ranging widely from February through May, depending on the season, region, and vertical location of capture. This variability among groups and locations likely reflects gradients in the timing of sea ice retreat, stock-specific differences, and different transport pathways from hatching to sampling locations due to ocean currents.

Estimated hatch dates ranged from as early as September through August of the following year. This protracted hatching pattern is consistent with previous studies (Bouchard and Fortier, 2011) and maximizes the chances that a least some offspring will hatch during favorable conditions. This bet-hedging strategy helps mitigate against annual variability in environmental conditions (Shama, 2015), which at high latitudes can impact the timing and magnitude of the spring algal bloom and thereby the timing and abundance of prey for Arctic Cod (LeBlanc et al., 2020). Because of these highly variable conditions, a bet-hedging strategy can impart some resilience to Arctic Cod in a changing environment, as long as some portion of a given year class encounters conditions within an acceptable range. The protracted hatching period can be a result of differences in incubation time or a difference in spawn timing among the regions. The large sizes (>50 mm) of some fish in our samples produced hatch dates that indicate spawning occurred outside of published literature dates (November—March) and prior to ice formation. At least two factors may have contributed to these results. First, early hatch dates could be an artifact of simulating ages based on lengths that exceeded the maximum length in our aging samples. The variance with the age-at-length model increased at a given length which could have produced unrealistic ages when extrapolating beyond the maximum length of the age-at-length relationship. The length range used to estimate the age-at-length relationship was limited due to the challenges associated with aging larger larvae. Second, some of the Arctic Cod in our length samples may have been age-1 fish. The oldest estimated ages and earliest mean hatch dates were associated with fish sampled in the SCS for both demersal and pelagic samples. Within this region, length varied considerably and fish in demersal samples may have included some age-1 individuals. Previous studies have reported age-1 Arctic Cod below the cutoff lengths used for this study (Norcross et al., 2017). Both the presence of age-1 fish and unrealistically large variances for the age of larger juveniles are likely causes of the seemingly unrealistic early hatch dates that we estimated. However, male Arctic Cod have been observed to have fully developed gonads by January and February (Nahrgang et al., 2015) and could potentially have spawned outside of the published literature dates.

### 1.5.1 Seasonal differences in hatch dates

Arctic Cod captured during the spring and summer had different mean hatch dates, with the spring-caught fish hatching later in the year. This was unexpected and suggests that spring-caught and summer-caught fish in the same region originated from two separate hatch events. Two separate hatch patterns among regions have been documented, specifically a short hatching event associated with ice break up and a protracted event extending from January to July (Bouchard and Fortier, 2008; LeBlanc et

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al., 2020). The protracted dates were associated with regions that do not experience significant freshwater influence similar to the Chukchi Sea and western Beaufort Sea (Bouchard and Fortier, 2008). Larval fish captured in the SCS in June had a mean hatch date in mid-May, suggesting that they originated relatively close to their sampling locations. Within the SCS, Kotzebue Sound has been hypothesized to be a hatching location for Arctic Cod due to the large number of larval fish captured in the outer Sound during the 2017 Arctic IES survey (Deary et al., 2021). In contrast, the summer-captured pelagic fish in the SCS had a mean hatch date of mid-February, ranging from January to June. The age of the summer SCS fish indicates that they were likely advected from southern regions as suggested by biophysical transport models (Deary et al., 2021; Vestfals et al., 2021).

Arctic Cod hatching locations in the Pacific Arctic are largely unknown, but they are believed to occur in several areas in the Bering Strait region, including the waters south of St. Lawrence Island, the Gulf of Anadyr, Kotzebue Sound, and areas along the Russian coast both south and north of the Bering Strait (A. Whiting, Native Village of Kotzebue, personal communication; Craig et al., 1982; Christiansen and Fevolden, 2000; Kono et al., 2016). In 2017, sea ice melt in the NBS began during late April and was complete by late May and 99% of estimated hatch dates occurred prior to May 25 with the mean hatch date occurring on April 6th. This suggests that Arctic cod in the NBS hatched prior to and during sea ice retreat. Spring-captured fish in the SCS had the latest mean hatch dates of all the regions in this study, possibly reflecting later ice melt in their hatching region. Two hatching sites have been proposed within the SCS; Kotzebue Sound in Alaska and the region along the Chukotka Peninsula in Russia (Deary et al., 2021). Both of these locations had later sea ice recession than the proposed hatching locations in the NBS. The delayed sea ice recession could explain why the spring SCS fish had a later mean hatch date than those caught in the NBS.

The length distribution of Arctic Cod sampled in spring 2017 was similar to those of collected during 2008 and 2013 from the NBS and SCS regions (Kono et al., 2016). In all three years, lower numbers of larger Arctic Cod were observed in the NBS compared to high abundances of smaller fish in the SCS. For example, the mean catch per unit effort in 2017 was 73 times higher in the SCS than in the NBS (this study). Although Kono et al. (2016) did not estimate hatch dates, the observed differences in size and relative abundance of Arctic Cod between the two regions suggest that earlier ice retreat, coupled with warmer waters in the NBS, is associated with earlier hatching (Kono et al., 2016). The earlier hatching larvae experienced a longer period where natural mortality can occur, which can explain their lower abundances in the NBS. Alternatively, larvae may have been advected out of the region prior to sampling.

In contrast to spring-captured fish, the mean simulated hatch date for all summer-captured fish occurred 87 days earlier, which suggests that they originated from a different hatching population than those caught in the spring. Summer-caught fish in the SCS displayed a wide range of hatch dates from January to May (Figure 1.8). The protracted range of hatch dates in the SCS may be an indication of multiple spawning events occurring at different times in different areas of the Bering and Chukchi sea. By the time of summer sampling, fish from multiple hatching events may have been advected into the Chukchi Sea, explaining the wide range of hatch dates observed. The earlier hatch dates of summer-captured fish indicate that they likely hatched south of Bering Strait when sea ice was still present in the region, before being advected with the prevailing northward currents through the Bering Strait into the Chukchi Sea (Berline et al., 2008; Vestfals et al., 2021). This is supported by the prevailing currents in the region and by simulations with an individual based particle tracking model, which suggests that age-0 Arctic Cod sampled in the northeast Chukchi Sea during 2012 and 2013 likely originated south of Bering Strait (Vestfals et al., 2021). This model also suggests that age-0 Arctic Cod in the Chukchi Sea must have originated in more southern, warmer waters to grow to the sizes observed during the summer surveys.

Alternatively, spawning could have occurred at similar times in the same region, but differences in development rates driven by environmental influences could have contributed to differences in hatch dates. For example, earlier hatching has been hypothesized to be associated with areas that receive an influx of fresh water such as the Mackenzie River (Bouchard and Fortier, 2008). This could warm the area enough to accelerate egg development and larval growth under the ice, giving juvenile Arctic Cod a physiological advantage over juveniles in colder waters because their increased size likely leads to increased feeding success and predator avoidance (Bouchard and Fortier, 2011; Laurel et al., 2015; Kent et al., 2016).

Another possibility for Chukchi Sea Arctic Cod is that spawning and hatching occurs in association with northern polynyas, such as the recurring polynyas in the eastern Chukchi Sea between Cape Lisburne and Icy Cape, AK. However, this polynya is characterized by high salinity and low but stable temperatures, despite reduced sea ice (Ladd et al., 2016). Thus, temperature conditions do not support the accelerated growth that would be necessary to achieve the observed sizes of summer caught larvae in the NCS. This contrasts with polynyas in the Beaufort Sea, which provide more favorable conditions for EBS Arctic Cod because of warmer temperatures (Bouchard and Fortier, 2011). Moreover, simulations suggest that larvae hatched in the northeast Chukchi Sea would be advected out of the region (Vestfals et al., 2021). Therefore, we conclude that the observed hatch date distributions in the

NBS and Chukchi Sea are most consistent with spawning and hatching occurring in the Bering Strait region or south of Bering Strait, with perhaps some contributions from Kotzebue Sound or other coastal areas in the Chukchi Sea. After hatching, larvae are advected northward and may be retained for extended periods over the northeast Chukchi Sea shelf due to wind and flow patterns that favor retention in the summer, before being advected northward off the shelf (Levine et al., 2021).

Bias could have been introduced since multiple gear types with different size selectivity were used to sample fish he bongo net may not have adequately sampled larger larvae in the spring (Shima and Bailey, 1994) and the Marinovich and IKMT may have excluded small larvae in the summer (De Robertis et al., 2017). This could have generated a bias towards later hatch dates in the spring because the bongo retains larvae that are smaller and younger on average than those in the water column. In contrast, summer samples could have been biased towards earlier hatch dates because the midwater trawls target larger and older larvae. Although these differences may partially explain the difference in hatch dates between the spring-and summer-caught Arctic Cod, they were unlikely to account for the large difference in mean hatch dates. Although the Bongo may have selected against larger larvae, larvae over 20 mm were absent from IKMT hauls during ASGARD. The similar size composition between the IKMT and bongo tows supports our assumption that the bongo tows provided an adequate representation of the larval Arctic Cod present in the region during the spring.

In addition to gear selectivity, natural mortality can also be size selective as smaller fish are typically more likely to be preyed upon then larger ones (Houde, 1987). Natural mortality of larval Arctic Cod is likely great (Marsh et al., 2020a) and size dependent. Feeding success and survival typically increase with size, thus faster growing larvae tend to have greater survival rates and may be overrepresented in the summer samples (Pepin et al., 2015). The selection against smaller Arctic Cod due to both natural mortality and gear selectivity may have caused our hatch date estimates to be biased towards earlier hatching, as well higher growth rate estimates for summer-captured fish.

Seasonal differences in hatch dates may have been further impacted by biased length measurements of preserved larvae. Two different methods for storing samples: summer-caught samples were frozen, whereas most spring-caught samples were preserved in 95% ethanol. Larval fish stored in ethanol have been shown to decrease in length over time. To our knowledge, the effect of ethanol on Arctic Cod has not been studied, but up to 1.8% reduction in length was observed in a similar gadid, Walleye Pollock, after preservation in 95% ethanol (Buchheister and Wilson, 2005). To assess the potential bias associated with using preserved fish for aging, we applied the upper shrinkage value

estimated for Walleye Pollock (5.7%) to correct the lengths of the spring-caught SCS samples and re-calculated the corresponding age-at-length relationship. Using the corrected relationship to convert field-measured lengths to ages resulted in younger ages at length and in mean hatch dates that were four days later than those based on uncorrected lengths. Because of the uncertain magnitude of the shrinkage and because overall conclusions were not affected, we presented the uncorrected results.

1.5.2    Regional differences in hatch dates

Differences in hatch dates among regions may be partially explained by the timing of sea ice recession, as well as other oceanographic differences among regions. Although sea ice formation may affect spawn times (Craig et al., 1982), we focus on the timing of hatch, which is influenced by ice coverage, water temperature (Kent et al., 2016), and possibly genetic differences. Sea ice retreat generally moves in a northerly direction, which is consistent with fish from the southern regions hatching earlier in the season relative to their counterparts in the North. Both pelagic and demersal age-0 Arctic Cod captured during summer had significantly earlier mean hatch dates in the SCS than in the NCS, possibly indicating that they originated from different spawning populations, although there was considerable overlap in hatch date distributions between the two regions. Similarly, pelagic and demersal age-0 fish in the WBS on average hatched earlier compared to those from the EBS. It is important to note that while the differences were significant, each region in the Beaufort Sea was sampled during different years, so these differences could also be the result of interannual variability.

Similar hatch dates for pelagic captured fish in the NCS and WBS are consistent with oceanographic connections between the two regions. The NCS and WBS are connected via the Alaska Coastal Current, which flows along the coast of Alaska from the Gulf of Alaska to the Beaufort Sea (Pickart et al., 2005) and has been hypothesized to transport larval and juvenile Arctic Cod from southern hatching locations into the WBS (Forster et al., 2020; Levine et al., 2021). Arctic Cod in the NCS and WBS appeared to be distinct from those in the EBS based on a gap in the spatial distribution of age-0 fish (Forster et al., 2020), genetic differences (Wilson et al., 2017, 2019; Nelson et al., 2020), and different elemental compositions of age-0 otoliths (Chapter 2; Frothingham et al., 2020). The differences in hatch dates observed here provide additional evidence that juvenile Arctic Cod in the WBS and EBS originate from two separate spawning populations.

1.5.3    Differences in hatch dates between demersal and pelagic juveniles

Hatch date distributions were different between demersal and pelagic age-0 Arctic Cod in most regions (Figure 1.8). Demersal fish were generally older than pelagic fish, supporting previous

observations that juveniles descend to the bottom in late summer and throughout the fall as they grow (Geoffroy et al., 2016). Differences in size and inferred hatch date distributions between pelagic and demersal fish are consistent with the general ontogenetic movements of age-0 fish from the surface into deeper waters and settlement to the bottom (Houde et al., 2002; Geoffroy et al., 2016). Arctic Cod in the Chukchi Sea migrate out of the epipelagic layer and into deeper waters when they reach lengths > 30 mm (Levine et al., 2021). In the Beaufort Sea, they to descend to depths >100 m and are completely out of the epipelagic zone by October (Geoffroy et al., 2011; Bouchard et al., 2015). Differences in hatch dates between demersal and pelagic fish were more pronounced in the Beaufort Sea, where the average station depth was 283 m deeper than in the Chukchi Sea where maximum sampling depths were 200 m for the IKMT and 1,000 m for bottom trawls. Deeper stations in the Beaufort Sea allowed for greater stratification of size classes between demersal and mid-water habitats. By contrast, in the NCS region the difference between mean hatch dates between pelagic and demersal fish were much smaller as demersal captured fish had mean hatch date slightly later than pelagic captured fish. This is likely due to the shallow depth (< 50 m) of the Chukchi Sea shelf, which is less than the depth ranges over which age-0 Arctic Cod are distributed in late summer in the Beaufort Sea (Geoffroy et al., 2016). Thus, daily vertical migrations of juvenile Arctic Cod on the Chukchi shelf are likely to extend to the bottom, limiting the vertical separation by size class.

1.5.4    Interannual variability in hatch dates

Samples in the EBS were collected over two years in 2013 and 2014, allowing for comparisons of hatch dates and growth rates among years as well as with previous studies. Previous estimates are available from Bouchard and Fortier (2011), who sampled pelagic age-0 Arctic Cod in 2005 and 2006 approximately 285 km east of our EBS sampling region and from Gallaway et al. (2017) who sampled the WBS and the EBS in 2011. These comparisons suggest some notable differences that may be due to annual differences in sea ice conditions, sea surface temperatures (SST), or salinity, all of which have been hypothesized to play a large role in Arctic Cod early life history (Doroshev and Arnovich, 1974; Graham and Hop, 1995; Geoffroy et al., 2011).  Peak hatching in 2011 occurred in late April (Gallaway et al., 2017), similar to 2005. The range of hatch dates was also similar among the four years, beginning in mid- to late-December and extending through mid-July. There were, however, some differences among years in the peak hatch dates. Specifically, the hatch date distributions in 2005 and 2006 were bimodal with one peak occurring in early April and the other in mid-May (Figure 1.7 in Bouchard and Fortier, 2011). These peaks were more pronounced in 2005, whereas the 2006 hatch dates were more broadly

distributed around these peak dates. In contrast, the hatch date distribution in 2013 showed a single, broad peak in late April (Figure 1.8), similar to 2011 (Gallaway et al., 2017). Differences in hatch timing could be due to interannual differences in environmental conditions such as sea ice coverage and freshwater influences. Mean hatch dates of samples from 2006 and 2013 were similar, differing by only one day, possibly because timing of sea ice retreat (Figure 1.9) was similar during those years.

Alternatively, hatch timing in the EBS may be explained by differences in freshwater discharge. The mean Mackenzie River discharge rate in March which coincides with the incubation period just prior to hatching was 25% higher in 2006 (4938 $m^3 s^{-1}$) than in 2005 (3672 $m^3 s^{-1}$; extracted from https://wateroffice.ec.gc.ca/mainmenu/historical_data_index_e.html on 10/1/2020), and was associated with earlier hatching in 2006. This is consistent with the hypothesis that increased freshwater discharge is associated with earlier hatching due to accelerated egg development in a freshwater lens that has slightly elevated temperatures compared to the surrounding seawater (Bouchard and Fortier, 2011). Thus, differences in freshwater discharge may be more important than the timing of sea ice retreat for determining the timing of hatching of Arctic Cod in areas near major river systems, whereas the timing of sea ice retreat may determine hatch timing in areas without strong freshwater influences such as the Chukchi Sea.

Mean hatch dates for demersal Arctic Cod in 2013 were 26 days earlier than those from 2014. These differences are consistent with the freshwater discharge hypothesis as the Mackenzie River discharge was considerably higher in 2014 (4655 $m^3 s^{-1}$) compared to 2013 (4022 $m^3 s^{-1}$). These differences were also consistent with the hypothesis that hatch timing is determined by the timing of sea ice retreat. In 2013, sea ice began to decrease in early June and some ice was still present in mid-August in the EBS, whereas ice began to recede in early May and was completely gone by the end of June in 2014 (Figure 1.9). Therefore, the observed differences in hatch timing between 2013 and 2014 could be explained by either differences in freshwater discharge, differences in the timing of ice retreat or both.

### 1.5.5 Arctic Cod growth rates

Region differences in growth rates may reflect differences in temperatures, food availability, or genetic composition. Arctic Cod growth rates range from 0.18 to 0.54 mm $d^{-1}$ for field-based estimates (Bouchard and Fortier, 2011; Deary et al., 2021; Levine et al., 2021; Vestfals et al., 2019), whereas laboratory estimates are generally lower, ranging from 0.11 to 0.19 mm $d^{-1}$ (Laurel et al., 2021; Koenker et al., 2018). The growth rates from our study fall within the range reported by Bouchard and Fortier

(2011) for the Beaufort Sea, with the exception of the spring NBS and summer EBS samples, which were lower (Table 1.2). Our low estimate for the EBS could reflect interannual differences but may also be due to the higher uncertainty in our length-age regression for the EBS ($R^2$ = 0.397) relative to other regions (Table 1.2) and to those reported by Bouchard and Fortier (2011).

Growth rates are positively correlated with SST across multiple Arctic seas (Bouchard and Fortier, 2011) and although we observed a similar correlation, there was an exception. Spring-caught fish in the NBS had lower growth rates than those in the SCS, despite being caught in warmer waters. The mean temperature across stations sampled for aging in the NBS was 5.57°C whereas the SCS had a mean temperature of 4.35°C across sampling stations. Slower growth in the NBS could be due to the temperature differences at capture not being representative of the average temperatures experienced by larvae since hatching. The differences in growth could also be an artifact of genetic differences between the stocks as well as differences in food availability (Koenker et al., 2018).

Estimates of growth rate of Arctic Cod could be affected by methodological differences. These estimates can be affected by size-selective mortality, gear selectivity, and sampling design. Size-selective mortality favors faster growing individuals; thus in situ growth rates based on the survivors may overestimate growth rate for a population (Bailey and Houde, 1989; Litvak and Leggett, 1992) and could have contributed to the higher estimated growth rates for summer-captured larvae in the SCS. While gear selectivity may also bias growth rate estimates by selecting against larger fish, the lack of Arctic Cod captured in the IKMT during the spring indicate larger fish were not present. In contrast, pelagic Arctic cod in the summer were sampled with a Marinovich trawl that may have selected against smaller larvae due to its large mesh size. These gear-related biases could have contributed to the apparent differences in growth rates between the spring and summer-caught Arctic Cod in this study.

Additionally, the sampling design can result in biased growth rate estimates. Many studies (e.g., Deary et al., 2021; Levine et al., 2021 for Arctic Cod) have estimated growth rates based on increases in mean length between successive surveys, making the strong assumption that the same population was sampled across surveys. This assumption may be justified if estimates are made over a relatively short time period (as in Levine et al., 2021), but are more difficult to justify when sampling is separated by several months in a highly advective environment (as in Deary et al., 2021). Our approach is based on data from single surveys and requires age and length samples that are representative of the population of interest. Samples from two different surveys in the SCS and NCS in 2017 (AMBON and Arctic IES II) had similar lengths and hatch dates, despite differences in sampling locations and gear type (Marinovich

vs. IKMT). Age samples were collected over a wide range of stations to minimize geographical biases but the extent to which they were representative of the populations in the study region is unclear as the population structure and the spatial distribution of different populations is largely unknown.

1.5.6    Arctic Cod early life history dynamics in a changing climate

Water temperatures in the Arctic are expected to increase 1.5°C by 2100 under current carbon emissions scenarios (Collins et al., 2013), which may impact Arctic Cod as well as the entire Arctic ecosystem. Warming conditions are expected to initially result in increased growth rates for juvenile Arctic Cod; however, once temperatures exceed 7.3°C, growth rates will probably begin to decrease (Laurel et al., 2015). Arctic Cod have a growth rate advantage over other gadid species at colder temperatures (< 5°C), but other gadids grow faster and have a higher thermal tolerance at warmer temperatures which may provide a competitive advantage. Arctic cod eggs will similarly be disadvantaged in a warming climate as they presumably depend on sea ice cover and experience high mortality and other developmental issues at temperatures above 3.5°C (Kent et al., 2016). If the abundance of Arctic Cod declines in a warming ocean, many predators will have to feed on other species that are not as lipid-rich as Arctic Cod.

In addition to changes in juvenile and larval growth rates, continued warming will affect other aspects of Arctic Cod early life history. Water temperature impacts the duration of the egg incubation period for Arctic Cod, which decreases from an average of 79 days at -1.5°C to as little as 29 days at 3.5°C (Aronovich et al., 1975; Kent et al., 2016; Laurel et al., 2018). Changes in incubation time can result in emergence and yolk sac depletion not coinciding with prey abundance. Decreased incubation time at warmer temperatures also results in decreased length at the time of hatch (Laurel et al., 2018), which limits the size of prey they can ingest and compromises their ability to avoid predation (Cowan et al., 1996). The impact of climate change on Arctic Cod is likely to be more severe at the southern limit of their range in the Bering Sea than in the Chukchi Sea as the former is warmer on average and has experienced more pronounced temperature anomalies in the fall (1.2°C) compared to the Chukchi Sea (0.7°C) (Danielson et al., 2020). Our results, combined with laboratory studies (Aronovich et al., 1975; Kent et al., 2016; Laurel et al., 2018) on size at hatch, indicate that as the Arctic continues to warm, Arctic Cod will hatch earlier and at a smaller size. These results on Arctic Cod provide a benchmark for Arctic Cod emergence and can be compared to future studies to understand how warming ocean conditions impact hatch dates and how changes in hatch dates impact their survival and recruitment to the spawning population.

### 1.5.7 Management considerations

Arctic Cod are recognized as a critically important forage species in the Arctic and in the US are managed and protected under the Arctic Fishery Management Plan (FMP) (NPFMC, 2009). The FMP requires periodic mapping of essential fish habitat, including potential spawning areas and nursery grounds that were largely unknown at the time the Arctic FMP was written (NPFMC, 2009). After more than a decade of research on Arctic Cod in the Pacific Arctic, much has been learned (Mueter et al., 2020), but direct observations of spawning or eggs are still lacking. Our hatch date estimates can provide essential information for biophysical transport models to refine estimates of likely hatching locations (Deary et al., 2021; Vestfals et al., 2021). More broadly, this study increases our understanding of the population dynamics of Arctic Cod to help predict how their life history and abundance will change under changing Arctic conditions. Important subsistence resources such as ringed seals and beluga whales rely on Arctic Cod as a lipid dense food source and changes in Arctic Cod distribution and abundance will impact the Indigenous people that depend on them (Magdanz et al., 2010; Crawford et al., 2015).

As Arctic waters become more accessible, oil exploration is likely to increase and a better understanding of spawning activity can inform measures to protect Arctic Cod and mitigate potential impacts from oil development. Larval Arctic Cod exposed to oil for only one hour have a greatly reduced chance of survival to age 1 and mortality would increase if physical or chemical dispersal methods were used to clean up an oil spill (Gallaway et al., 2017; Word et al., 2011). Artic cod eggs may be even more susceptible to oil contamination because their buoyancy increases the chance of interaction with a spill (Laurel et al., 2019). When exposed to low concentrations of crude oil during the larval growth period of Arctic Cod it can disrupt the normal development of the jaw and heart, as well as change lipid metabolism (Laurel et al., 2019, Bender et al., 2021). This can degrade the health of Arctic Cod, increase mortality rates, and ultimately impact predators that rely on them. The impacts of an oil spill will be further exacerbated when it occurs in warmer waters (Bender et al., 2021).

Bioaccumulation after oil exposure is also a concern as Arctic Cod are a key component of the food web. Indirect exposure to oil thru the consumption of contaminated prey such as *Calanus* spp. can lead to bioaccumulation within Arctic Cod (Agersted et al., 2018), as well as in the predators that consume them – many of which are important subsistence resources. Models used to predict the impact of oil spills on the Arctic ecosystem focus on how larval and juvenile Arctic Cod are affected because of their importance to the arctic ecosystem (Word et al., 2014). By predicting how oil spills will impact the

recruitment of adult females to the spawning population they can predict the ecological impact by extrapolating the loss of critical prey to the Arctic food web. Thus, Arctic Cod can serve as a key indicator for the potential ecological impacts from anthropogenic disturbances.

Transport ships are also known to negatively impact of Arctic Cod. Noise pollution created by vessel traffic can cause Arctic Cod to move away from vessels and can alter their behavior (Ivanova et al., 2020). The presence of moored and moving vessels caused Arctic Cod to spend more time moving away from the source of the noise and less time searching for food. During the summer, when food is most abundant, the trade off in energetics from feeding to fleeing can have a negative impact on Arctic Cod achieving optimal size prior to the winter season (Ivanova et al., 2020). It is important that management strategies account for potential anthropogenic impacts on Arctic Cod, as well as to their essential habitat, to preserve Arctic ecosystem functions.

1.6     Conclusions

The wide range of hatch dates estimated here provides strong evidence that Arctic Cod use a bet-hedging strategy that distributes offspring over a wide range of environmental conditions by spawning over a protracted time period across multiple locations in the Pacific Arctic. Our results align with previous findings and indicate that regional and interannual variation in hatch dates and growth rates are associated with the timing of sea ice retreat and freshwater discharge, highlighting the sensitivity of Arctic Cod to changing environmental conditions in the Pacific Arctic. Earlier sea ice retreat and increased freshwater discharge under climate warming suggest that Arctic Cod will hatch earlier in the future, with unknown consequences for their early growth and survival. Any impacts of climate change on Arctic Cod have the potential to negatively affect upper trophic level species that rely on them or their consumers as a resource, including humans. Regional and seasonal differences in Arctic Cod hatch dates documented here provide evidence for the existence of multiple spawning populations in the Pacific Arctic. Although our results provide an initial assessment, additional genetic and biological information is required to help differentiate putative populations or sub-populations. Finally, the improved understanding of hatch timing and spawning dynamics can inform the development of measures to protect Arctic Cod during their early life history. Continued monitoring and additional research on Arctic Cod will be required to fully understand how climate change will impact their distribution and abundance and the consequences of these changes for the Arctic ecosystem.

## 1.7    Acknowledgements

Figure 1.1: Locations where Arctic Cod for aging were captured. Color change from dark to light represents the number of specimens captured at a station, where N=7 denotes 7 or more specimens. Study regions are the northern Bering Sea (NBS), southern (SCS) and northern Chukchi Sea (NCS) and western (WBS) and eastern Beaufort Sea (EBS). No samples were obtained from the WBS.
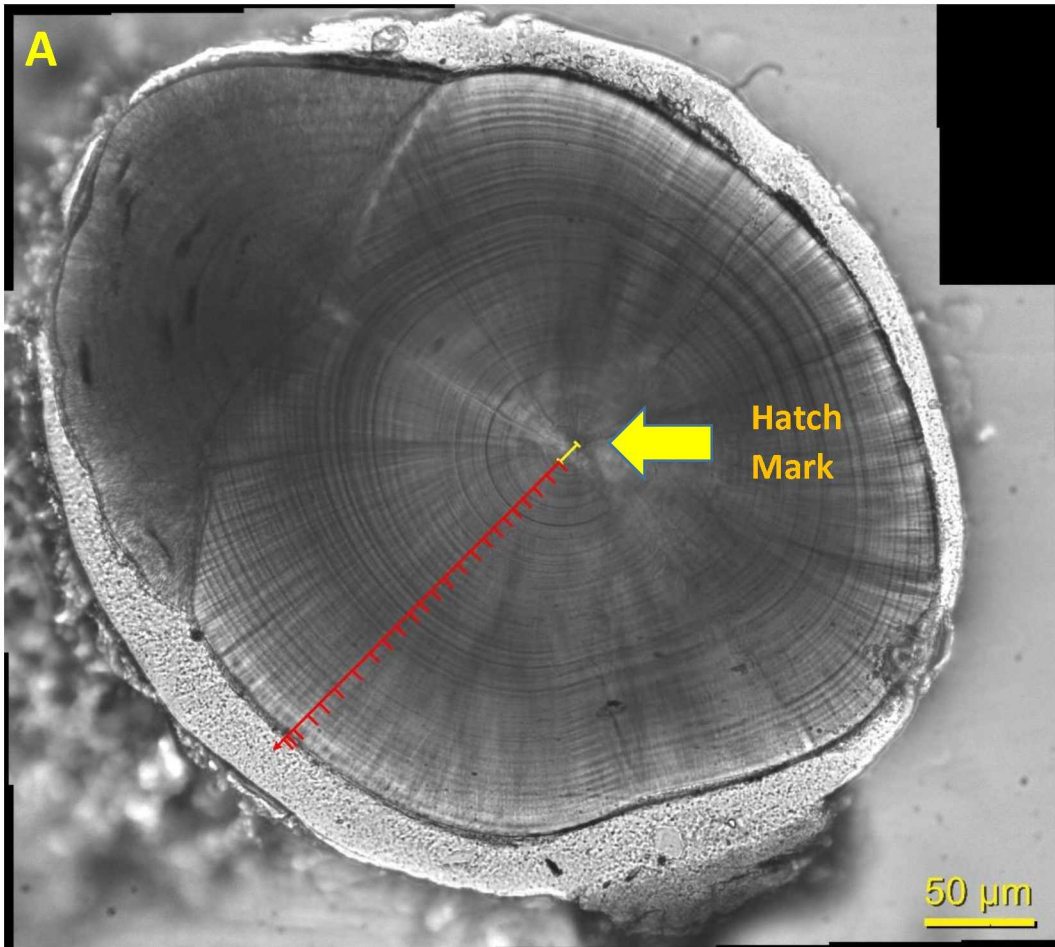
Figure 1.2: (A) Polished sagittal otolith at 40x magnification with daily growth increments marked at every 5th increment. (B) Cropped image of the same otolith showing the hatch mark. (C) Sagittal otolith at 100X magnification from a lab raised Arctic Cod with the hatch mark outlined.
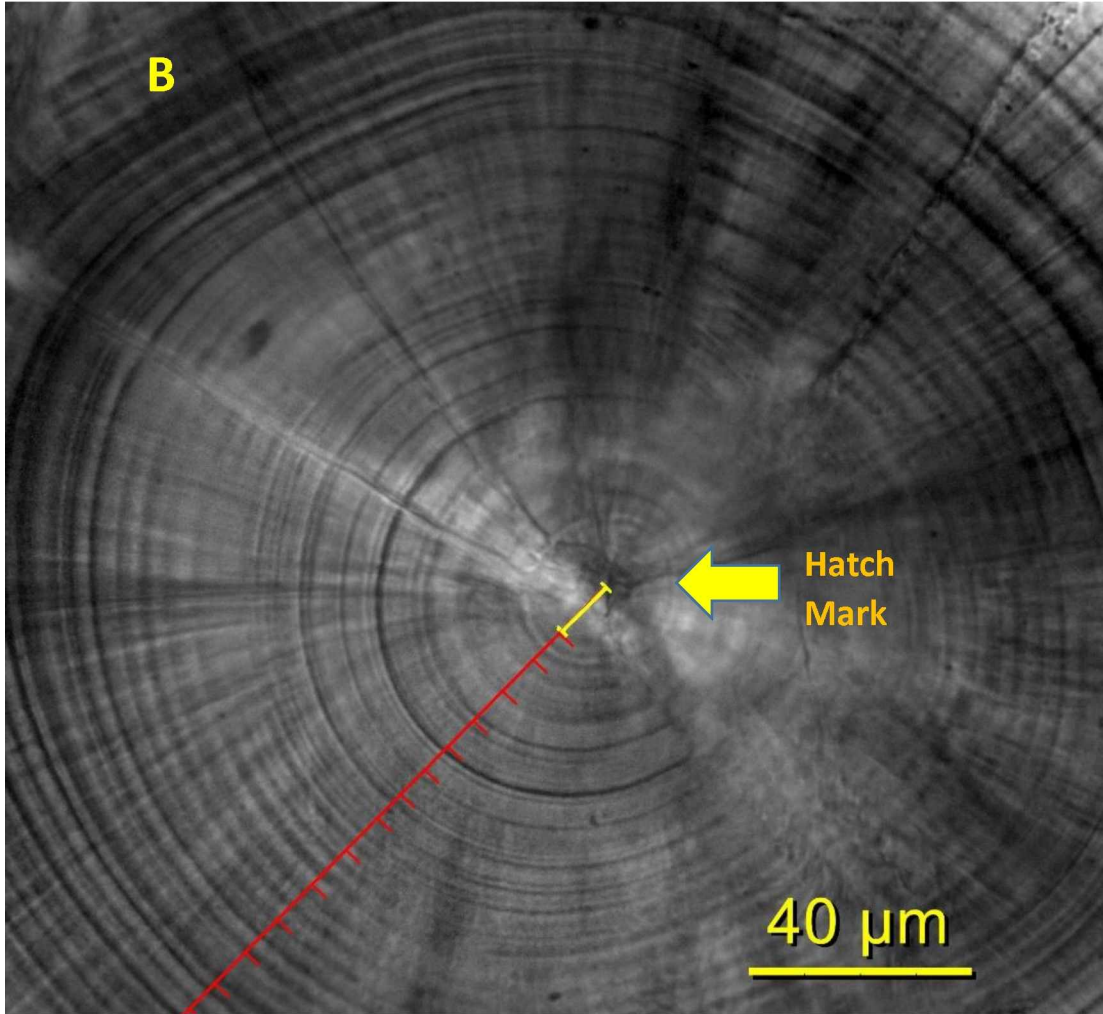
Figure1.2: (Continued)
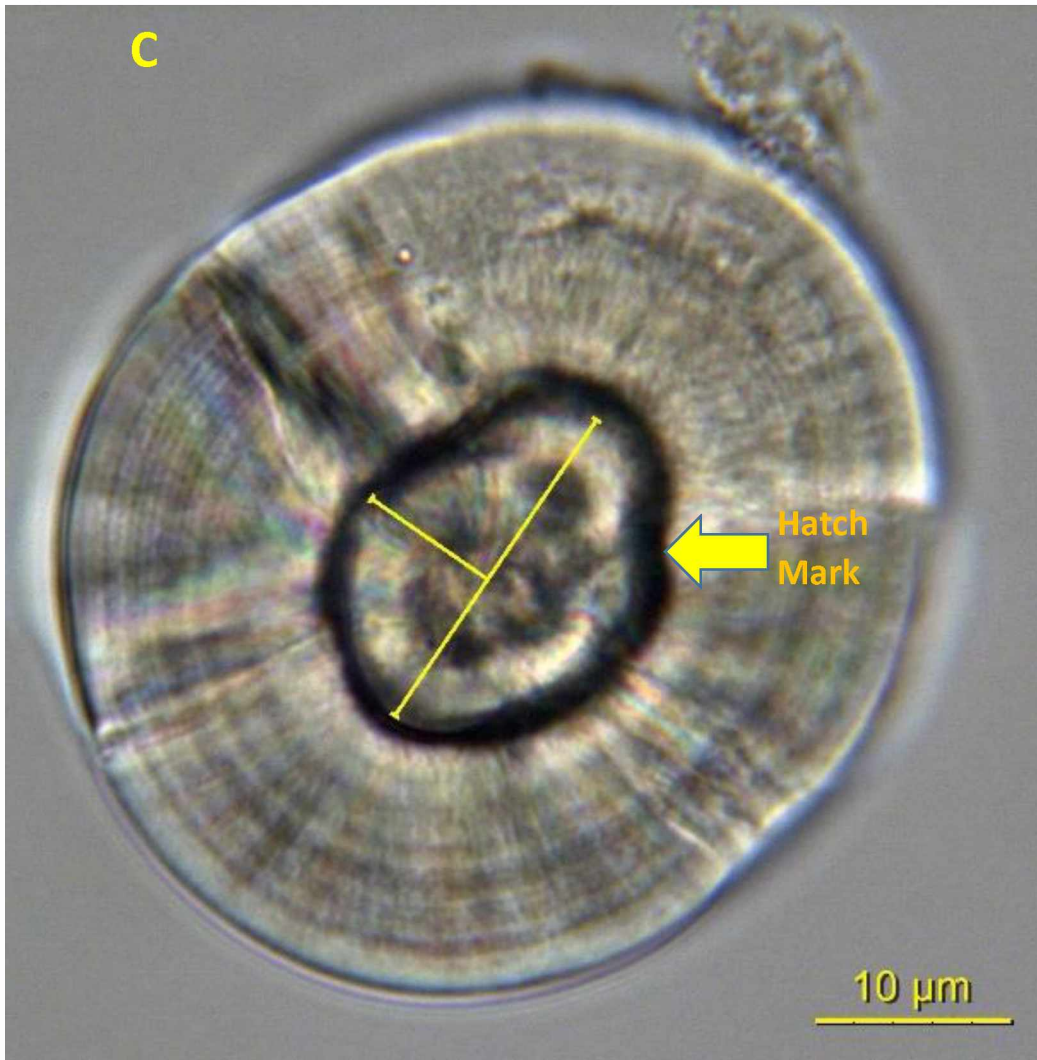
Figure 1.2: (Continued)
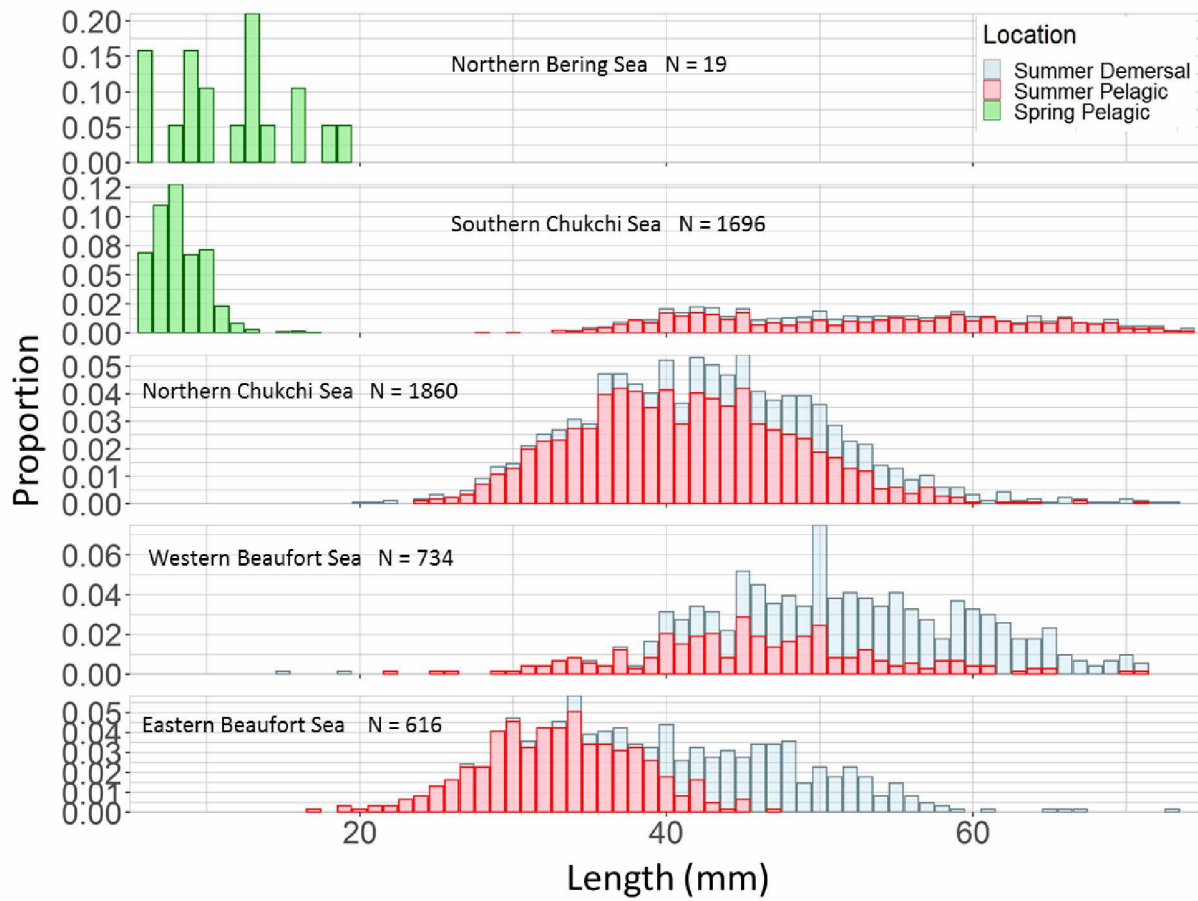
Figure 1.3: Length frequency distributions (x-axis, in mm) of age-0 Arctic Cod sampled in each region by season and vertical location in the water column. The number of individuals measured in each region is indicated (N).
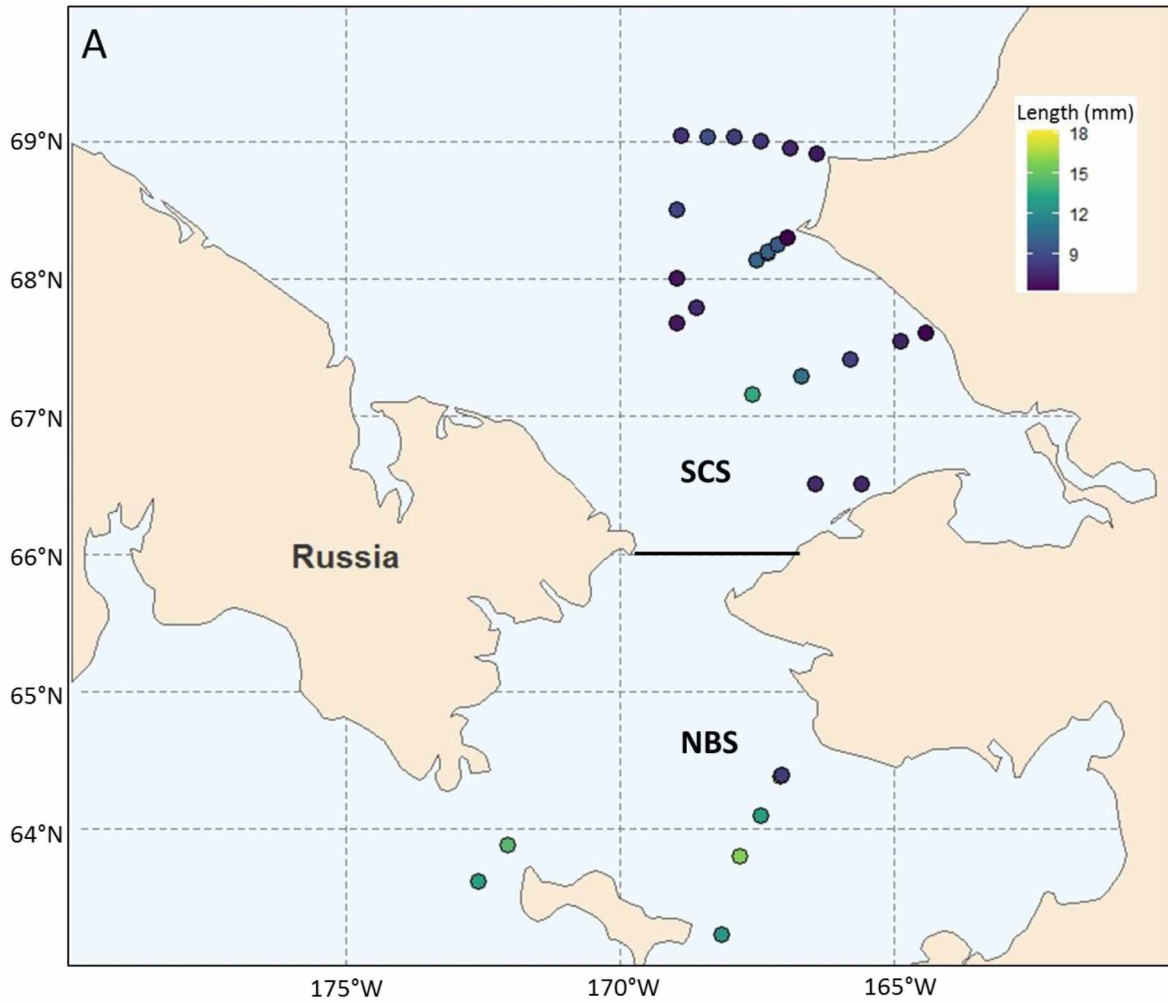
Figure 1.4: Stations with length data for Arctic Cod. Shading denotes average length (mm) by station for Arctic Cod captured during the spring survey in the water column (bongo nets, B) and during the summer survey in the water column (pelagic trawl, B) and on the bottom (demersal trawl, C).
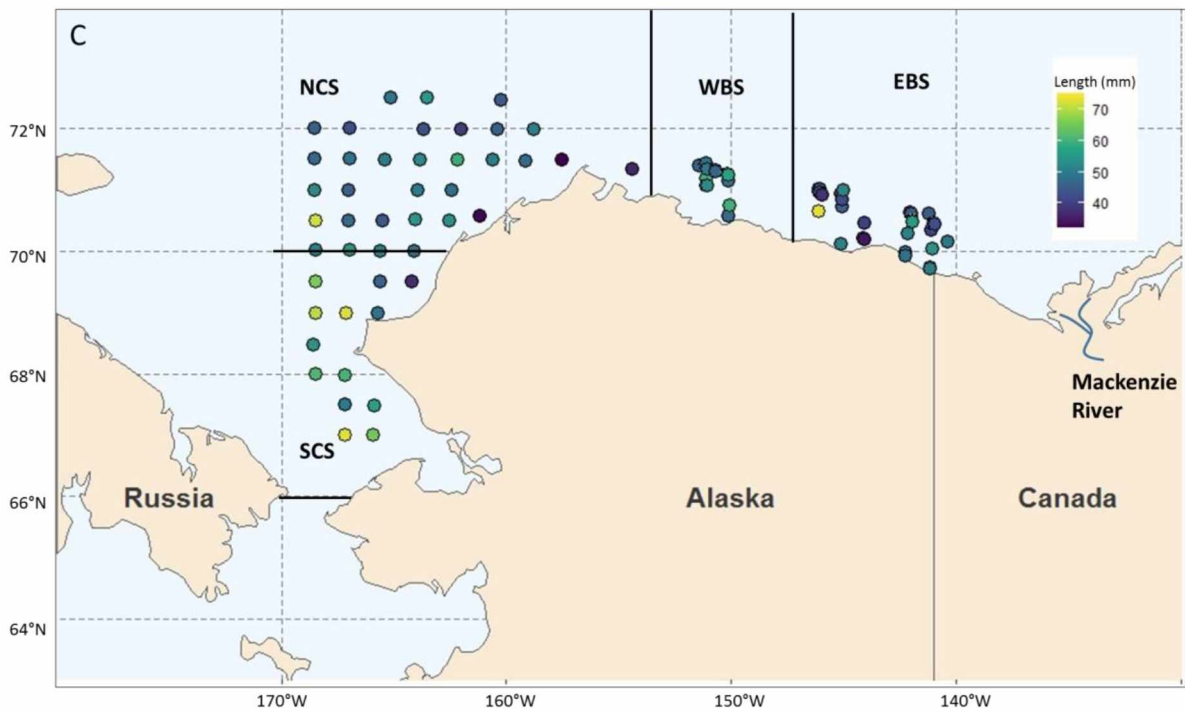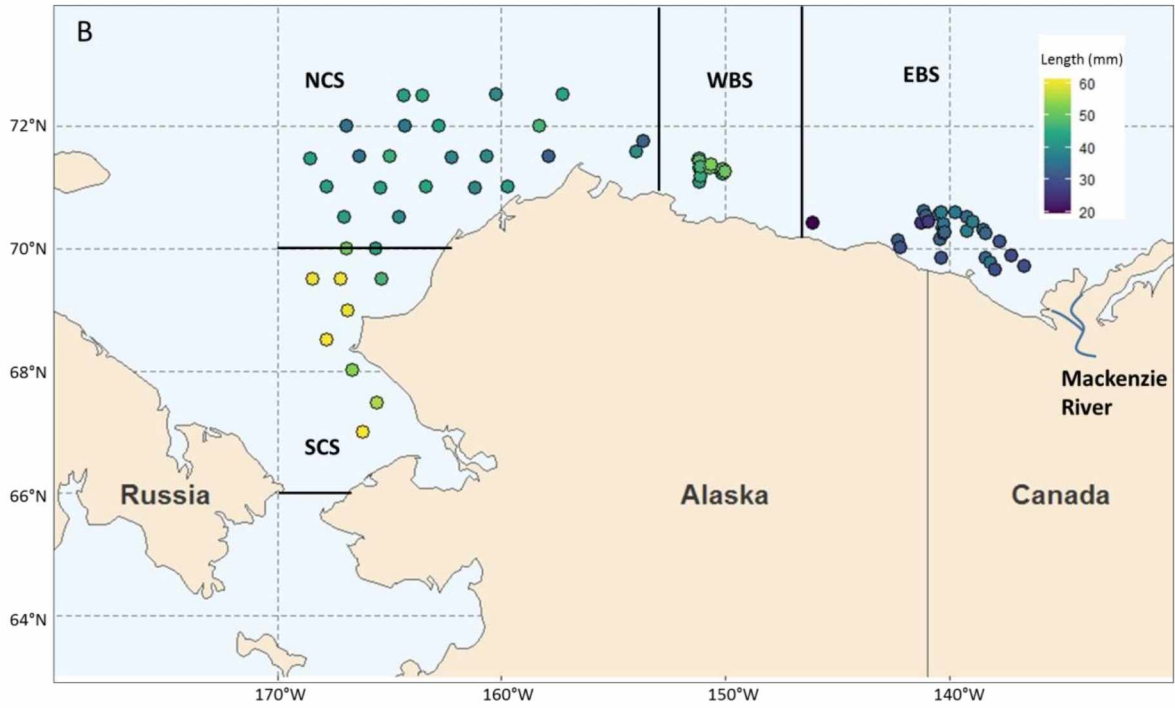
Figure 1.4: (continued)
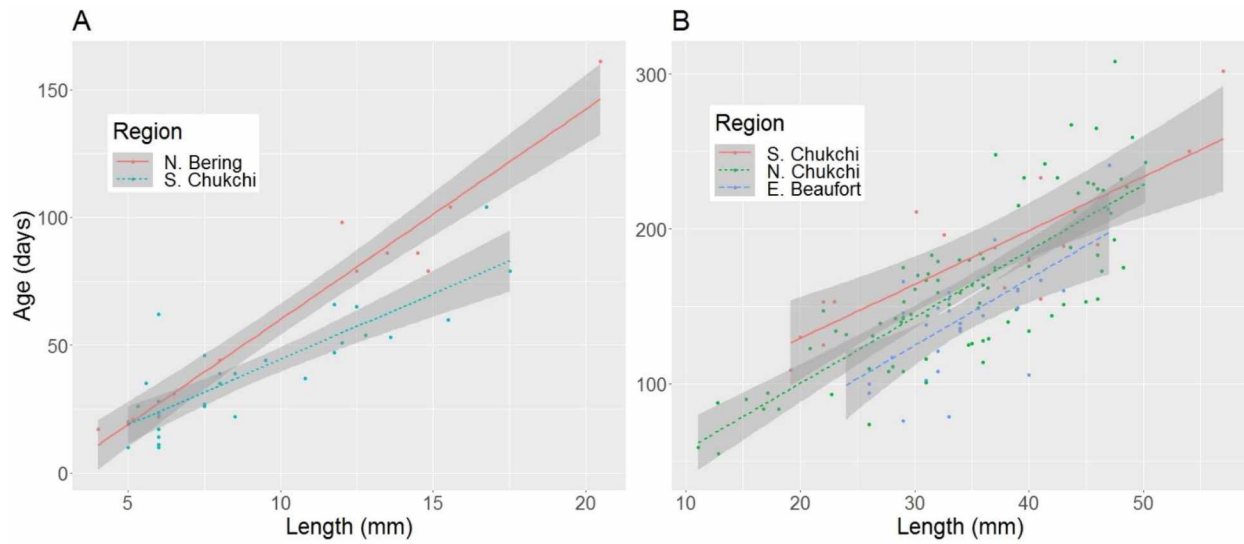
Figure 1.5: Age-at-length regressions by sampling region for spring (A) and summer (B) captured Arctic Cod with 95% confidence bands. Lengths ranged from 5 to 20 mm in spring (A) and from 10 to 55 mm in summer (B). Ages ranged from 0 to 150 days (A) and 100 to 300 days (B) in the spring and summer, respectively.
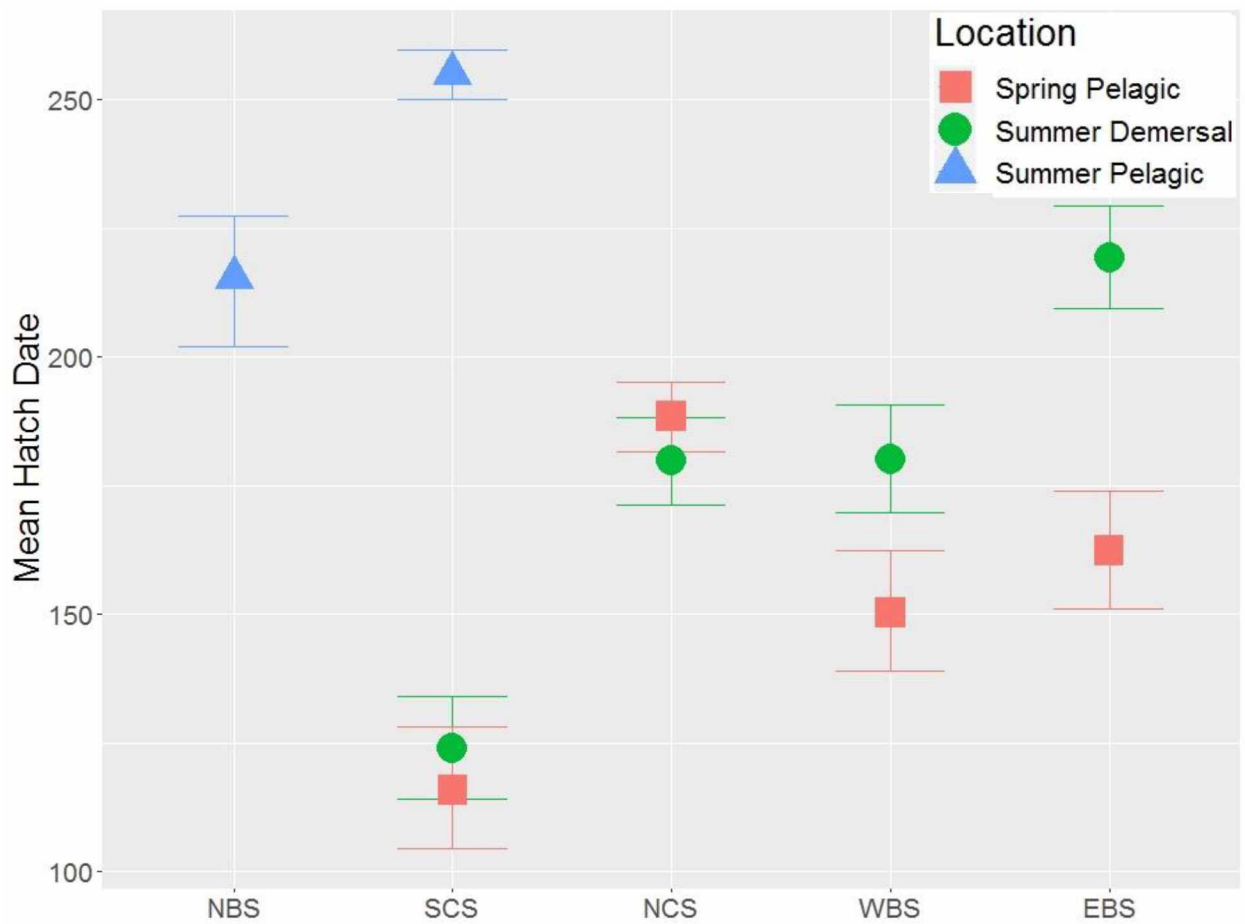
Figure 1.6: Estimated mean hatch date with 95% confidence limits for each region (northern Bering Sea (NBS), southern Chukchi Sea (SCS), northern Chukchi Sea (NCS), western Beaufort Sea (WBS), and eastern Beaufort Sea (EBS)) and vertical location (pelagic or demersal). Mean hatch date 100 corresponds to December 12 and hatch date 240 corresponds to May 11.
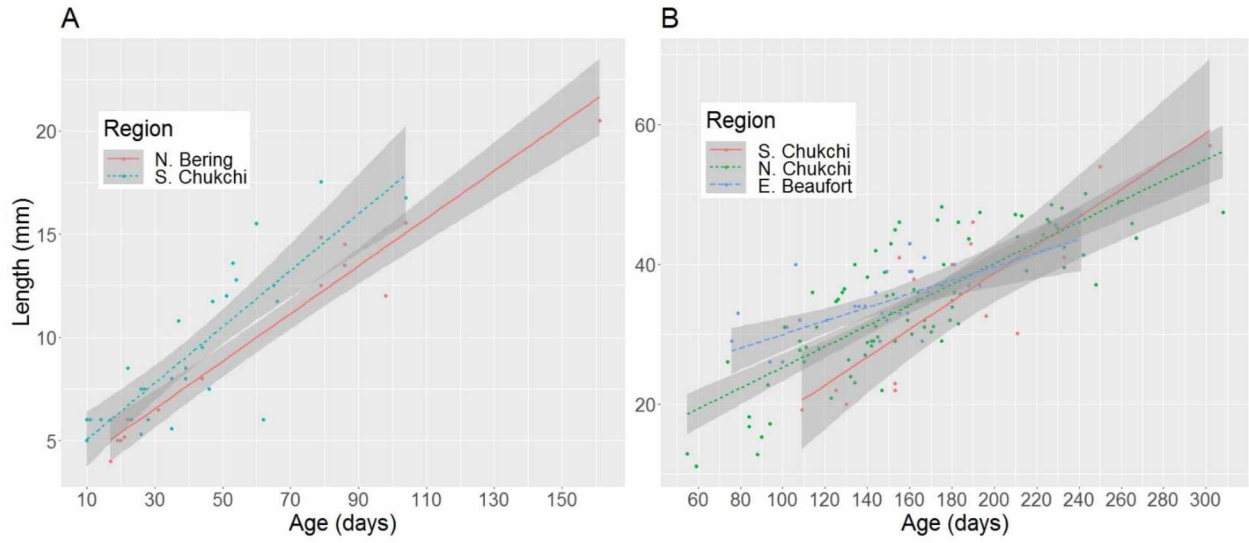
Figure 1.7: Regressions of length on age to estimate growth rates for spring (A) and summer (B) captured age-0 Arctic Cod.
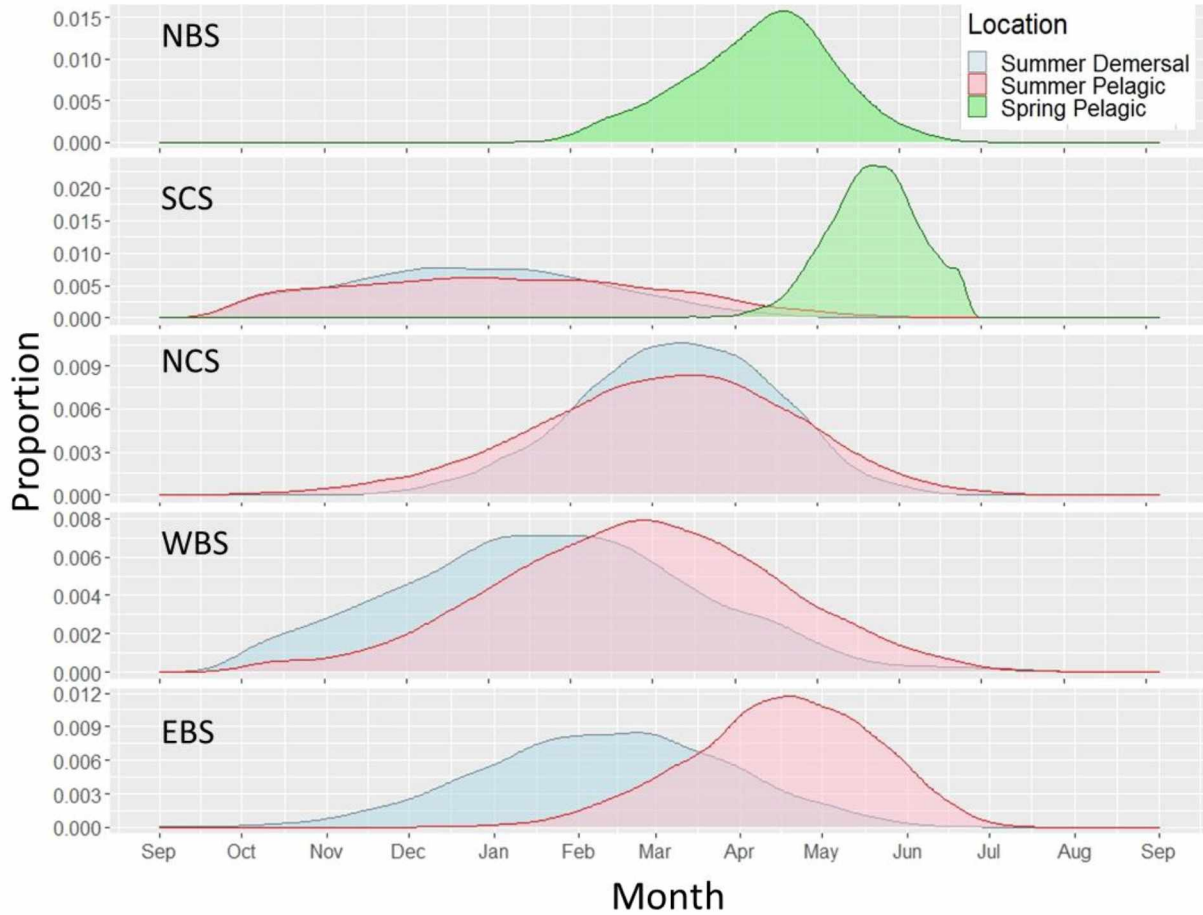
Figure 1.8: Estimated hatch date distributions by location in the water column for each region. Northern Bering Sea (NBS); southern Chukchi Sea (SCS); northern Chukchi Sea (NCS); western Beaufort Sea (WBS); eastern Beaufort Sea (EBS).
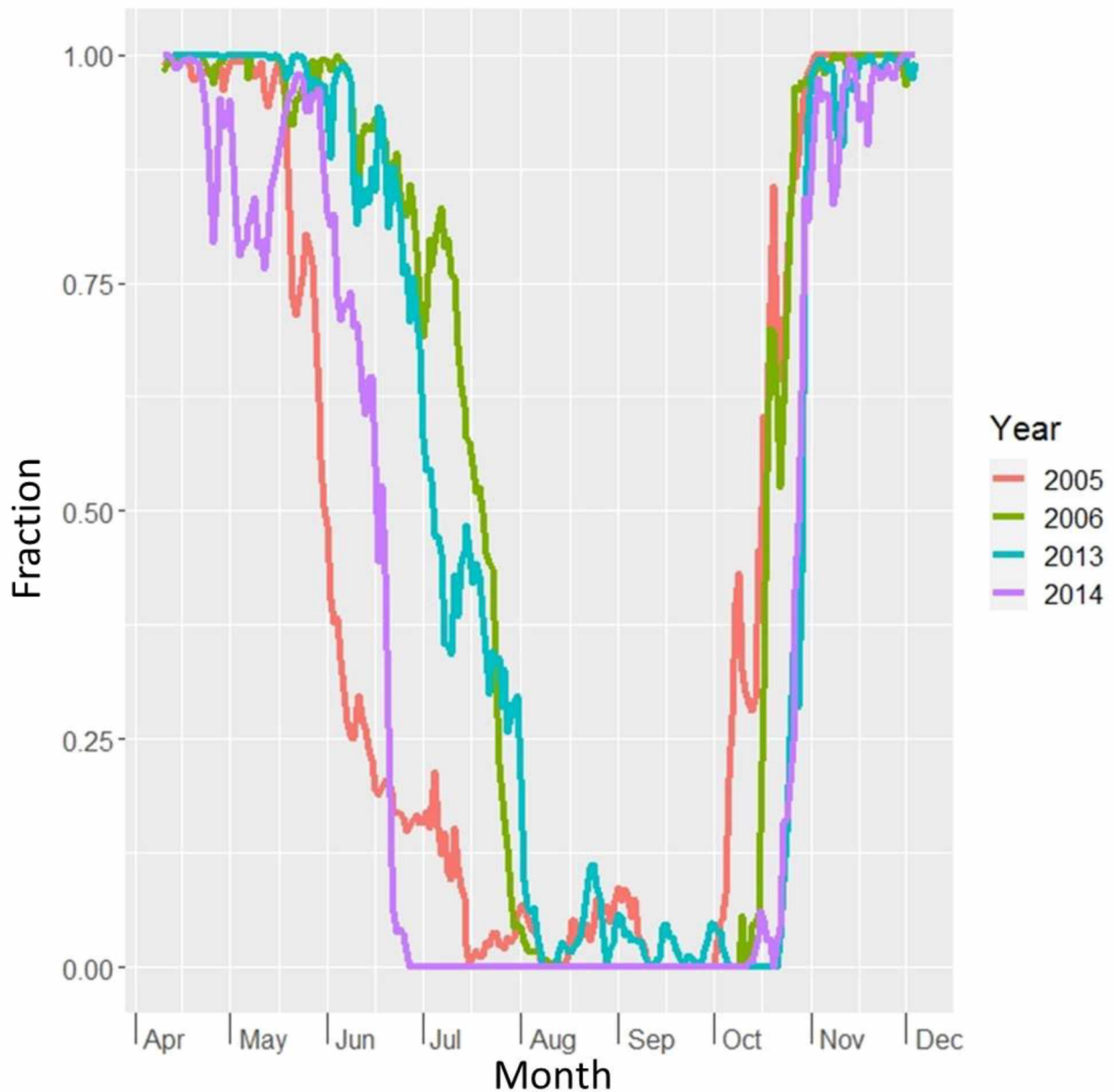
Figure 1.9: Seasonal trends in sea ice concentration (fraction of area with > 15% sea ice) for the eastern Beaufort Sea sampling region for four selected years with hatch date information. Sea ice concentrations are the fraction of the maximum daily extent of sea ice observed on the eastern Beaufort Sea shelf between 69.6 and 79.1°N and between 138°W and 134°W. Data from the NOAA National Snow and Ice Data Center (Peng et al., 2013; Meier et al., 2017).

1.9    Tables:

Table 1.1: Season, cruise, region, and sampling year with the dates, gear type, number of stations and number of Arctic Cod that were captured with their length range and mean length (mm). Regions are the northern Bering Sea (NBS), southern Chukchi Sea (SCS), northern Chukchi Sea (NCS), western Beaufort Sea (WBS) and eastern Beaufort Sea (EBS). Bongo, Isaacs-Kidd Midwater Trawl (IKMT), and Marinovich trawls sampled fish in the pelagic zone and bottom trawls sampled fish in the demersal zone. Cruises included the Arctic Shelf Growth, Advection, Respiration and Deposition (ASGARD) rate measurement survey, the Arctic Marine Biodiversity Observation Network (AMBON) survey, the Arctic Integrated Ecosystem Survey (Arctic IES II) and the Transboundary Surveys in 2012 (TB12) 2013 (TB13) and 2014 (TB14). See text for details.

| Season | Cruise | Region | Year | Sampling Dates | Gear Type | # of Stations | Arctic Cod Captured | length range (mm) | mean length (mm) |
|--------|--------|--------|------|----------------|-----------|---------------|---------------------|-------------------|------------------|
| Spring | ASGARD | NBS | 2017 | Jun-10 — Jun-28 | Bongo | 7 | 19 | 6—19 | 12 |
| Spring | ASGARD | SCS | 2017 | Jun-28 — Jun-23 | Bongo | 23 | 838 | 5—17 | 8 |
| Summer | AMBON | SCS | 2017 | Aug-6 — Aug-21 | IKMT | 4 | 91 | 18—56 | 37 |
| Summer | AMBON | SCS | 2017 | Aug-6 — Aug-21 | Bottom Trawl | 11 | 584 | 28—68 | 50 |
| Summer | AMBON | NCS | 2017 | Aug-18 — Aug-22 | IKMT | 6 | 1076 | 21—51 | 29 |
| Summer | AMBON | NCS | 2017 | Aug-9 — Aug-22 | Bottom Trawl | 50 | 2841 | 11—73 | 38 |
| Summer | Arctic IES | SCS | 2017 | Sep-13 — Sep-27 | Marinovich | 9 | 666 | 28—74 | 52 |
| Summer | Arctic IES | SCS | 2017 | Sep-12 — Sep-27 | Bottom Trawl | 14 | 190 | 34—74 | 52 |
| Summer | Arctic IES | NCS | 2017 | Aug-13 — Sep-13 | Marinovich | 19 | 1256 | 25—71 | 42 |
| Summer | Arctic IES | NCS | 2017 | Aug-10 — Sep-14 | Bottom Trawl | 30 | 463 | 22—73 | 48 |
| Summer | TB12 | WBS | 2012 | Sep-21 — Sep-29 | Bottom Trawl | 12 | 480 | 15—71 | 54 |
| Summer | TB12 | WBS | 2012 | Sep-22 — Sep-30 | IKMT | 13 | 254 | 22—71 | 46 |
| Summer | TB13 | EBS | 2013 | Sep-15 — Sep-30 | Bottom Trawl | 11 | 15 | 30—42 | 35 |
| Summer | TB13 | EBS | 2013 | Aug-13 — Aug-30 | IKMT | 27 | 351 | 17—47 | 33 |
| Summer | TB14 | EBS | 2014 | Aug-19 — Aug-31 | Bottom Trawl | 27 | 264 | 27—73 | 46 |

Table 1.2: Number of age-0 Arctic Cod aged by season and region with the range of standard lengths (mm) and estimated ages (days), and parameters of the length-at-age regressions with the slope representing estimated growth rates in mm d$^{-1}$.

| Season | Region | Total aged | Length range (mm) | Age range (days) | Length-age regressions | | |
|--------|--------|-----------|-------------------|------------------|-------|-----------|-------|
| | | | | | Slope | Intercept | R$^2$ |
| Spring | N. Bering | 14 | 4—21 | 17—161 | 0.115 | 3.075 | 0.943 |
| Spring | S. Chukchi | 29 | 5—18 | 10—104 | 0.183 | 1.804 | 0.912 |
| Summer | S. Chukchi | 15 | 19—57 | 109—302 | 0.200 | -1.290 | 0.673 |
| Summer | N. Chukchi | 77 | 11—50 | 55—308 | 0.149 | 10.435 | 0.631 |
| Summer | E. Beaufort | 31** | 24—47 | 76—241 | 0.097 | 20.250 | 0.397 |

** 27 were aged from 2013 survey and four were aged from 2014 survey

Table 1.3: Model coefficients, their estimates and standard errors, and Wald's t-test results for regressions of age in days on standard length (mm) by season. Region-specific intercepts ($\alpha$) and slopes ($\beta$) were estimated in the spring; a common slope was estimated in the summer. Subscript for regions are Northern Bering Sea (NBS), southern (SCS) and northern Chukchi Sea (NCS) and eastern Beaufort Sea (EBS).

| Season | Region | Coefficient | Estimate | Std. Error | t-value | P value |
|--------|--------|-------------|----------|-----------|---------|---------|
| **Spring** | NBS | $\alpha_{NBS}$ | -21.967 | 6.148 | -3.573 | 0.001 |
| | NBS | $\beta_{NBS}$ | 8.210 | 0.541 | 15.185 | 0.001 |
| | SCS | $\alpha_{SCS}$ | -11.483 | 5.371 | -2.138 | 0.039 |
| | SCS | $\beta_{SCS}$ | 5.485 | 0.538 | 10.197 | 0.001 |
| **Summer** | SCS | $\alpha_{SCS}$ | 41.489 | 10.545 | 3.935 | 0.001 |
| | NCS | $\alpha_{NCS}$ | 20.896 | 7.331 | 2.850 | 0.005 |
| | EBS | $\alpha_{EBS}$ | 2.069 | 9.003 | 0.230 | 0.819 |
| | All | $\beta$ | 4.092 | 0.238 | 17.178 | 0.001 |

Table 1.4: Mean and range of hatch dates (HD) by season, region and vertical location in water column. Dates marked with * are from the previous year. Regions are Northern Bering Sea (NBS), southern (SCS) and northern Chukchi Sea (NCS) and western (WBS) and eastern Beaufort Sea (EBS).

| Season and region | Vertical location | Mean HD | Earliest | Latest |
|---|---|---|---|---|
| **Spring** | | | | |
| NBS | Pelagic | Apr—06 | Jan—02 | Jun—09 |
| SCS | Pelagic | May—16 | Feb—26 | Jun—22 |
| **Summer** | | | | |
| SCS | Pelagic | Jan—04 | Sep—27* | Jul—22 |
| SCS | Demersal | Dec—27* | Sep—27* | Jun—07 |
| NCS | Pelagic | Mar—01 | Sep—27* | Aug—06 |
| NCS | Demersal | Mar—10 | Oct—07* | Jun—28 |
| WBS | Pelagic | Mar—02 | Sep—30* | Jul—21 |
| WBS | Demersal | Jan—31 | Sep—29* | Jul—19 |
| EBS | Pelagic | Apr—10 | Nov—01* | Jul—14 |
| EBS | Demersal | Feb—12 | Sep—02* | Jun—26 |

Table 1.5: Pairwise comparisons of mean hatch dates among regions by season and vertical location in the water column with bootstrap-based p-values and estimated difference in mean hatch dates. Negative differences in mean HD imply that the first region had an earlier hatch date than the second region.

| Regional comparisons | Season | Vertical Location | P-value | Difference in Mean HD |
|---|---|---|---|---|
| **Spring** | | | | |
| NBS — SCS | Spring | Pelagic | 0.002 | - 40 |
| **Summer** | | | | |
| SCS — NCS | Summer | Pelagic | 0.002 | - 55 |
| SCS — WBS | Summer | Pelagic | 0.002 | - 56 |
| SCS — EBS | Summer | Pelagic | 0.002 | - 95 |
| NCS — WBS | Summer | Pelagic | 0.957 | 0 |
| NCS — EBS | Summer | Pelagic | 0.002 | - 39 |
| WBS — EBS | Summer | Pelagic | 0.002 | - 39 |
| SCS — NCS | Summer | Demersal | 0.002 | - 72 |
| SCS — WBS | Summer | Demersal | 0.002 | - 34 |
| SCS — EBS | Summer | Demersal | 0.002 | - 46 |
| NCS — WBS | Summer | Demersal | 0.002 | 38 |
| NCS — EBS | Summer | Demersal | 0.002 | - 26 |
| WBS — EBS | Summer | Demersal | 0.059 | - 12 |

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Chapter two: Arctic Cod (*Boreogadus saida*) otolith microchemistry supports regional differences in hatching habitats off Alaska

2.1     Abstract

The early life history of fish is often poorly understood because of the difficulties observing spawning and sampling early life stages. This is especially true for Arctic Cod (*Boreogadus saida*), which may hatch in remote areas of the Arctic, making direct observations difficult. Although larval and early juvenile Arctic Cod have been collected throughout the Alaskan Arctic, their spawning and hatching locations remain unknown. One way to identify habitat conditions associated with hatching is through the analysis of otolith microchemistry. I measured the ratio of seven trace elemental concentrations relative to Calcium near the hatch marks in otoliths of Artic Cod from the Chukchi and Beaufort seas using laser ablation inductively coupled mass spectrometry. Trace elemental concentrations were compared among three regions of sampling to estimate the water characteristics during the time of hatching using previously established relationships between elemental ratios and water properties. The results showed no difference in elemental signatures near the hatch mark between the northern Chukchi Sea and the western Beaufort Sea, whereas four of the seven trace elemental ratios differed significantly between the eastern Beaufort Sea and the western regions. Differences in trace elemental ratios indicated that Arctic Cod from the eastern Beaufort Sea hatched in less saline waters than those from the western regions, possibly a result of hatching within the freshwater plume from the Mackenzie River. Some samples from the northern Chukchi Sea had elevated levels of Zinc near the hatch mark, which could be due to hatching in Kotzebue Sound where Zinc concentrations may be elevated due to high natural concentrations as well as from mining. These results expand knowledge of Arctic Cod early life history and showed promise for using otolith microchemistry to improve understanding of Arctic Cod hatch locations.

2.2     Introduction

Arctic Cod (*Boreogadus saida*) have a circumpolar distribution in the Arctic and are a primary food source for many predators. They have a narrow thermal range and are sensitive to high temperatures, especially during their larval stage (Laurel et al, 2015; Kent et al, 2016), making them vulnerable to a changing climate. Because of this vulnerability and their importance to the Arctic ecosystem, understanding their early life history is critical to management and conservation (NPFMC, 2009). Several important aspects of the early life history of Arctic Cod in the Pacific Arctic, however,

remain poorly understood, especially with regards to hatching and spawning locations. Here I examine the usefulness of elemental signatures in otoliths to differentiate stocks and infer habitat characteristics of Arctic Cod during hatching.

Environmental variables such as water temperature and salinity can be reconstructed by analyzing the elemental composition of fish otoliths (Campana, 1999). Otoliths are acellular and metabolically inert and therefore store many elements in proportion to their concentration in the water masses they inhabit (Campana, 2005). Because otoliths are not reabsorbed, they provide a permanent record of environmental conditions encountered during an individual's lifetime (Campana, 1999). The concentrations of many trace elements differ among water masses and these differences are recorded in the elemental composition of otoliths. Moreover, the rate at which certain elements are incorporated into the otoliths may depend on environmental conditions such as temperature and salinity, as well as somatic growth rate (Bath et al., 2000; Bath Martin and Thorrold, 2005). Incorporation of Barium and Strontium occurs independent of growth rates, whereas Manganese and Magnesium can be dependent.

Elements used commonly for otolith analyses include Barium (Ba), Lithium (Li), Magnesium (Mg), Manganese (Mn), and Strontium (Sr) because change in these elements can be used to infer changes in temperature and salinity (Bouchard et al., 2015). Concentrations of Ba, Li, Mg, and Sr from the otolith edge are positively correlated with salinity, whereas Mn has a negative relationship with salinity (Bouchard et al., 2015). Water temperature also plays a role in the elemental composition of the otolith as Li and Mn concentrations are both negatively correlated with water temperature (Bouchard et al., 2015). Other elements such as Zinc (Zn) and Copper (Cu) can be used to reconstruct environmental conditions, as they may be associated with freshwater runoff from drainages or mines. For example, relatively large Zn concentrations have been correlated with acid mine tailings from a Cu and Zn mine and associated with freshwater (Saquet et al., 2002). Zinc can also be incorporated naturally from ingesting prey species and from freshwater influences near shore within the early stages of life for Arctic Char (*Salvelinus alpinus*) (Halden et al., 2000). Using the concentrations of these elements within otoliths may therefore help identify various water masses fish inhabited during different life stages.

The elemental composition of otoliths provides a timeline of environmental conditions because of the incremental nature of otolith development. The elemental composition of growth rings along the current edge of the otolith reflects the ambient water mass within a few days of capture (Miller, 2011). Similarly, the elemental composition near the hatch mark reflects conditions at the time of hatching when the mark formed. The identification of possible hatch locations requires calibrating the elemental

composition by relating elemental concentrations in the otolith to environmental characteristics such as temperature and salinity. Ideally, calibration should be performed using larvae captured shortly after hatching. These calibrations can subsequently be used to determine the origin of juveniles or adults caught at later stages. For example, elemental signatures in otoliths from recently hatched Capelin (*Mallotus villosus*) were used to establish a relationship between these otolith signatures and environmental characteristics including temperature and salinity (Davoren et al., 2015). If larvae from known spawning locations or different known populations are unavailable, calibration may be performed using relationships between the elemental composition at the otolith edges of juvenile or adult samples and the water masses at the capture location. However, applying water mass associations established for juvenile or adult stages to identify hatch locations based on their core elemental composition can be problematic if larvae occur at different water depths with varying elemental compositions (Bouchard et al., 2015). Trace elemental composition may also change seasonally and the rate of uptake of trace elements may change with ontogeny (Hüssy et al., 2020). Nevertheless, establishing relationships between environmental conditions and elemental composition can provide valuable information about potential hatching areas and habitat use during early life (Bouchard et al., 2015).

Several studies of Arctic Cod have used otolith microchemistry to identify relationships between elemental composition and environmental conditions. Trace elemental composition along the edge of Arctic Cod otoliths has been used to infer bottom water mass occupancy across the Chukchi Sea with high confidence, indicating the potential for using trace elements to determine habitat associations of early life stages (Gleason et al., 2016). Gleason et al. (2016) demonstrated that benthic temperatures played a larger role than salinity in differentiating among demersal habitats based on otolith elemental compositions of Arctic Cod because temperature is typically more variable among masses than salinity. Significant differences in environmental conditions must exist among habitats if the composition of otoliths is to be useful for making inferences about habitat use and distribution. Trace elemental composition along the edge and in the core of Arctic Cod otoliths differed between each other and among six different regions across the circumpolar Arctic (Bouchard et al., 2015). Trace elemental concentrations at the edge were related to salinity and temperature at the capture locations. Using this relationship between composition and salinity at the otolith edge, trace elemental composition near the core was used as a proxy for salinities at the time of hatching to predict the extent to which hatching occurs in locations influenced by freshwater discharge.

In the Pacific Arctic, the stock structure of Arctic Cod and their spawning and hatching locations are not well known. Therefore, the goal of this study was to assess the use of elemental signatures from the hatch mark of otoliths for differentiating possible hatching events of Arctic Cod in the Pacific Arctic. Specifically, the objectives were to (1) compare the trace elemental composition near otolith hatch marks among three different regions of the Alaskan Arctic and (2) infer habitat differences of early larval stages and possible hatching locations based on previously established relationships between the otolith elemental composition of Arctic Cod and surface water salinity and temperature.

## 2.3    Methods

### 2.3.1    Study area

Arctic Cod were sampled in three distinct regions; the northern Chukchi Sea (NCS), the western Beaufort Sea (WBS), and the eastern Beaufort Sea (EBS) (Figure 2.1), following the rationale in Chapter 1. Samples in the NCS were collected from the shallow shelf north of 70° latitude and west of 155° longitude. Individuals from the WBS were collected east of 155°W, whereas EBS samples were collected near the U.S Canadian Border near 140°W.

### 2.3.2    Sample collection

Arctic Cod were collected during three different surveys: The 2017 Arctic Integrated Ecosystem Survey (Arctic IES II) which sampled the NCS and WBS and the 2013 (TB13) and 2014 (TB14) US/Canada Transboundary Surveys which sampled the EBS (Table 2.1). Arctic IES II survey samples were collected using a Marinovich Trawl, the TB13 samples were collected using an Isaacs-Kidd Midwater Trawl (IKMT), and the TB14 samples were collected with a Plumb-Staff Beam Trawl; see Chapter 1 for more details on sampling gear. All larval and juvenile gadids used for otolith analysis were stored in 95% ethanol or frozen. Arctic IES II samples were shipped to the University of Alaska Fairbanks (UAF) lab in Juneau, Alaska. Approximately 60% of the specimens were examined by larval taxonomists from the National Oceanic and Atmospheric Administration (NOAA) to confirm species identifications. The remaining fish were identified by the author after being trained by NOAA larval taxonomists. The TB13 and TB14 samples were identified, measured for length and weight, had sagittal otoliths removed, and archived at the Fisheries Oceanography lab at UAF. Archived otoliths were shipped to the UAF lab in Juneau, Alaska, for this analysis. All otoliths were extracted, cleaned, mounted to glass slides with thermoplastic cement, and polished in preparation for laser ablation (see Chapter 1 for details on sample processing).

### 2.3.3  Otolith microchemistry

I measured the concentration of Calcium ($Ca^{40}$), Lithium ($Li^7$), Magnesium ($Mg^{24}$), Manganese ($Mn^{55}$), Copper ($Cu^{64}$), Zinc ($Zn^{65}$), Strontium ($Sr^{88}$), and Barium ($Ba^{137}$) along a transect from the center to the edge of 133 otoliths (Figure 2.2), but only the region near the hatch mark was used for this study. Elemental concentrations near the hatch mark (approximately 11.5 μm from otolith's core) were used to reconstruct environmental conditions at the time of hatching and identify possible hatch locations. The identification of the hatch mark was validated using otoliths from laboratory raised Arctic Cod from the Hatfield Marine Science Center in Newport, Oregon as a visual reference (Figure 2.3; Benjamin Laurel, NOAA Alaska Fisheries Science Center, pers. comm.) The ablation at 11.5 μm did not include the hatch mark but was within the first week of life and therefore indicative of environmental conditions just after hatching. Each ablation transect began at a point just outside of the core to minimize the possibility of incorporating any maternal elemental signatures. Otoliths were analyzed at the University of Alaska Fairbanks Advanced Instrumentation Laboratory using a New Wave UP213 Laser with an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (LA-ICP-MS). Nine otoliths were placed on a slide and processed in a single run. Prior to each run, Nist610 and Febs pellets were ablated to ensure a consistent standard. The transects were ablated at 5 μm/s, using a beam width of 25 μm with a pulse frequency of 10 Hz at 55% laser power. Data from the LA-ICP-MS were processed using Igor Pro version 6.37® (WaveMetrics) and Iolite software package version 3.0®. To ensure data quality, all transect profiles were analyzed to identify and eliminate unrealistic elemental spikes caused by elemental fractionation, which is the transport of aerosol particles from the ablation chamber to the ICP and is not representative of the actual elemental abundance (Limbeck et al., 2015). For statistical analysis all measurements were expressed as ratios relative to Ca to standardize the results for the amount of material ablated.

### 2.3.4  Statistical analysis

Multivariate and univariate statistical analyses were conducted to compare otolith elemental compositions near the hatch mark among collection regions. Trace elemental ratios were log-transformed to achieve approximate multivariate normality and were standardized to a mean of zero and a standard deviation of one. Pairwise Euclidean distances among samples were calculated to visualize differences in the elemental composition of the hatching location among sampling regions using a multivariate ordination based on a Principle Components Analysis (PCA). Multivariate analysis of variance (MANOVA) was used to test for overall differences in elemental composition among regions.

When overall differences were significant ($p < 0.05$), these were followed by ANOVAs for each elemental ratio to determine which ratios differed among regions and, for those that did, Tukey Honest Significant Difference (HSD) post hoc tests to determine which pairwise differences among regions were significant. All analyses were performed using the R statistical computing environment version 3.5.3 (R Core Team, 2019).

## 2.4    Results

The elemental composition of otoliths around the time of hatching differed between the EBS and the two western regions, but not between the WBS and NCS. Within the EBS, no significant differences were found between 2013 and 2014 for most trace elemental ratios, except for Mg/Ca (t-test: $p = 0.001$) and Ba/Ca ($p=0.042$). Because of this, the 2013 and 2014 EBS samples will be pooled and referred to as EBS. The PCA ordination showed considerable overlap existed in trace elemental compositions among regions (Figure 2.4), however, there was an overall statistical difference in the mean elemental composition among regions (MANOVA: Pillai's trace = 0.299, $p = 0.004$). Specifically, elemental ratios for Mn, Zn, Sr and Ba differed among regions (ANOVAs: $p = 0.012$, 0.003, 0.002 and 0.035, respectively, Figure 2.5). The Mn/Ca ratio was significantly higher in the EBS than the NCS ($p = 0.011$). Zn/Ca was significantly lower in the EBS than in the NCS ($p = 0.008$) and the WBS ($p = 0.013$). The following elemental ratios were marginally different as Sr/Ca, and Ba/Ca were all lower in the EBS than in the NCS ($p = 0.008$ and $p = 0.070$) and WBS ($p = 0.009$ and $p = 0.074$, respectively). In summary, there were no significant differences for any elemental ratio near the hatch mark between the NCS and the WBS; in contrast, seven out of 15 pairwise comparisons between the EBS and the other two regions were (Table 2.2).

## 2.5    Discussion

The elemental analyses suggest considerable overlap in the trace-elemental ratios of the hatch mark in otoliths from the NCS, WBS, and EBS. Nevertheless, small but statistically significant differences in individual elemental ratios among regions were found, likely reflecting differences in the range of habitats where hatching occurs within each region. Similarly, Bouchard et al. (2015) found significant differences in several of the same trace elements (Mn, Sr, and Ba) across multiple circumpolar regions. They reported that Mn/Ca was negatively correlated with salinity, whereas Sr/Ca and Ba/Ca were positively correlated with salinity. In this study, I found a higher ratio for Mn and reduced ratios for Ba and Sr in the EBS relative to the other regions, suggesting that EBS Arctic Cod hatched in waters with

lower salinity than those in the WBS and NCS. Arctic Cod captured in the EBS may have hatched in nearshore waters off the Mackenzie River (Bouchard and Fortier, 2008), a region that receives large amounts of freshwater runoff and is characterized by low salinities (Lansard et al., 2012), consistent with the elemental signature of larval Arctic Cod from the EBS. The signature in the otoliths of larvae sampled in the other two regions suggests that they originated in areas with higher salinity, consistent with their hypothesized origin near the Bering Strait region (Vestfals et al., 2021).

Elemental differences between Arctic Cod in the EBS and western portions of the Chukchi Sea have been documented previously. Frothingham et al., (2020) found differences between EBS and Chukchi Sea Arctic Cod for both the core and edge, but their Chukchi Sea samples were collected near Wrangel Island in Russian waters. The difference in elemental composition between these two locations was more pronounced than in this study, likely because of their greater geographical separation, whereas the smaller distances among samples from this study may have resulted in some mixing of Arctic Cod populations among regions. Regardless, the differences in otolith microchemistry support the existence of at least two unique spawning populations in the Beaufort and Chukchi seas, consistent with previous studies on Arctic Cod genetics (Wilson et al., 2017, 2019, Nelson et al., 2020), spatial distributions (Forster et al, 2020) and hatch date distributions (chapter 1).

Differences between the EBS, which was sampled in 2013/14, and the two western regions, which were sampled in 2017, could have resulted from inter-annual variability in environmental conditions associated with freshwater inputs since the elemental signatures for both years were consistent with low salinity origins. The greater Mg and Ba signatures in 2014 compared to 2013 may reflect lower discharge from the Mackenzie River during the April–June hatching period (12.57 thousand $m^3$/s on average) compared to 2013 (14.00 thousand $m^3$/s) (https://wateroffice.ec.gc.ca/mainmenu/historical_data_index_e.html). However, other environmental variables could have resulted in the observed differences in elemental compositions between these years such as rain, ice melt, and wind mixing.

The similarity in elemental signatures between Arctic Cod sampled in the NCS and in the WBS during the 2017 survey suggests that they originated from waters with similar elemental compositions. This supports the hypothesis that they are part of the same spawning population, consistent with similarities in hatch dates (Chapter 1), oceanographic connectivity between the two regions (Forster et al., 2020), and model results suggesting transport of larvae from the Chukchi Sea into the western Beaufort Sea (Levine et al. 2021). Although elemental ratios in the NCS and WBS were significantly

different from those in the EBS, there was high variability within each region, suggesting that hatching occurs across multiple locations within each region.

Although the mean concentrations of several elements that reflect salinity variations differed among regions, there was considerable overlap in elemental composition among regions. In particular, the concentrations of Lithium, a trace element that primarily reflects temperature variations in the environment (Bouchard et al., 2015), did not differ among regions. Temperature affects otolith composition by modifying the uptake of trace elements from the water into the otolith (Collingsworth et al., 2010), thus the lack of significant differences in Li suggest similar temperature conditions across regions at the time of hatching. This is not surprising considering that hatching typically occurs under the ice at temperatures near freezing (Bouchard and Fortier, 2011). Like Li, Mn tends to be negatively correlated with temperature, but also has a negative relationship with salinity (Bouchard et al., 2015). Therefore, the lack of difference in Li in the otoliths among regions suggests that the observed elevated Mn concentrations are due to lower environmental Mn concentrations related to lower salinities in the EBS. Alternatively, the observed difference in Mn could be due to slightly elevated temperatures in the EBS region associated with the Mackenzie River freshwater plume. Arctic Cod hatching in freshwater plumes are hypothesized to experience slightly elevated temperatures and increased growth rates compared to regions without a strong freshwater influence such as the NCS and WBS (Bouchard et al., 2015). However, the lack of a difference in Li concentrations among regions and lower growth rates in the EBS (Chapter 1) are not consistent with the hypothesis that EBS larvae experienced elevated temperatures at the time of hatching compared to larvae in the NCS and WBS. It is also possible that previously observed relationships between Li and water temperature were spurious and that Li is not a good indicator of water temperature.

Differences in most elemental ratios provide some insight regarding salinity and temperature conditions at the time of hatching, but trace elements such as Zn may be associated with anthropogenic sources. Although Zinc occurs naturally in freshwater and seawater, increased levels of Zn in otolith hatch marks of fishes can be caused by Zn runoff from mine tailings near the hatching area (Saquet et al., 2002; Halden and Friedrich, 2008). In the current study, Zn levels from the NCS and WBS Arctic Cod were significantly higher than those captured in the EBS. One of the hypothesized hatching areas for Arctic Cod is Kotzebue Sound (Deary et al., 2021), which is located about 50 miles downstream from the world's largest Zinc mine where high concentrations of Zinc occur, possibly explaining the higher mean levels of Zn in NCS and WBS Arctic Cod. More targeted sampling of environmental Zinc as a tracer and of

Arctic Cod at a range of distances from the potential discharge location are required to support this tentative conclusion and would also help determine the relative importance of Kotzebue Sound as a hatch location.

2.6      Conclusion

The results of this study provide further evidence that at least two separate populations exist within the Pacific Arctic. The EBS Arctic Cod hatched in waters with a lower salinity than those from the western regions. This is consistent with the theory that they hatch within the Mackenzie River plume. The elevated levels of Zinc in the NCS and WBS fish may have been present because hatching occurred in Kotzebue Sound downstream from the Red Dog mine. Although this study has provided greater insight into Arctic Cod populations within Alaska's waters and where they may hatch, more studies will be needed to identify hatching locations of Arctic Cod. Applying the methods used in this study to a larger sample size collected over an expanded geographic range could help identify the spawning populations within Alaskan waters.
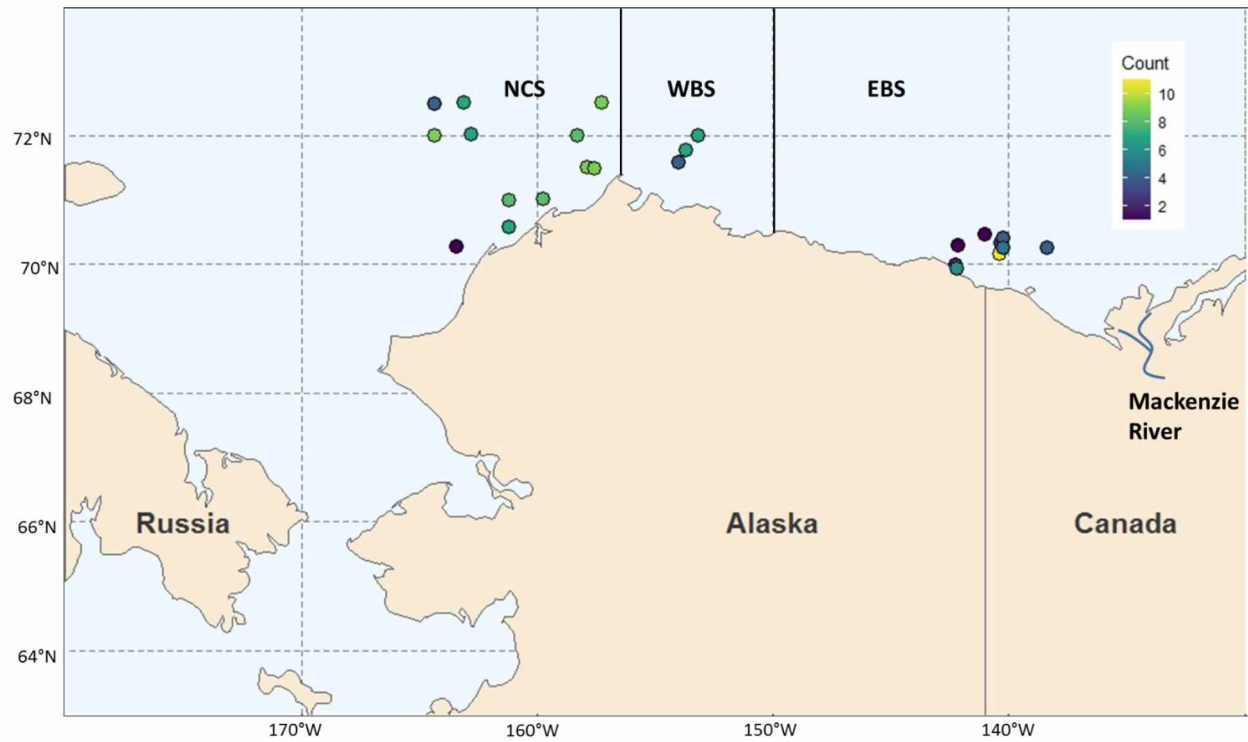
Figure 2.1: Location of samples from which otoliths were collected for microchemistry analysis from the Arctic IES and Transboundary Surveys in the northern Chukchi Sea (NCS), western Beaufort Sea (WBS) and eastern Beaufort Sea (EBS). The number of samples analyzed from each station is indicated by the color change from cool (blue) to warm (yellow).
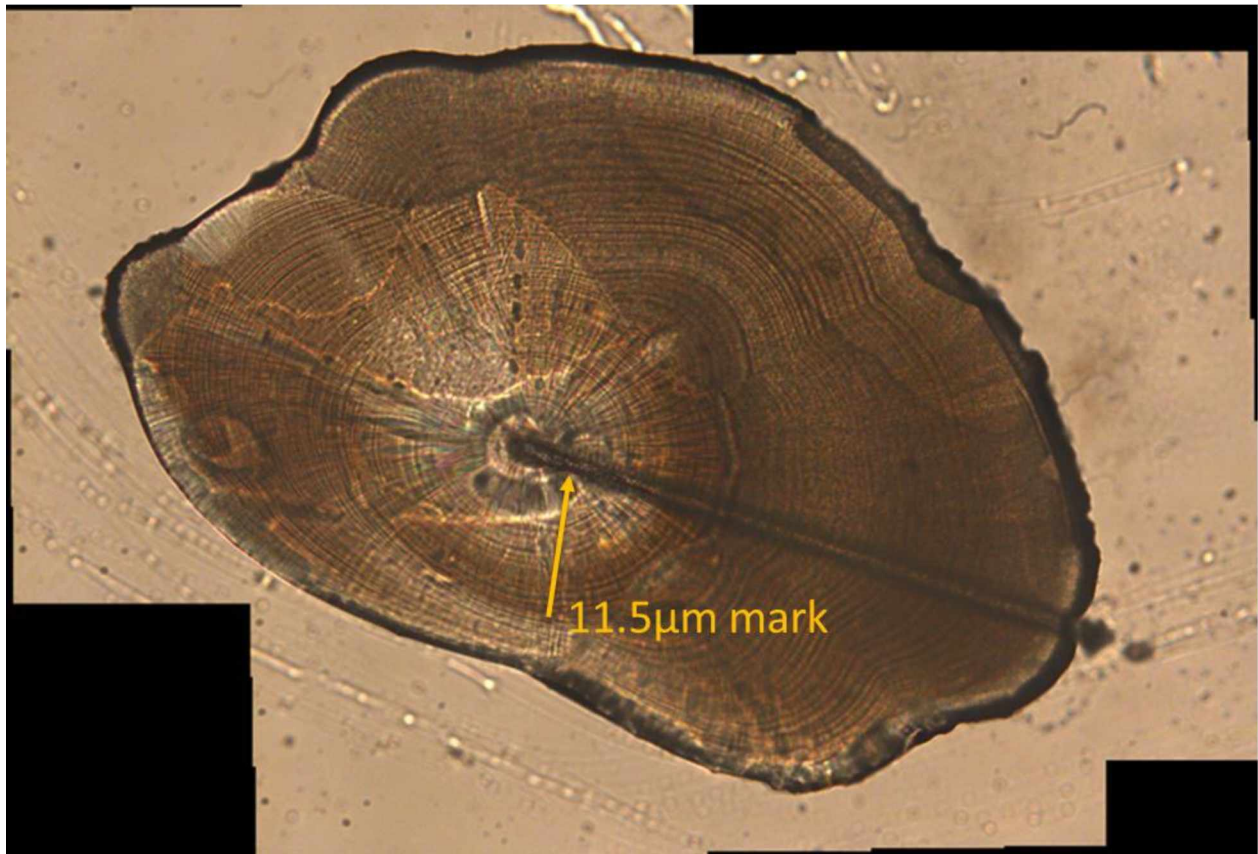
Figure 2.2: Polished otolith with ablation scar from laser ablation inductively coupled plasma mass spectrometer for determining trace elemental concentrations. The ablation point used for analysis was at 11.5 µm from the center just past the hatch mark.
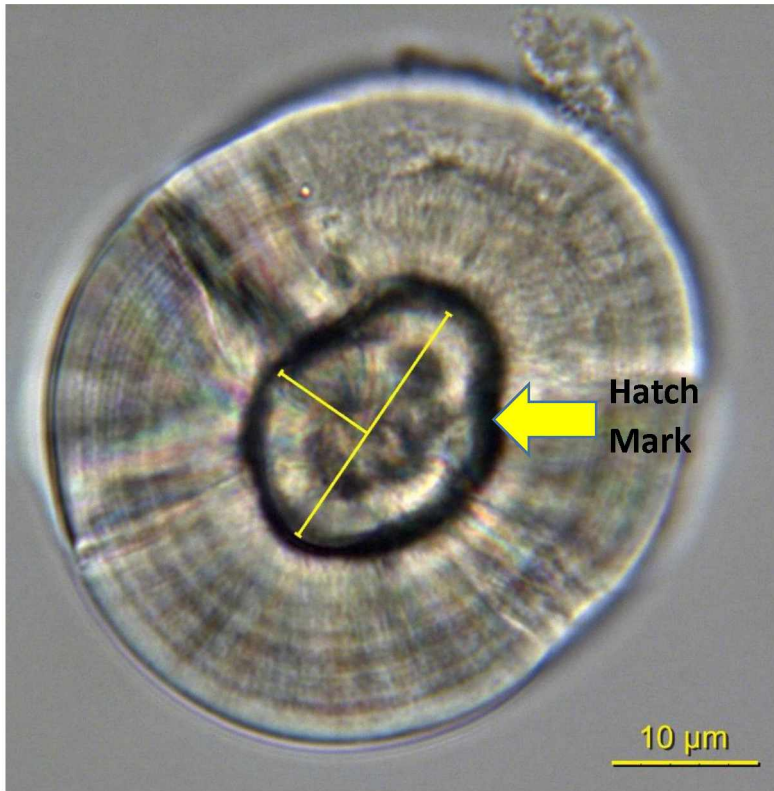
Figure 2.3: Sagittal otolith at 100X magnification from a lab-raised Arctic Cod with the hatch mark outlined.
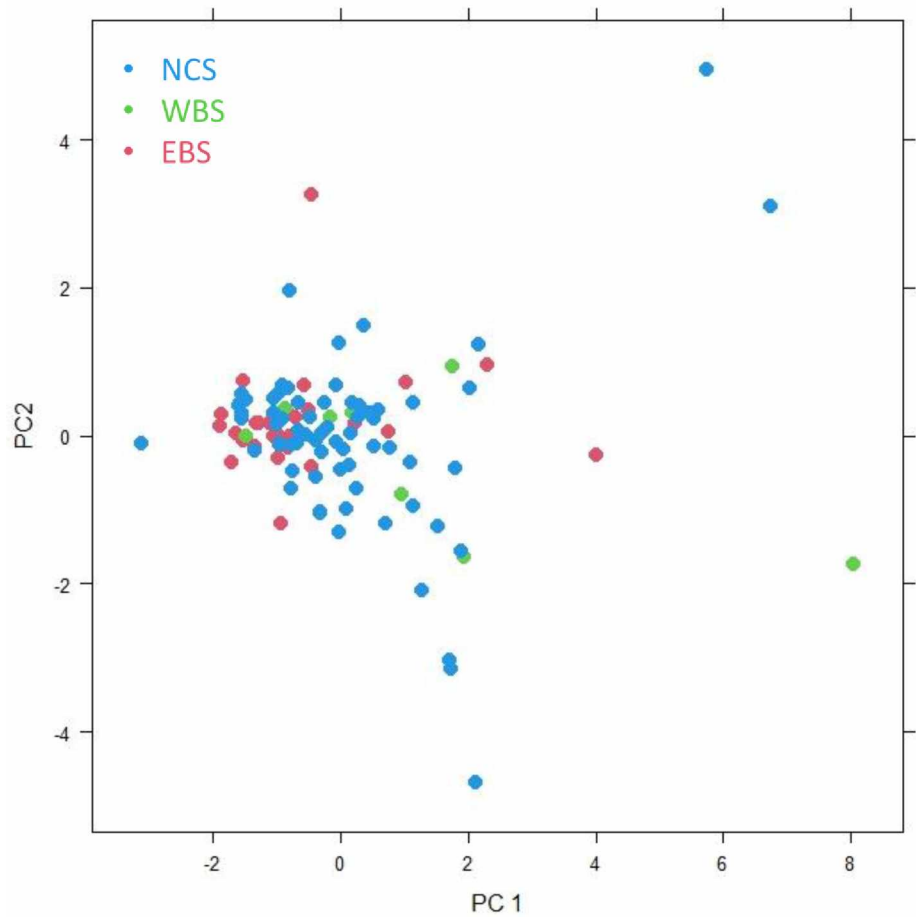
Figure 2.4: Principal components analysis ordination of 104 individual otoliths collected in the northern Chukchi Sea (NCS, blue), western Beaufort Sea (WBS, green), and eastern Beaufort Sea (EBS, red) based on the concentrations of seven trace elements standardized relative to Ca.

Figure 2.5: Log-transformed elemental ratios (black dots, jittered along x-axis) with estimated mean (blue bar) and 95% confidence intervals (grey shading) for the four ratios that differed significantly among three regions (Northern Chukchi Sea (NCS), Western Beaufort Sea (WBS), and Eastern Beaufort Sea (EBS)).

## 2.8    Tables

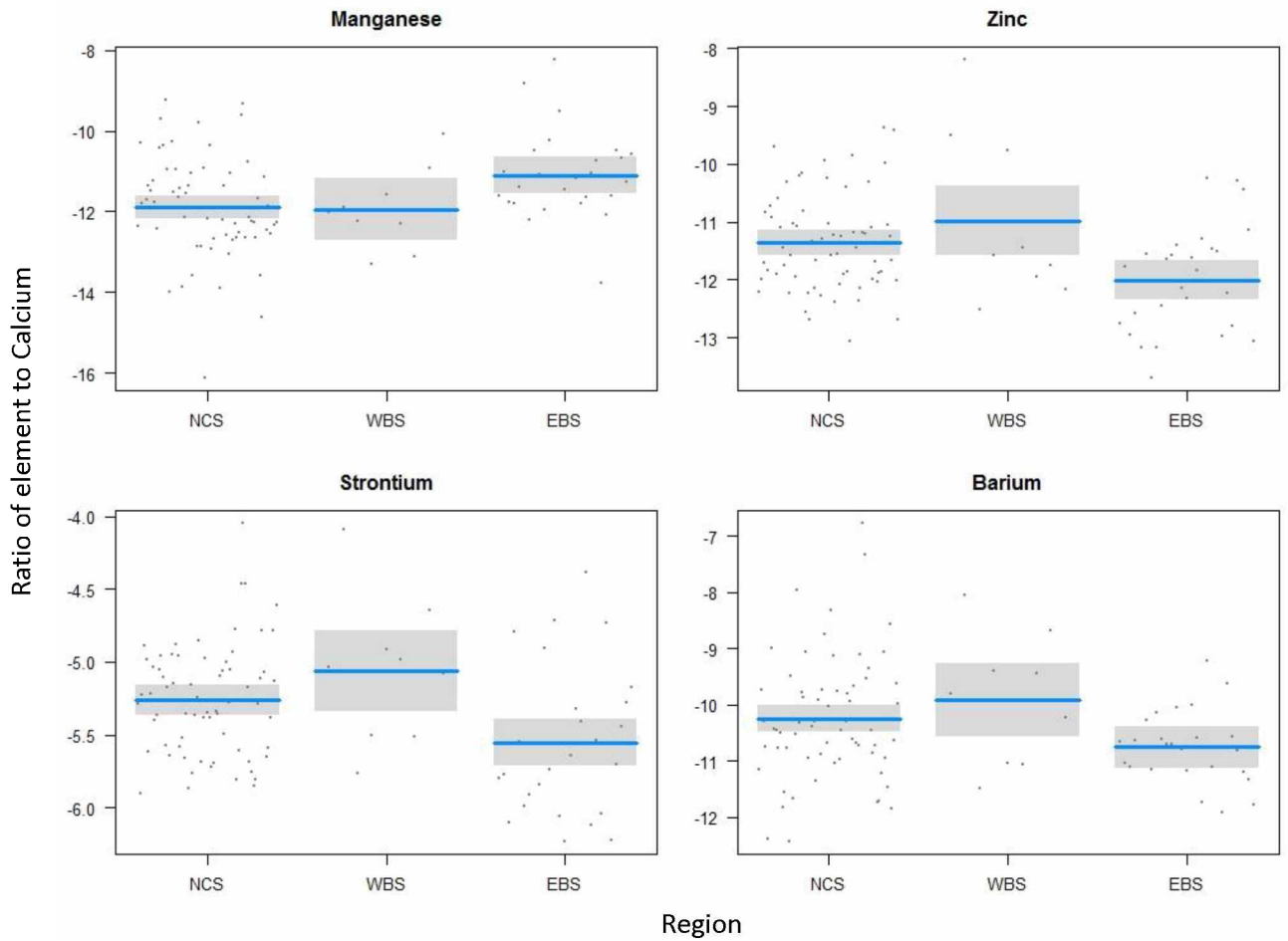Table 2.1: Summary of the sample size, mean, standard deviation, median, and range of the log-transformed elemental ratios for fish captured in the northern Chukchi Sea (NCS), western Beaufort Sea (WBS) and eastern Beaufort Sea (EBS).

| Element Ratios | Region | Sample Size | Mean | SD | Median | Min | Max |
|---|---|---|---|---|---|---|---|
| Li/Ca | NCS | 68 | -12.279 | 0.898 | -12.091 | -15.502 | -10.544 |
| Mg/Ca | NCS | 68 | -7.590 | 0.686 | -7.570 | -8.926 | -5.9132 |
| Mn/Ca | NCS | 68 | -11.812 | 1.226 | -11.908 | -16.114 | -9.214 |
| Cu/Ca | NCS | 68 | -10.905 | 1.677 | -10.987 | -13.891 | -7.314 |
| Zn/Ca | NCS | 68 | -11.338 | 0.934 | -11.452 | -13.069 | -8.196 |
| Sr/Ca | NCS | 68 | -5.212 | 0.402 | -5.195 | -5.899 | -4.050 |
| Ba/Ca | NCS | 68 | -10.129 | 1.069 | -10.292 | -12.423 | -6.774 |
| Li/Ca | WBS | 9 | -12.450 | 1.228 | -12.060 | -15.502 | -11.458 |
| Mg/Ca | WBS | 9 | -7.538 | 0.434 | -7.517 | -8.286 | -6.843 |
| Mn/Ca | WBS | 9 | -11.937 | 1.009 | -12.009 | -13.287 | -10.065 |
| Cu/Ca | WBS | 9 | -10.295 | 1.202 | -9.883 | -12.290 | -8.406 |
| Zn/Ca | WBS | 9 | -10.985 | 1.468 | -11.568 | -12.506 | -8.196 |
| Sr/Ca | WBS | 9 | -5.059 | 0.504 | -5.039 | -5.767 | -4.093 |
| Ba/Ca | WBS | 9 | -9.909 | 1.153 | -9.789 | -11.489 | -8.055 |
| Li/Ca | EBS | 27 | -12.453 | 1.053 | -12.363 | -14.992 | -10.007 |
| Mg/Ca | EBS | 27 | -7.777 | 0.507 | -7.816 | -8.923 | -6.795 |
| Mn/Ca | EBS | 27 | -11.093 | 1.085 | -11.174 | -13.783 | -8.231 |
| Cu/Ca | EBS | 27 | -11.370 | 1.372 | -11.369 | -14.094 | -8.720 |
| Zn/Ca | EBS | 27 | -12.004 | 0.915 | -11.839 | -13.689 | -10.249 |
| Sr/Ca | EBS | 27 | -5.554 | 0.506 | -5.639 | -6.229 | -4.384 |
| Ba/Ca | EBS | 27 | -10.754 | 0.621 | 10.740 | -11.923 | -9.209 |

Table 2.2: Pairwise Tukey HSD comparison of elemental ratios between regions for four trace elements that showed significant differences among regions (ANOVA). All elemental concentrations are expressed as a ratio relative to calcium. Regions are the northern Chukchi Sea (NCS), western Beaufort Sea (WBS), and eastern Beaufort Sea (EBS). Differences that were significant at the 90% significance level are highlighted.

| Element Ratio | Regions | P-Value |
|---|---|---|
| Manganese | WBS-NCS | 0.995 |
| | EBS-NCS | **0.011** |
| | EBS-WBS | 0.160 |
| Zinc | WBS-NCS | 0.481 |
| | EBS-NCS | **0.008** |
| | EBS-WBS | **0.013** |
| Strontium | WBS-NCS | 0.389 |
| | EBS-NCS | **0.008** |
| | EBS-WBS | **0.009** |
| Barium | WBS-NCS | 0.602 |
| | EBS-NCS | **0.070** |
| | EBS-WBS | **0.074** |

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General Conclusions:

The early life history of Arctic Cod is difficult to sample (Eriksen et al., 2020), but with the use of otolith analysis I was able to gain a better understanding of the timing and location of hatching of Arctic Cod in the northern Bering, Chukchi, and Beaufort seas. I used daily increment counts on otoliths of larval Arctic Cod to estimate age and established relationships between length and age to estimate hatch dates and growth rates by region. Using otolith microchemistry, I showed differences in the trace elemental composition of larval Arctic Cod otoliths at the time of hatching between the Chukchi and Beaufort seas and inferred water characteristics at the time of hatching. Using both the morphology (growth increments) and elemental composition of otoliths, I gained insights into hatching dynamics that are otherwise very difficult to obtain. This study, combined with previous research, has expanded our knowledge regarding Arctic Cod populations in the Alaskan Arctic. By combining these results with transport models, we may be able to identify discrete hatching locations and such information could be used to inform policy and management to best protect this vital species.

In Chapter 1, I identified the mean hatch dates of young-of-year Arctic Cod captured on the bottom or in the water column from several regions off Alaska. I found a general pattern of earlier hatching in more southern regions within a given season. Unexpectedly, the spring-captured fish in the southern Chukchi Sea (SCS) hatched much later in the year than those captured in the summer in either the SCS or northern Chukchi Sea (NCS). Their young ages and recent hatch dates indicated they hatched close to their capture location, consistent with proposed hatching in nearby Kotzebue Sound, associated with a late ice break-up in this nearshore region (Deary et al., 2021). The summer-captured fish usually hatched earlier in the year than spring-captured fish although there was some overlap. The presence of high concentrations of larvae and juveniles in the NCS that hatched over a period of several months suggests that they may have originated from multiple spawning events occurring at different times and in different places, and that they were transported to shared nursery grounds where multiple hatching populations mix and are retained for much of the summer (Levine et al. 2021). This prolonged hatching pattern is consistent with Bouchard and Fortier (2008), who showed that in many regions Arctic Cod hatching occurs from December through July. This is also consistent with biophysical modeling for the Chukchi Sea, which indicate that larvae from multiple hatch locations in the northern Bering Sea (NBS) and Bering Strait region are advected into the northern Chukchi Sea (Vestfals et al., 2021). This is the first time hatch dates have been estimated for the Bering and Chukchi seas, and provides additional hatch dates for Alaskan waters of the Beaufort Sea (Gallaway et al., 2017). I found some evidence of

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interannual variability in hatch dates that may be associated with the timing of ice retreat, but additional monitoring of hatch dates over multiple years will be needed to understand the possible impact of climate warming on hatch times of Arctic Cod.

The second goal of Chapter 1 was to estimate growth rates of Arctic Cod derived from field samples and to compare growth rates among regions. I found that growth rates differed among regions along a north-south gradient. The spring captured fish in the SCS had faster growth rates than the NBS fish, whereas the opposite pattern was seen for summer captured fish with southern fish having the greater growth rates. These results show that each region has its own set of factors that may alter the growth rate of these fish such as temperature and prey availability. These differences can also be attributed to hatch timing as northern fish hatched later and had a slower growth rate. The northern fish likely experienced later ice retreat and reared in colder temperatures than the southern regions. The first chapter demonstrates the power of otolith analysis as a tool to better understand the early life history of a species living in inaccessible waters.

The objectives for Chapter 2 focused on comparing the elemental ratios near the hatch mark in otoliths among regions to reconstruct environmental conditions at the time of hatching. Although there was considerable overlap in elemental compositions due to high variability, means differed significantly among regions. Several trace elemental ratios differed between the eastern Beaufort Sea (EBS) and the western regions, but not between the NCS and WBS. These results are consistent with differences in mean hatch dates and growth rates between the EBS and other regions (Chapter 1) as well as with previous studies showing distinct spatial distributions and genetic differences (Wilson et al., 2017, 2019; Forster et al., 2020; Nelson et al., 2020). Differences in elemental ratios between young-of-year Arctic Cod sampled in the EBS and those from the NCS and WBS further support the idea that these two groups have different origins. Elemental signatures in otoliths of EBS fish were consistent with a strong freshwater influence, suggesting that they hatched in the Mackenzie River plume, which may provide an important hatching and nursery area for Arctic Cod (Bouchard and Fortier, 2011).

The results from this work, along with previous studies, suggest that at least two major populations of Arctic Cod exist off Alaska: 1) a southern population spawns and hatches from the northern Bering Sea to the southern Chukchi Sea and supplies larvae to a major nursery area that is concentrated in the northeast Chukchi Sea and may extend into the western Beaufort Sea (De Robertis et al., 2017; Levine et al., 2021; Vestfals et al., 2021) and 2) a northern population spawns and rears in the Canadian Beaufort Sea, extending into the Alaskan portion of the eastern Beaufort Sea. The extent

to which these two populations may mix during later life stages is not known. Within each region, there are likely to be local differences in spawning and hatching as suggested by the protracted distribution of hatch dates present within each region. For example, I identified a relatively short hatching event in the SCS that may reflect a distinct spawning event in Kotzebue Sound associated with a late ice break-up (Deary et al., 2021). Possible hatching in or near Kotzebue Sound, which is located 50 miles downstream from the world's largest Zinc mine, was further supported by elevated levels of Zinc in Arctic Cod sampled in the Chukchi Sea. This example shows that the combination of physical and elemental characteristics of otoliths can provide much needed data to better understand Arctic Cod early life history.

Gaining a better understanding of the early life history of a keystone species such as Arctic Cod is crucial to more informed management. This is especially true in the rapidly changing Arctic environment. Arctic Cod have a narrow temperature tolerance and projected temperatures for the southern extent of their range will likely be exceeded in the future. Subartic fishes are already moving north into the northern Bering Sea (Lauth et al., 2019) and into the Chukchi Sea (Orlov et al. 2020). This increased borealization of the fish community in the Pacific Arctic is expected to continue with further expansions of southern species into the Chukchi Sea (Alabia et al. 2018). Many of these migrating species have a growth advantage at warmer temperatures and may outcompete Arctic Cod for resources as temperatures continue to rise (Laurel et al., 2015, Marsh and Mueter, 2020). Increased competition with southern species, coupled with a decrease in Arctic Cod body condition, can lead to a shift in the ecology of Arctic food webs. Projections indicate that southern portions of the Arctic Ocean will be ice free year-round by 2050 (Onarheim et al., 2018) which could have significant impact on the spawning and hatching of Arctic Cod that occurs near what is currently the southernmost extent of sea ice in the region. The response of Arctic Cod to further reductions in sea ice is an active area of study and the information gained through this thesis will contribute to research models for Arctic Cod and developing appropriate management strategies. The data presented here provide a baseline for future monitoring, but more field research and continued monitoring will be needed to resolve how Arctic Cod will cope with a changing environment.

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Appendices

Appendix A: Determining age-0 cutoff length.
Length frequency plots from the Chukchi and Beaufort seas-captured Arctic Cod used to determine the cutoff for age-0 fish by looking for the gap or dips in length.
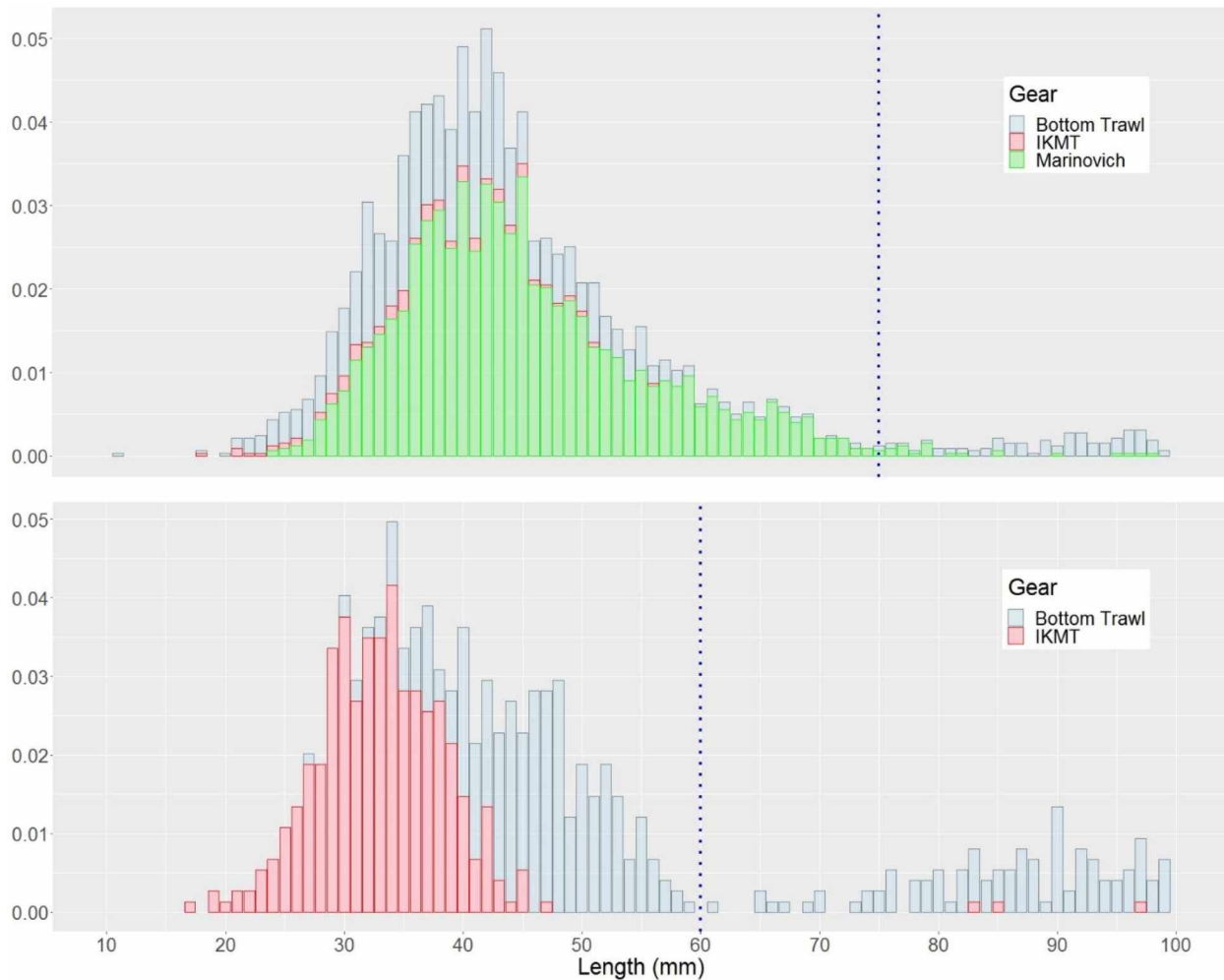


Figure A.1: Full length (mm) distributions from the Chukchi Sea (top) and Beaufort Sea (bottom) with a vertical line indicating length cutoff for age-0 Arctic cod. The gear type used for sampling is indicated by color with blue representing bottom trawl, red representing Isaacs-Kidd Midwater Trawl (IKMT), and green representing Marinovich trawl.

## Appendix B: Determining age for otoliths with damaged core

During the process of grinding otoliths to reveal daily increments, the innermost section of the otolith would occasionally become unreadable. This unreadable zone had a maximum length of 92 μm with a mean length of 52 μm. To predict the number of increments for the otoliths with an unreadable zone (n=38), a regression model was developed to predict the cumulative number of growth increments as a function of distance from the core and other available covariates. The relationship between the cumulative number of increments and distance from the otolith core was approximately linear for all otoliths with complete counts (Figure A1). We therefore fit a linear regression of the number of cumulative increments (cum_incr) as a function of distance from the core for all otoliths with complete increment counts. Regressions were fit to all increments between the hatch mark and 100 μm from the core. There was high variability in mean increment width across otoliths because of variations in individual growth and because increments were counted along transects with different locations on the otoliths. Therefore, we included a measure of mean increment width for a given otolith as a covariate in the regression. Mean increment width was computed for each otolith over a section of the otoliths from 100 μm to either 150 μm or the edge of the otolith, whichever was shorter. The slope of cum_incr on distance from the core was allowed to vary among regions as a fixed region effect and among individuals within regions as a random effect of individual, resulting in the following model:

$$cum\_incr_{r,i,k} = \alpha_r + a_i + \beta 1_r * dist_{r,i,k} + b_i * dist_{r,i,k} + \beta 2 * MIW_{r,i} + \beta 3 * (MIW_{r,i} * dist_{r,i,k})$$
$$+ \varepsilon_{r,i,k}$$

where $cum\_incr_{r,i,k}$ is the cumulative increment k, corresponding to age in days, for otolith *i* in region *r*, $dist_{r,i,k}$ is the corresponding observed distance from the core, $MIW_{r,i}$ is the mean increment width between 100 and 150 μm for otolith *i* in region *r*, $\alpha_r$ and $\beta_r$ are the intercept and slope of the mean relationship between distance and number of increments for region *r*, $a_i$ and $b_i$ are random intercepts and slopes of this relationship for otolith *i* that are assumed to be normally distributed with mean 0, variances $\sigma_a^2$ and $\sigma_b^2$ and covariance $\sigma_{ab}^2$, and the $\varepsilon_{r,i,k}$ are residuals that are assumed to be normally distributed with mean 0 and variance $\sigma_\varepsilon^2$. Region, mean increment width between 100 and 150 μm and distance from core to the first readable increment were then used to predict the number of missing increments for otoliths with incomplete increment counts using the estimated parameters.

The predicted increments for each otolith were combined with the visible increments to estimate the age of the individual.
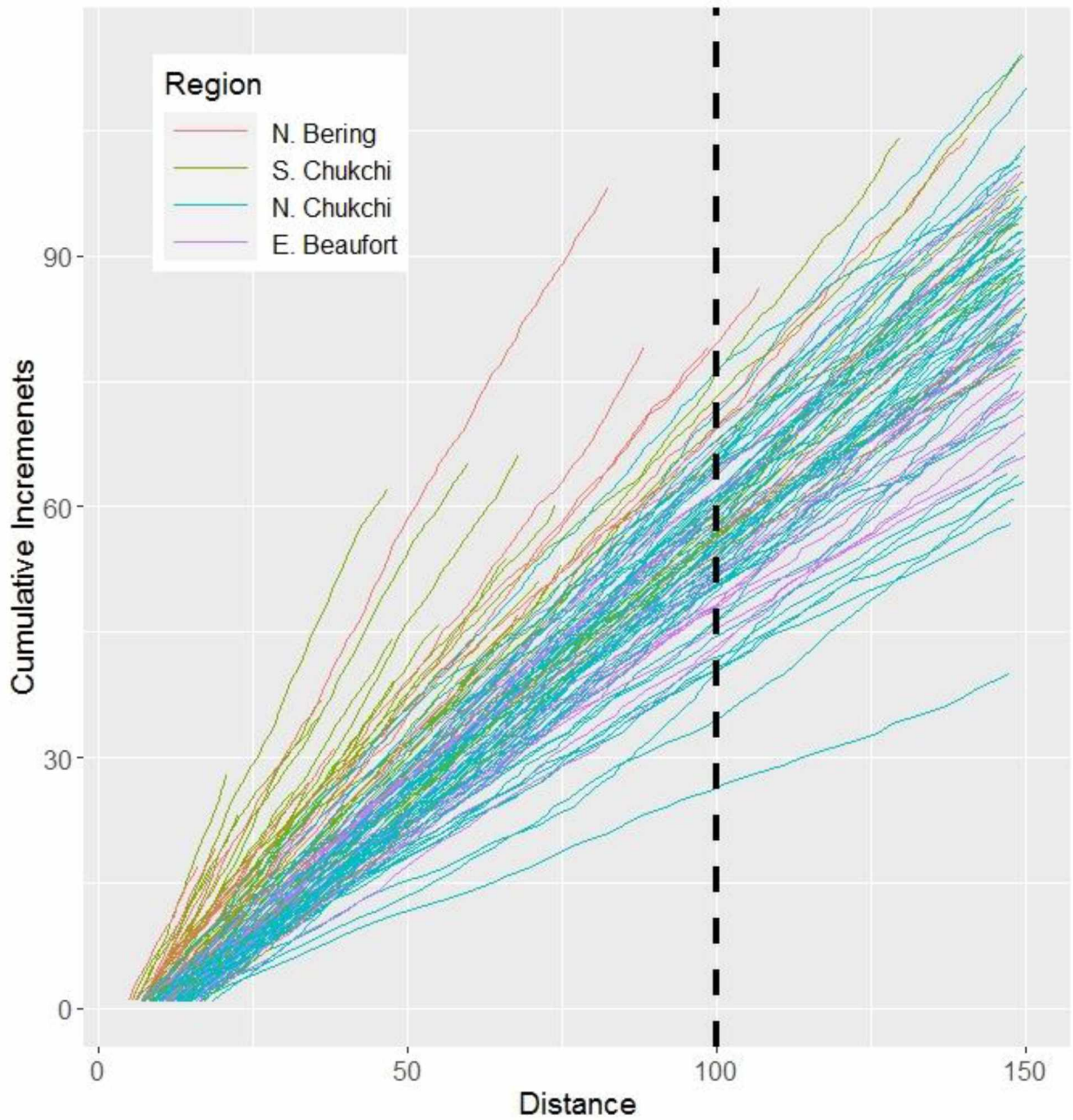


Figure B.1: Number of otolith's increments against cumulative distance (< 150 μm) from the otolith core for all otoliths with complete increment counts by region.