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The Influence of Water Flow, Water Conditions, and Seasonality on Fish Communities in Estuarine Nearshore Habitats in Kachemak Bay, Alaska

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Identifying Hatch Dates and Potential Hatch Location of Arctic Cod (*Boreogadus saida*) through Otolith Analysis

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Abstract

Estuarine and nearshore habitats are critical nurseries and feeding grounds for juvenile fish. The coastal habitats of the northern Gulf of Alaska are home to numerous juvenile fish species that serve as forage, recruitment populations, and ecological role-players in the broader Alaskan marine coastal ecosystem. River mouths are a particularly important coastal nearshore habitat type used by these fish. This project examined how water flow and other environmental conditions (water temperature, salinity, dissolved oxygen, and turbidity) influence the seasonality of estuarine nearshore fish communities in Kachemak Bay, a large embayment in Cook Inlet in the Gulf of Alaska. This goal was addressed by (1) characterizing seasonal trends in water flow and water conditions at six estuarine nearshore sites, (2) characterizing seasonal trends in fish community structure at those sites, and (3) determining the strength of influence that water flow and other environmental water conditions have on estuarine nearshore fish community structure. To assess fish community structure, beach seining was conducted at three ‘high flow’ and three ‘low flow’ sites throughout the summer and fall of 2018. Water flow and other water conditions were characterized at each site and then correlated to fish community structure. Forage species (e.g., Pacific sand lance, Pacific herring, smelts) were found to associate with high flow sites. Benthic-dwelling species (e.g., crescent gunnels, snake pricklebacks) and multi-year rearing salmon (e.g., coho, sockeye, and chinook salmon) associated more with low flow sites. Many fish groups had seasonal occurrences, including salmonids, smelt, cods, and small flatfish and sculpin. All of the factors studied here were significantly correlated to variability in fish species abundance. Water flow, dissolved oxygen, and seasonality explained a relatively larger proportion of the variance in fish community structure compared to temperature, salinity, and turbidity.

Introduction

Globally, estuarine and nearshore habitats are regarded as critical nursery and feeding grounds for juvenile fish (Simenstad et al. 1982; Bennett 1989; Blaber et al. 1995). These habitats enhance juvenile recruitment to adult populations, many of which are valued for cultural or economic reasons (Beck et al. 2003; Magnusson and Hilborn 2003; Dahlgren et al. 2006). River mouths (herein, referred to as the estuarine nearshore) are recognized as an important habitat within the nearshore seascape 'mosaic' (Nagelkerken et al. 2015) because they have high lower-trophic production and provide favorable water conditions for fish (St. John et al. 1992; Hoem-Neher et al. 2013; Brodeur and Morgan 2016). In the coastal marine ecosystem, these findings come from studies that have mostly focused on forage species (Pikitch et al. 2014). Forage fish are a critical link between lower-trophic production and upper-trophic predators, such as larger fish, marine mammals, and sea birds (Springer and Speckman 1997). The juvenile fish that are not preyed upon may recruit into adult populations that depend on forage species for food (Sheaves et al. 2015)**.** In the case of Pacific salmon, juvenile and adult populations utilize the same estuarine nearshore habitat at different times, driven by the seasonality in their life history traits (Quinn et al. 1992). In addition to supporting forage and adult populations, the estuarine nearshore is home to a range of predator types and ecological role players (e.g., flatfish, sculpins, snailfish, gunnels). Whole system functions, such as the cycling of nutrients and trophic transfer of biomass, are afforded by the collective members of the estuarine nearshore fish community who, together, constitute the overall value of these habitats (Sheaves et al. 2015).

Fish in the estuarine community can be categorized into marine, freshwater, estuarine, and diadromous types based on the varied functional uses exhibited by each species (Elliot et al. 2007; Potter et al. 2015). In addition to nursing and feeding, fish utilize estuarine habitats as refugia and as migration corridors that connect with other coastal habitats (Simenstad et al. 1982; Gillanders et al. 2003). While marine and freshwater fish are more stenohaline or freshwater in nature, respectively, they may also be in estuarine waters as facultative opportunists (e.g., for feeding purposes) or accidently (e.g., caught on the other side of a salt wedge). Fish in the estuarine category are obligate users and include those species found solely in estuaries; however, estuarine fish can also be adapted to marine or fresh waters. Estuarine fish also include larval 'migrants' that leave for some reason (e.g., tidal flushing) and later return. Diadromous fish encompass all those considered anadromous and catadromous and those with partial migration routes attributed to either life history. These are different than estuarine migrants in that diadromous fish migrate specifically for spawning-related purposes. Finally, certain fish are viewed as estuary-dependent (Ray 2005), which typically includes estuarine and diadromous fish. The extent to which marine and freshwater fish can be deemed estuary-dependent is neither clear nor agreed upon (Potter et al. 2015); however, this does not preclude them from membership in the estuarine fish community.

The species composition and related spatial and temporal variability of estuarine fish communities is well-documented around the world (Potter et al. 1997; Abookire et al. 2000; Simenstad et al. 2006; Boughton et al. 2007). Species composition is influenced by the environmental variability of the estuary, particularly salinity (Wolanski and Elliott 2015), and other water characteristics like temperature, dissolved oxygen, and turbidity (Benfield and Minello 1996; Abookire et al. 2000; Boughton et al. 2007); anthropogenic disturbances (Perez-Dominguez et al. 2012); and water flow patterns (Poff and Zimmerman 2010). Anthropogenic disturbances and water flow patterns can alter the variability of other conditions and their influence on fish (Simenstad et al. 2006; Kornis et al. 2017). Due to the mixing of fresh and marine waters, the water quality of any particular estuarine system can exhibit a wide range of conditions, most notably a variance in salinity. Typically, the influx of marine water results in decreased water temperature and increased dissolved oxygen content, and the influx of fresh water results in increased turbidity and decreased salinity (Wolanski and Elliott 2015). The estuarine nearshore is considered brackish, but it can be strongly influenced by nearshore forces (e.g., extreme tidal flux), allowing stenohaline species to appear in close-to-shore habitats (Elliott et al. 2007). Human-caused disturbances (e.g., shoreline hardening, landscape alterations, oil spills) are a major concern for highly trafficked habitats like estuaries and can alter the aforementioned conditions (Kornis et al. 2017) and influence the structure of estuarine fish communities. Finally, water flow patterns have been shown to influence fish populations (Bunn and Arthington 2002; Simenstad et al. 2006; Elliott et al. 2007; Langerhans 2008). Determining a system’s ‘natural flow’ regime is often the aim for research and ecosystem-based management efforts in rivers and estuaries (Poff et al. 1997, Poff and Zimmerman 2010; Jickells et al. 2014). Since the timing and force of waters flowing in the estuary are always changing (Wolanski and Elliott 2015), land managers aim to define a system’s natural flow variability as a specific regime (Poff et al. 1997) that can be referenced as a mitigation or restorarion measure against anthropogenic disturbances (Poff and Zimmerman 2010; Toft et al. 2018). While flow regimes are useful in this way, few studies have directly examined how natural flow conditions drive fish community structure (Maes et al. 2004; Selleslagh and Amara 2008; Ferguson et al. 2013).

The structures of nearshore fish communities in the northern Gulf of Alaska have been well documented (Blackburn 1980; Robards et al. 1999a; Abookire et al. 2000); however, given the temporal variability expected for these fish communities (Robards et al. 1999a), their current structure may differ from past studies. Furthermore, prior studies did not specifically examine estuarine nearshore habitats. This study provided benchmark data on the species abundance and seasonal occurrence of estuarine nearshore fish in the study area and examined how water flow influences estuarine nearshore fish communities.

The study area for this project focused on the southern coast of Kachemak Bay, a large embayment of Cook Inlet in the northern Gulf of Alaska. Recent oil and gas lease sales in Cook Inlet (Lease Sales Report, BOEM 2018) have raised concerns about impacts should oil disperse into the coastal environment. Understanding the structure of estuarine nearshore fish communities would help forecast how these systems might be affected. Under natural conditions, water flow was hypothesized to be a stronger influencer of these communities than salinity, temperature, dissolved oxygen, and turbidity.

Objectives

1) Characterize seasonal trends in water flow and water conditions (temperature, salinity, dissolved oxygen, turbidity) at six estuarine nearshore sites.
2) Characterize seasonal trends in fish community structure (species composition and abundance) at six estuarine nearshore sites.
3) Determine the strength of influence that water flow has on estuarine nearshore fish community strcuture among other water conditions and seasonal variability.

Methods

Study Area

Kachemak Bay, Alaska (59°34’00” N, 151°33’00” W) is a large estuarine system located on the eastern coast of lower Cook Inlet in the northern Gulf of Alaska (Figure 1). The southern coast exhibits heterogeneous coastal formations, with deep fjords in the outer bay and more bight-like formations in the inner bay. The coastline is interspersed with rivers that vary in nearshore water quality because the associated watersheds differ in catchment size, elevation, gradient, and water source (e.g., glacial, snowmelt, and precipitation) (Hood and Scott 2008; O’Neel et al. 2015). The area also has along-bay oceanographic gradients (e.g., temperature, salinity, dissolved oxygen, and turbidity) caused by marine waters flowing in from the Gulf of Alaska and an accumulation of river input as water moves inward to the head of the bay (Burbank 1977; Bentz et al. 2018). The physical habitat along the southern coast is also heterogeneous and includes steep sand and gravel beaches and low-gradient mudflats (Harney et al. 2008).

Site Selection

The study focused on six estuarine nearshore sites associated with different watersheds along the southern coast of Kachemak Bay (Figure 1). Diurnal tides in the area have a vertical range exceeding eight meters (Adams et al. 2007); thus, the water depth and physical habitat available to inshore fish can vary dramatically within a single tide exchange. Since no data were available on flow conditions, the sites were selected *a priori* using broad-scale sea surface circulation patterns mapped for the area (Bentz et al. 2018). Three low flow sites (Halibut Cove, Tutka Bay, and Seldovia Harbor) were selected within ‘less exposed’ coastal embayments that were expected to have weaker flow patterns and, for contrast, three high flow sites (Glacier Spit, China Poot, and Barabara) were selected that were ‘more exposed’ to the bay’s circulation patterns. The six sites were dispersed along the bay’s southern coast to account for along-bay water quality gradients, some of which have been related to nearshore fish distribution (Abookire et al. 2000; Speckman et al. 2005). Since the sites were selected based on an assumption of local flow patterns, the sampling design was confirmed with field measurements of water flow.

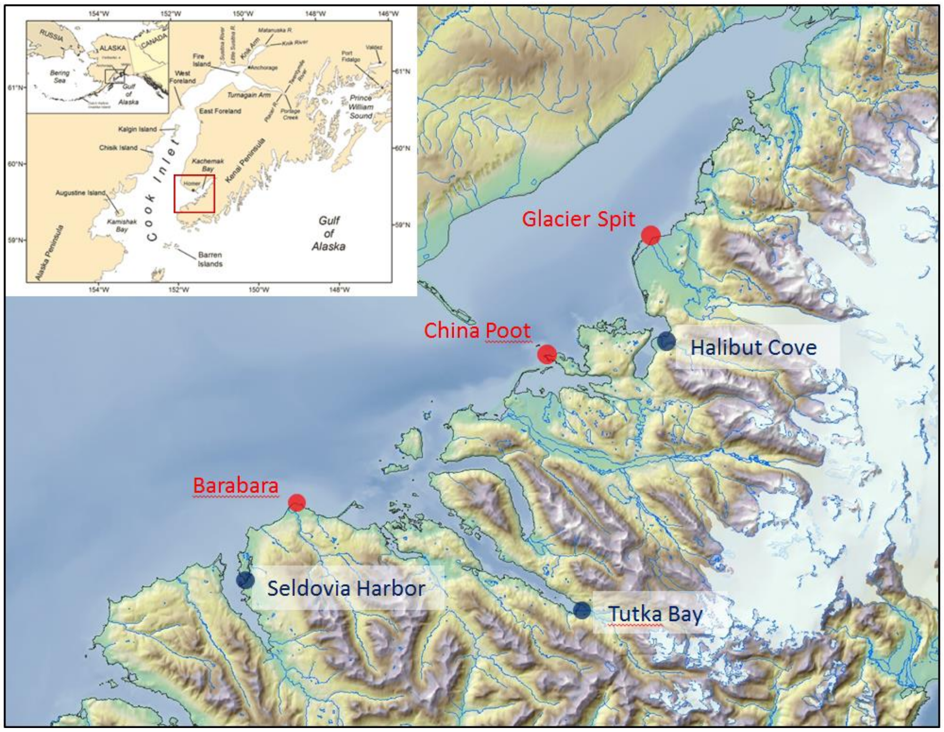

Figure 1. Map of Kachemak Bay and sampling sites (red=high flow, blue=low flow). Topographical attributes include glacial coverage (white) and major hydrological features (blue).

Sampling

Fish collection

Study sites were sampled during the same tide series and approximtely every two weeks from late-June to mid-October 2018. Fish were collected using a 34 m tapered nylon mesh beach seine. The net's bunt dimensions were 8 m width by 5 m depth with 3 mm mesh, which is similar to gear that was used in prior studies (Blackburn et al. 1980; Robards et al. 1999a; Abookire et al. 2000). Each set of the beach seine was considered a single replicate. A set involved deploying the net parallel to shore then hauling it in with 30 m ropes attached to each end. During a site visit, up to four replicates were conducted depending on the time required for processing the catch from the previous set. The linear distance from the middle of the bunt to the shoreline was considered the sampling reach. Since the number of sets per site visit could differ, the combined sampling reach was used to measure effort per site visit. Beach seining was restricted to within two hours of the low slack tide to consistently sample the subtidal habitat and reduce effects related to tidal stage. All fish caught were identified to the lowest taxonomic level (usually species), counted, and released live.

Water characteristics

Water characteristics were measured immediately after each beach seine at the location where the bunt of the net was initially deployed. Surface measurements for temperature (°C), salinity, dissolved oxygen (mg/L) and turbidity (FNU) were made using a multi-parameter water quality sonde (YSI, EXO1 Water Quality Sonde) at one-meter depth or half the distance to the sea floor. The probes used to measure each parameter were calibrated monthly using manufacturer software (YSI, KOREXO v1.59).

Water flow

Water flow was measured using continuously recording data loggers (Tilt Current Meter 1, Lowell Instruments LLC) placed near the mouth of each site's outflow channel and submerged at a standard depth 2 m below the mean lower low water (MLLW) tide level. The rod-shaped instruments are designed to attach at one end by an anchor while the other end remains buoyant, resulting in a vertical orientation. Water drag causes the meter to tilt away from its neutral axis. Within each current meter, an accelerometer was programmed to make a 20-second 'burst' data recording every 15 minutes from May 23 to June 25, 2018. Insufficient data were collected during the rest of the summer/fall (from late-June to mid-October) for water flow to be used as a continuous variable in analyses. Post data collection, a single vectorized water speed (cm/s) was calculated for each 15-minute data record using manufacturer software (Lowell Instruments LLC). Water flow measurements were used to calculate a 'flow score' for each site and to confirm the high flow versus low flow sampling design (i.e., 'flow class').

Data Analyses

Data analyses were performed using the statistical software R (R Core Team 2019) and the R package vegan (Oksanen et al. 2019). Species abundance was measured as a catch-per-unit-effort (CPUE) calculated as the number of individuals per linear meter of seining reach. Average species abundance was calculated using all seine replicates from a site visit, and a site visit was considered a sample of the fish community in multivariate analyses. Species CPUE were treated with a fourth-root transformation prior to analyses due to certain species having extremely high abundances. Environmental variables consisted of seasonality (as Julian day), site flow score, and salinity, temperature, salinity, dissolved oxygen, and turbidity averaged by site visit. All environmental variables were standardized to a mean of zero and a standard deviation of one prior to analyses. Turbidity data were treated with a natural log transformation due to the raw data being strongly right skewed. The total number of site visits was less than anticipated due to incomplete sampling caused by inclement weather and equipment malfunction.

The influence of environmental variables (i.e., water flow, water characteristics, and seasonality) on estuarine nearshore fish community structure (i.e., species composition and abundance) was tested using multivariate methods for examining patterns in community data (Clarke 1993). Ecological distances were calculated for fish community structure using Bray-Curtis distance and for environmental variables using Euclidean distance. Variability in fish community structure was visualized using a non-metric multidimensional scaling (NMDS) ordination. The relationship between environmental variability and fish community structure was interpreted using an indirect gradient analysis (BIOENV, Clarke and Ainsworth 1993). This relationship was then examined with randomization tests (i.e., Mantel), which provided a 'best set' of explanatory environmental variables based on Spearman rank correlation. Homogeneity of multivariate dispersion (beta-diversity) was examined among sampling periods and flow groupings to assess whether variability differed among the periods and groups being compared (BETADISPER, Anderson et al. 2006). Variance in fish community structure was partitioned among environmental variables and evaluated for significance using permutation-based multivariate analyses of variance models (PERMANOVA, McArdle and Anderson 2001). A sequential (‘Type I’) PERMANOVA model was used to account for the effect of seasonality, then flow score, and then the rest of the environmental variables. The least effect of water flow on fish community structure was determined with a marginal (‘Type III’) PERMANOVA model that first accounted for all other significant variables. The species that contributed most to differences in variability of fish communities between flow class groups were identified with a test for dissimilarity percentage based on Bray-Curtis distances (SIMPER, Clarke 1993).

Results

Seasonal Variability in Fish Community

A total of 33,866 fish from 46 species were collected from June 25 to October 16, 2018. Across all seasons and sites, the most abundant group of fish were sculpins (Cottidae) (Table 1). Other major fish groups (i.e., groups composing more than five percent of the total CPUE across all sites and seasons) included cods (Gadidae), flatfish (Pleuronectidae), salmonids (*Salmonidae*), greenlings (*Hexagrammodae*), and Pacific sand lance (*Ammodytidae*). Within each major group, a single species contributed disproportionately to the total CPUE. These included great sculpin (*Myoxocephalus polyacanthocephalus*), saffron cod (*Eleginus gracilis*), rock sole (*Lepidopsetta spp.*), Dolly Varden (*Salvelinus malma*), white spotted greenling (*Hexagrammos stelleri*), and Pacific sand lance (*Ammodytes hexapterus*). Saffron cod was the most abundant species, followed by great sculpin, rock sole, sand lance, and Dolly Varden.

Table 1. Complete list of all fish collected. Taxa are sorted by the proportional contribution of each group (family) to the total catch-per-unit-effort (CPUE) of all fish across all sites and seasons, then listed in descending order of species abundance.

FAMILY	%CPUE[1]	COMMON NAME	SCIENTIFIC NAME	CPUE
Cottidae	24.2	Great Sculpin	*Myoxocephalus polyacanthocephalus*	48.4
		Micro Sculpin[2]		21.5
		Buffalo Sculpin	*Enophrys bison*	18.9
		Staghorn Sculpin	*Leptocottus armatus*	17.3
		Tidepool Sculpin	*Oligocottus maculosus*	10.4
		Scalyhead Sculpin	*Artedius harringtoni*	6.1
		Armorhead Sculpin	*Gymnocanthus galeatus*	3.9
		Padded Sculpin	*Artedius fenestralis*	1.7
		Red Irish Lord	*Hemilepidotus hemilepidotus*	1.4
		Shorthorn Sculpin	*Myoxocephalus scorpius*	1.0
		Sharpnose Sculpin	*Clinocottus acuticeps*	1.0

FAMILY	%CPUE[1]	COMMON NAME	SCIENTIFIC NAME	CPUE
Gadidae	17.5	Saffron Cod	*Eleginus gracilis*	55.4
		Pacific Cod	*Gadus macrocephalus*	20.3
		Tom Cod	*Microgadus proximus*	15.1
		Walleye Pollock	*Gadus chalcogrammus*	4.2
Pleuronectidae	16.1	Rock Sole	*Lepidopsetta spp.*	46.0
		Starry Flounder	*Platichthys stellatus*	17.8
		English Sole	*Parophrys vetulus*	10.8
		Micro Flatfish[2]		7.9
		Sand Sole	*Psettichthys melanostictus*	4.8
Salmonidae	10.4	Dolly Varden	*Salvelinus malma*	30.2
		Pink Salmon	*Oncorhynchus gorbuscha*	8.6
		Chum Salmon	*Oncorhynchus keta*	7.4
		Coho Salmon	*Oncorhynchus kisutch*	6.0
		Sockeye Salmon	*Oncorhynchus nerka*	3.7
		Chinook Salmon	*Oncorhynchus tshawytscha*	0.7
Ammodytidae	6.6	Pacific Sand Lance	*Ammodytes hexapterus*	35.9
Hexagrammodae	6.5	White Spotted Greenling	*Hexagrammos stelleri*	16.8
		Lingcod	*Ophiodon elongatus*	8.6
		Masked Greenling	*Hexagrammos octogrammus*	6.9
		Kelp Greenling	*Hexagrammos decagrammus*	1.9
		Rock Greenling	*Hexagrammos lagocephalus*	1.3
Pholidae	5.0	Crescent Gunnel	*Pholis laeta*	26.3
		Penpoint Gunnel	*Apodichthys flavidus*	0.6
Clupeidae	4.7	Pacific Herring	*Clupea pallasii*	25.6
Stichaeidae	2.9	Snake Prickleback	*Lumpenus sagitta*	14.4
		Slender Eelblenny	*Lumpenus fabricii*	1.2
Osmeridae	2.2	Capelin	*Mallotus villosus*	8.7
		Surf Smelt	*Hypomesus pretiosus*	2.0
		Longfin Smelt	*Spirinchus thaleichthys*	1.2
Liparidae	1.1	Snailfish	*Liparis spp.*	6.2
Larval[3]	1.1	Larval[3]		5.8
Agonidae	0.7	Sturgeon Poacher	*Podothecus accipenserinus*	2.4
		Tubenose Poacher	*Pallasina barbata*	1.4
Gasterosteidae	0.5	Three Spine Stickleback	*Gasterosteus aculeatus*	2.9
Hemitripteridae	0.3	Silver Spotted Sculpin	*Blepsias cirrhosus*	1.8
Trichodontidae	0.2	Sandfish	*Trichodon trichodon*	1.0

[1] '%CPUE' was calculated as the proportional contribution of each group (family) to the total CPUE across all sites and seasons.
[2] 'Micro' sculpin and flatfish were individuals less than 30 mm in total length and unidentifiable beyond family.
[3] 'Larval' fish were individuals less than 20 mm and indiscernible to any particular group or family.

Average CPUE was variable throughout the sampling period for almost all fish species (Figure 2). Except for a few extremely high catches in cod and sand lance at Barabara and China Poot, respectively (Figure 2), there was a noticeable reduction in catches for major fish groups (i.e., groups composing more than five percent of the total CPUE across all sites and seasons) during the fall months (September–October) (Figure 3). Average cod CPUE increased throughout the sampling period before October. Average salmonid CPUE was temporally consistent except for in October. Average CPUE remained temporally consistent for other major groups, including sculpin, flatfish, and greenlings. Average sand lance CPUE was the only major fish group to increase in October.

Beta-diversity in fish community structure, which is a measure for homogeneity of multivariate dispersion, was not significantly different when comparing across months (ANOVA, $F = 1.815$, $p = 0.151$). On coarser seasonal scales, a significant difference in beta-diversity was found amongst early (June–July), mid (August), and late (September–October) sampling periods (ANOVA, $F = 4.224$, $p = 0.024$). Tukey pairwise comparisons revealed a significant difference between mid and late sampling periods ($p = 0.033$), not early and mid periods ($p = 0.928$). The difference between early and late periods had a weak significance ($p = 0.053$). Thus, early and mid periods were combined into a summer period (June–August) to be compared to a fall period (September–October) in subsequent SIMPER tests.

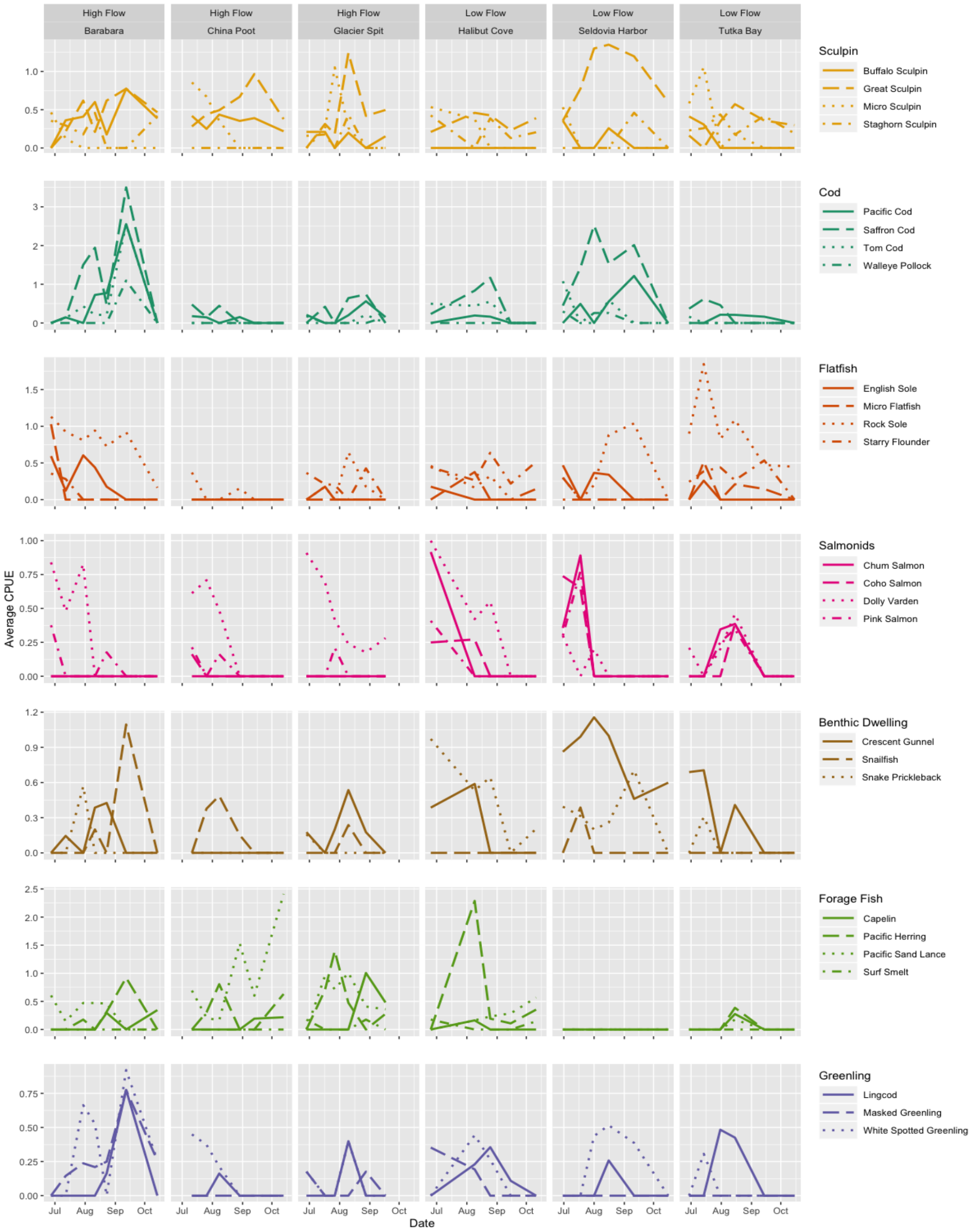

Figure 2. Average CPUE for the three to four most abundant species (or with CPUE ≥ 2) within select fish groups across all seasons (see also Table 1). Graphs show high flow sites (left three columns), low flow sites (right three columns), CPUE trends over collection dates (line), and select species (line-type). Error values were not included for visualization purposes.

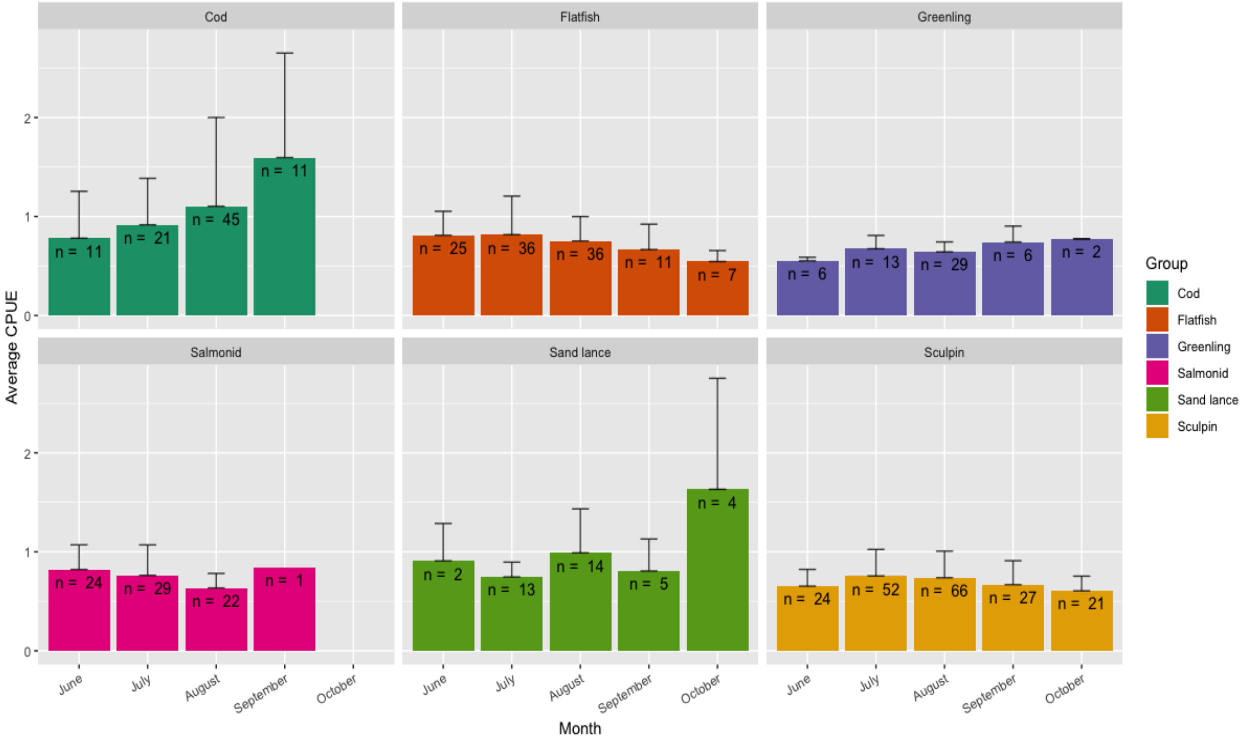

Figure 3. Average CPUE of major fish groups (groups > 5% of total CPUE across all sites and seasons) by month. Bars are colored by fish group and labelled with the number of instances that species within a group were caught that month. Error bars represent one standard deviation of the mean CPUE per group and month.

Water Flow, Fish Community, and Water Conditions

There was a significant difference in variance of water speed between the sites with different ‘flow classes’ (ANOVA, F = 4314.3, $p < 0.001$). A flow score was calculated at each site as the average site water speed over time for the period when water flow data overlapped for all sites from May 23 to June 25, 2018 (Figure 4). A post hoc Tukey test revealed that the mean water speeds of all sites were significantly different from each other ($p < 0.001$), so site comparisons in flow measurements were deemed appropriate to relate flow to fish community.

In general, the variability in fish community structure based on Bray-Curtis distances of average CPUE had no obvious pattern between ‘flow class’ groups (Figure 5). Beta-diversity was not significantly different when comparing groups based on ‘flow class’ (ANOVA, F = 1.199, p = 0.281) or when comparing among groups based on ‘flow score’ (ANOVA, F = 0.222, p = 0.951). Thus, subsequent permutation-based models were primarily testing differences in group location rather than distances to the group centroid. When tested alone, ‘flow class’ explained a significant proportion of the variance in fish community structure based on centroid location (PERMANOVA, F = 3.476, $p < 0.001$). Similarly, flow score alone explained a significant proportion of the variance in fish community (PERMANOVA, F = 4.825, $p < 0.001$).

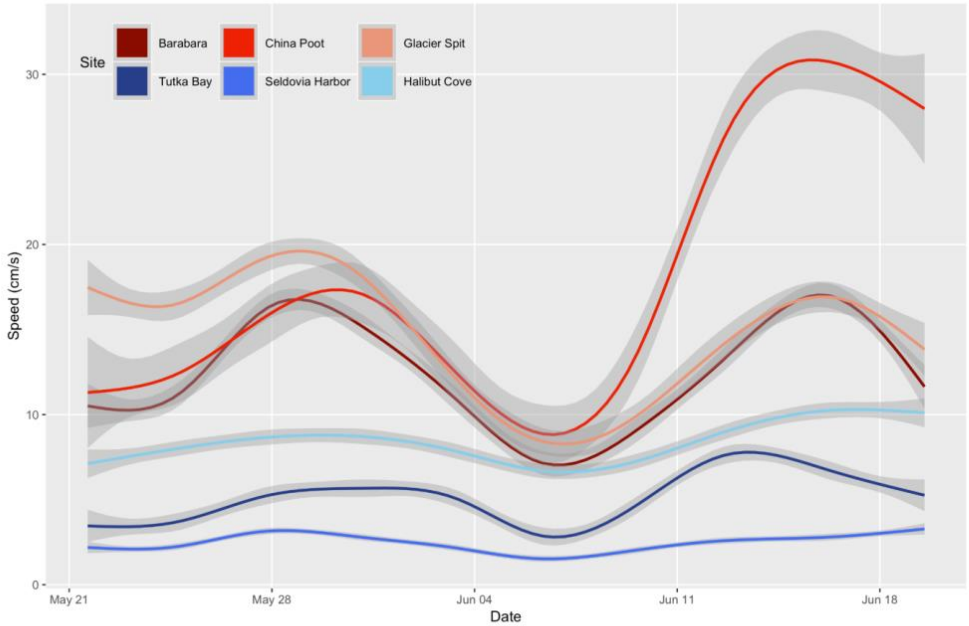

Figure 4. Water speed (cm/s) for all sites from May 23 to June 25, 2018. Data were smoothed using a generalized additive model. Red and blue hues represent high flow and low flow sites, respectively. Shaded areas represent the 95% confidence interval around the smoothed line.

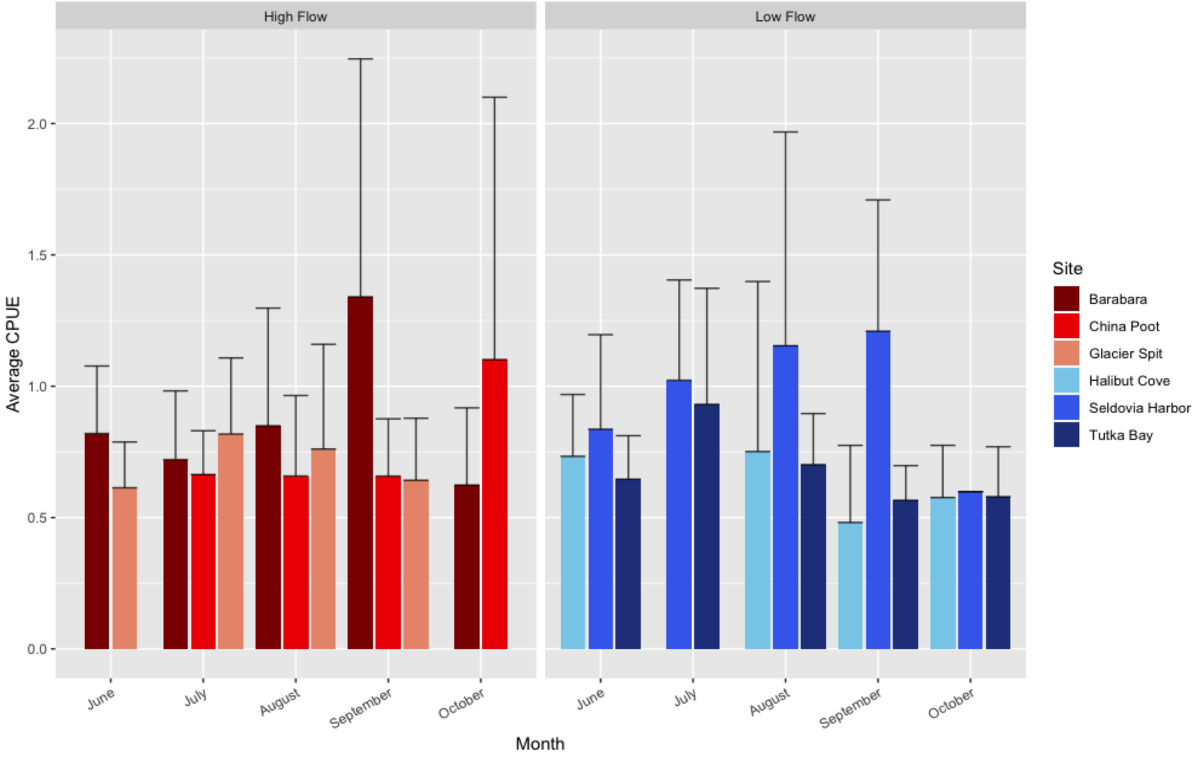

Figure 5. Average CPUE for each site by month comparing high flow (left panel) and low flow sites (right panel). Error bars represent one standard deviation of the mean CPUE per site and month.

An nMDS ordination of fish community structure revealed a moderate separation of flow class groups in two dimensions (red hue versus blue hue points in Figure 6). Kruskal's stress score was relatively high for interpreting the ordination in two dimensions (k = 0.224); however, the ordination in two dimensions was deemed no more or less informative compared to the ordination plots generated using three dimensions, which resulted in a lower stress score. Even though the ordination of community samples in two (or three) dimensions did not definitively separate between high and low flow sites, this pattern should not be translated to multivariate space. The ordination was found useful for interpreting environmental correlations with fish community, which were visualized with vectors superimposed on the nMDS (labeled arrows in Figure 6). The combination of variables that resulted in the highest correlation between environmental distances and fish community was seasonality ('Day' in Figure 6) and flow score (BIOENV, Mantel's r = 0.545, p < 0.001). When temperature, salinity, dissolved oxygen, and turbidity were included in the analyses, the correlation decreased but remained significant (Mantel's r = 0.357, p < 0.001).

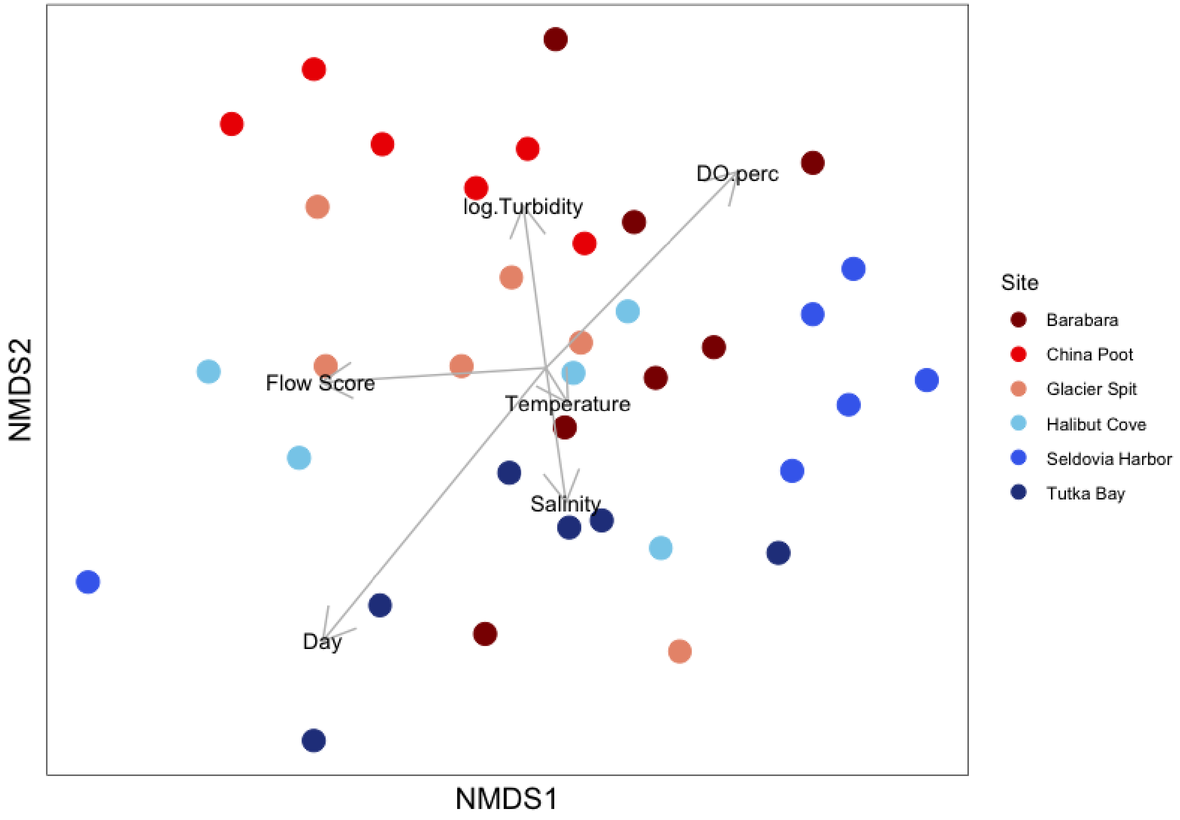

Figure 6. Non-metric multidimensional scaling (nMDS) ordination of fish community structure. Number of dimensions = 2, Kruskal's stress = 0.23. Points are site visit samples as nMDS scores based on Bray-Curtis distances of average species abundance (fourth-root transformed CPUE). Superimposed arrows are environmental vectors based on correlations (Spearman rank) in Euclidean space (ENVFIT). Red and blue hues represent high flow and low flow site visits, respectively.

There were seasonal, site-level, and within-site variances evident in water conditions (Figure 7), and overall, these conditions had weak correlations amongst each other. Water temperatures increased throughout the summer months then declined in the fall. Salinity did not exhibit clear patterns across seasons, but there were notable differences in variability among sites. Barabara and Seldovia Harbor had the highest average salinity levels and were the least variable across seasons. By contrast, Tutka Bay and Glacier Spit exhibited large variability across seasons and within site visits (see trends beginning mid-August in Figure 7). There was a decrease in dissolved oxygen at all sites during the sampling period; this was evident in a correlation test between dissolved oxygen and seasonality (rho = -0.65, $p < 0.01$). Dissolved oxygen did not result in a similarly high correlation with fish community structure as did seasonality in a Mantel test (BIOENV). There were generally low levels of turbidity during the sampling period, except for exceptionally high levels at Glacier Spit in late-July and late-August (Figure 7), which corresponded with low Glacier Spit temperatures. Turbidity had a moderate negative correlation with salinity (rho = -0.57, $p < 0.01$), and a weakly positive correlation with flow score (rho = 0.41, $p = 0.01$).

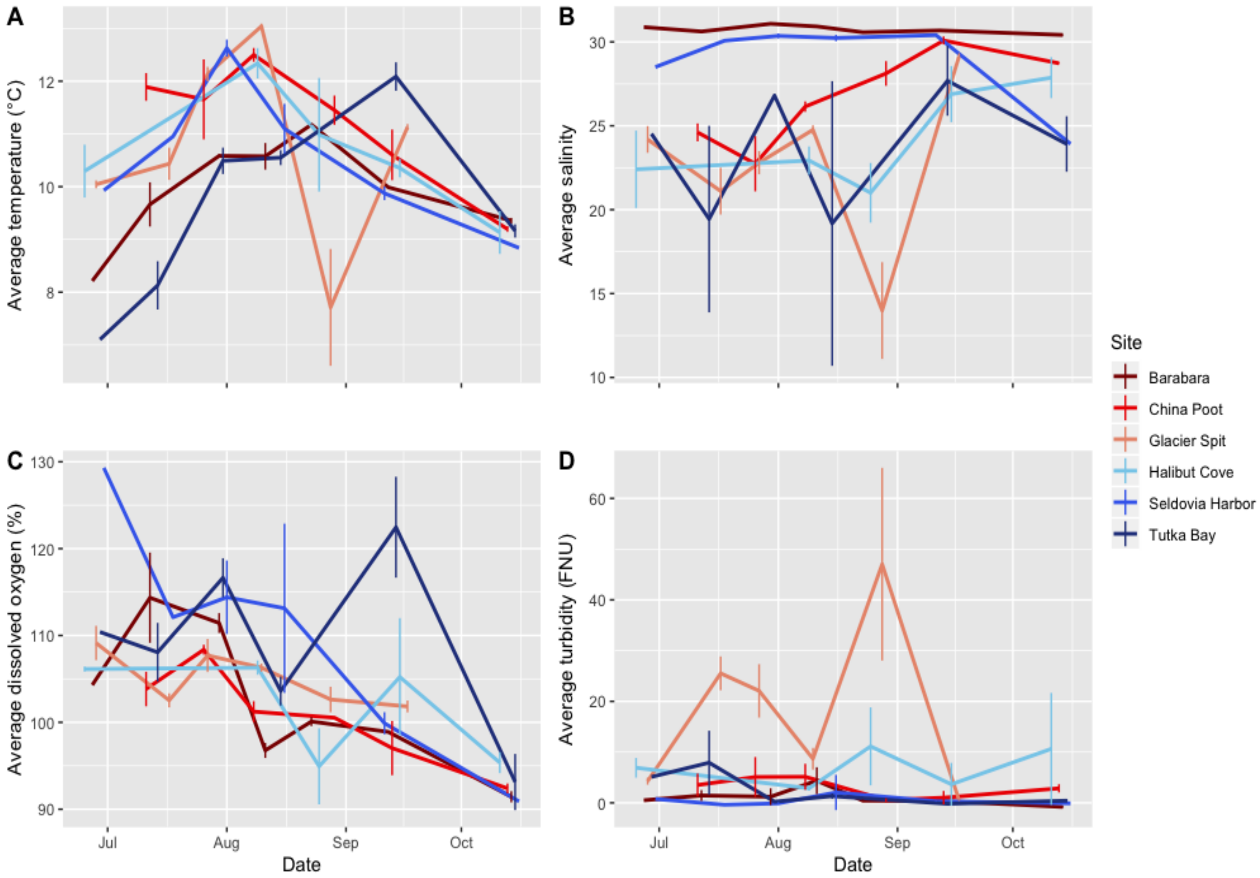

Figure 7. Site water conditions for each site during the study the period (June–October). Lines represent trends among collection dates for (A) temperature, (B) salinity, (C) dissolved oxygen, and (D) turbidity, averaged by site visit. Error bars represent the data range during a site visit. Red and blue hues represent high flow and low flow sites, respectively. Data were not treated for this figure.

When tested sequentially in PERMANOVA models, seasonality and flow score both accounted for a relatively large proportion of the variance in fish community structure compared to other environmental factors (Table 2). Although temperature, turbidity, and salinity had significant p-values, their sums of squares (SS) values contributed minimally to the reduction of residual SS. Dissolved oxygen was excluded from PERMANOVA tests due to its high correlation with seasonality. After all other significant variables had been accounted for, the marginal effect of flow score was still a relatively large proportion of the total variability (Table 3). Similar to the sequential Type I model, turbidity and salinity had relatively small SS scores and weakly significant pseudo-F scores in the Type III model.

Table 2. Type I sum of squares ('SS') computed with a permutation-based analysis of variance in fish community structure partitioned by environmental measurements.

	Df	SS	MeanSqs	F.Model	R2	Pr(>F)
Temperature	1	0.323	0.323	1.824	0.039	0.046 *
Seasonality	1	1.075	1.075	6.077	0.129	<0.001 ***
Turbidity	1	0.432	0.432	2.441	0.052	0.007 **
Salinity	1	0.354	0.354	1.999	0.043	0.031 *
Flow Score		0.825	0.825	4.663	0.099	<0.001 ***
Residuals	30	5.308	0.177		0.638	
Total	35	8.316			1.000	

Number of permutations: 4999
Significance: *** < 0.001 ** < 0.01 * < 0.05 # < 0.1

Table 3. Type III sum of squares ('SS') computed with a permutation-based analysis of variance in fish community structure partitioned by environmental measurements.

	Df	SS	R2	F	Pr(>F)
Temperature	1	0.267	0.032	1.508	0.114
Turbidity	1	0.296	0.036	1.674	0.074 #
Salinity	1	0.313	0.038	1.771	0.057 #
Seasonality	1	0.933	0.112	5.273	<0.001 ***
Flow Score		0.825	0.099	4.663	<0.001 ***
Residuals	30	5.306	0.638		
Total	35	8.316	1.000		

Number of permutations: 4999
Significance: *** < 0.001 ** < 0.01 * < 0.05 # < 0.1

Species Contributions to Community Dissimilarity

The species that contributed most to community dissimilarity between high flow and low flow sites were those that were also highly abundant across all seasons, such as Saffron cod, Pacific herring, and Pacific sand lance (Figure 2; Tables 4 and 5). Saffron cod were more abundant at low flow sites during summer, then became more abundant at high flow sites in the fall (Figure 8). Conversely, Pacific herring were more abundant at low flow sites during summer, then became more abundant at high flow sites in the fall. Pacific sand lance were more abundant in high flow sites no matter the season. Although highly abundant throughout the sampling period, rock sole and great sculpin did not contribute significantly to site differences. In the summer months (June to August), crescent gunnels (*Pholis laeta*) and snake pricklebacks (*Lumpenus sagitta*) were significant contributors to low flow sites (Table 4). Similarly, three Pacific salmon species, chum salmon (*Oncorhynchus keta*), coho salmon (*O. kisutch*), and sockeye salmon (*O. nerka*), were significant contributors to low flow sites; notably, those species were not caught in fall. During the fall, capelin (*Mallotus villosus*), buffalo sculpin (*Enophrys bison*), and Dolly Varden were significant contributors to high flow sites (Table 5). These species and rarer gadids, such as tom cod (*Microgadus proximus*) and walleye pollock (*Gadus chalcogrammus*), were absent from low flow sites in the fall (Figure 8).

Table 4. SIMPER results for most important species composing up to 80% of the cumulative contribution of the dissimilarity (Bray-Curtis) between high flow (High) and low flow (Low) sites during summer months (June–August 2019). ‘Avg’ is the species contribution, ‘sd’ is the standard deviation, ‘Sum’ is the relative contribution adding to a total of one, and ‘p’ is the permutational p-value.

Common Name	Avg	sd	Avg/sd	High	Low	Sum	p
Saffron Cod	0.048	0.043	1.12	0.684	1.449	0.076	0.042 *
Pacific Sand Lance	0.032	0.021	1.51	0.830	0.156	0.127	<0.001 ***
Pacific Herring	0.027	0.028	0.96	0.454	0.313	0.169	0.535
Snake Prickleback	0.025	0.018	1.41	0.095	0.586	0.208	0.003 **
Tom Cod	0.024	0.025	0.97	0.216	0.579	0.246	0.074 #
Micro Sculpin	0.023	0.020	1.19	0.440	0.466	0.283	0.921
Crescent Gunnel	0.023	0.017	1.34	0.304	0.685	0.320	0.032 *
Rock Sole	0.023	0.020	1.14	0.541	0.764	0.355	0.607
Pacific Cod	0.021	0.017	1.25	0.431	0.452	0.388	0.875
Pink Salmon	0.020	0.021	0.97	0.204	0.414	0.420	0.175
Buffalo Sculpin	0.020	0.015	1.37	0.513	0.275	0.452	0.052 #
Starry Flounder	0.020	0.016	1.23	0.251	0.494	0.483	0.106
English Sole	0.019	0.018	1.09	0.286	0.396	0.514	0.638
Chum Salmon	0.019	0.025	0.74	0.000	0.410	0.543	<0.001 ***
White Spotted Greenling	0.017	0.016	1.11	0.318	0.346	0.571	0.881
Dolly Varden	0.017	0.015	1.15	0.586	0.507	0.598	0.990
Coho Salmon	0.017	0.019	0.87	0.035	0.388	0.625	0.004 **
Great Sculpin	0.016	0.015	1.13	0.643	0.723	0.651	0.915
Staghorn Sculpin	0.016	0.014	1.10	0.285	0.351	0.676	0.554
Capelin	0.016	0.034	0.46	0.279	0.120	0.701	0.988
Tidepool Sculpin	0.015	0.017	0.90	0.242	0.190	0.725	0.963
Lingcod	0.014	0.015	0.96	0.112	0.305	0.748	0.053 #
Sockeye Salmon	0.014	0.018	0.76	0.043	0.285	0.769	0.008 **
Scalyhead Sculpin	0.013	0.015	0.83	0.190	0.183	0.789	0.996
Larval Fish	0.013	0.013	0.94	0.151	0.235	0.809	0.692

Number of permutations: 4999

Significance: *** < 0.001 ** < 0.01 * < 0.05 # < 0.1

Table 5. SIMPER results for most important species composing up to 80% of the cumulative contribution of the dissimilarity (Bray-Curtis) between high flow (High) and low flow (Low) sites during fall months (September–October 2019). ‘Avg’ is the species contribution, ‘sd’ is the standard deviation, ‘Sum’ is the relative contribution adding to a total of one, and ‘p’ is the permutational p-value.

Common Name	Avg	sd	Avg/sd	High	Low	Sum	p
Pacific Sand Lance	0.088	0.101	0.88	0.818	0.336	0.115	0.234
Buffalo Sculpin	0.052	0.018	2.96	0.566	0.000	0.183	0.004 **
Saffron Cod	0.047	0.071	0.66	0.700	0.335	0.245	0.731
Capelin	0.046	0.032	1.45	0.416	0.000	0.305	0.004 **
Pacific Cod	0.044	0.045	0.97	0.602	0.284	0.362	0.847
Staghorn Sculpin	0.042	0.029	1.42	0.126	0.542	0.416	0.185
Pacific Herring	0.040	0.035	1.12	0.477	0.161	0.467	0.232
Rock Sole	0.036	0.033	1.09	0.281	0.403	0.514	0.996
Tidepool Sculpin	0.029	0.029	1.01	0.246	0.240	0.552	0.912
White Spotted Greenling	0.026	0.032	0.81	0.339	0.129	0.585	0.281
Great Sculpin	0.025	0.019	1.35	0.738	0.638	0.618	0.197
Starry Flounder	0.024	0.026	0.91	0.000	0.248	0.649	0.422
Tom Cod	0.024	0.048	0.49	0.510	0.000	0.680	0.125
Snake Prickleback	0.023	0.035	0.65	0.000	0.338	0.709	1.000
Crescent Gunnel	0.023	0.036	0.63	0.000	0.253	0.739	0.954
Masked Greenling	0.022	0.030	0.72	0.310	0.000	0.767	0.052 #
Walleye Pollock	0.020	0.026	0.78	0.310	0.000	0.793	0.050 #
Dolly Varden	0.018	0.038	0.47	0.169	0.000	0.817	0.048 *

Number of permutations: 4999
Significance: *** < 0.001 ** < 0.01 * < 0.05 # < 0.1

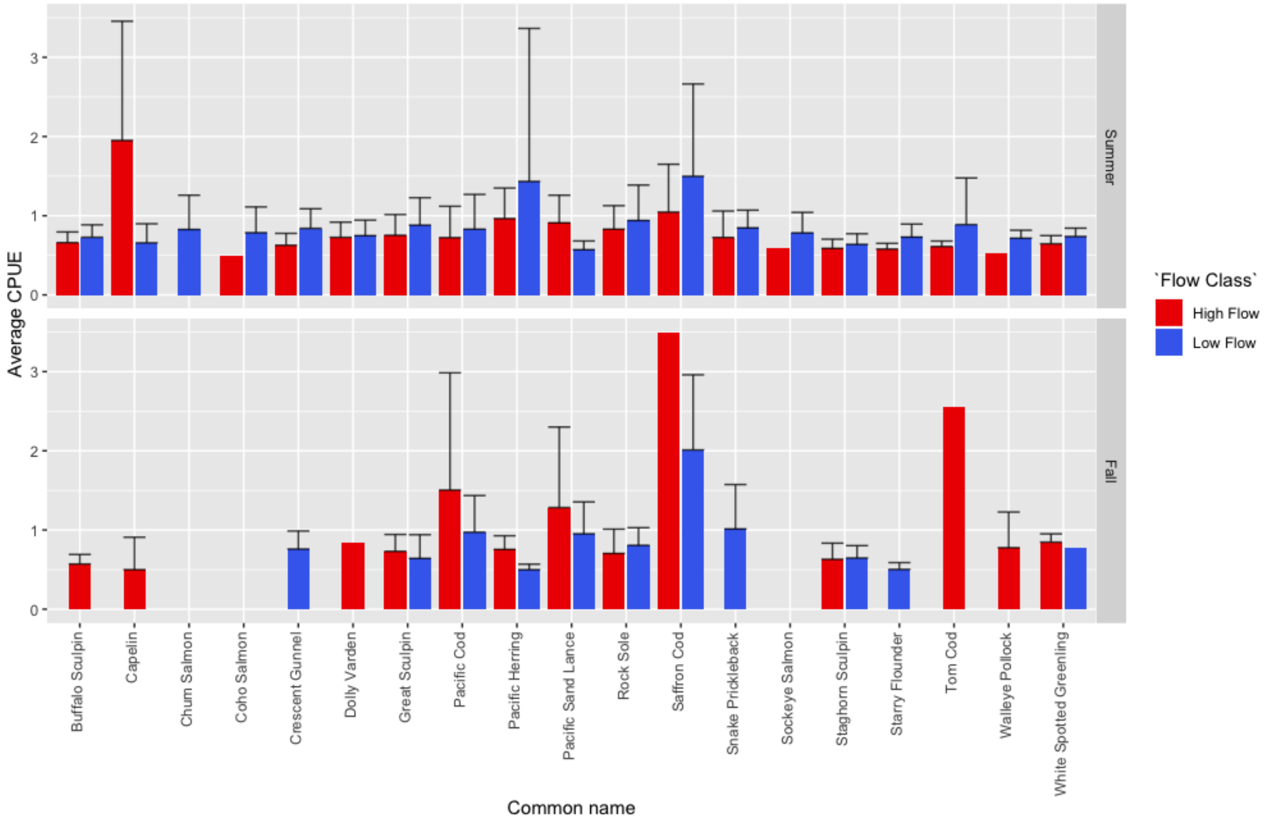

Figure 8. Average CPUE of select species sampled during June–August (Summer, upper panel) and September–October (Fall, lower panel) based on Bray-Curtis dissimilarites in community between high flow (red) and low flow (blue) sites. Error bars represent one standard deviation of the mean CPUE for each species.

Discussion

Seasonal Variability in Fish Community

Of the factors examined in this project, seasonality had the strongest influence on estuarine nearshore fish community structure. In general, seasonal variances of environmental conditions in the estuarine nearshore are well reported (Potter et al. 1997; Abookire et al. 2000; Simenstad et al. 2006; Boughton et al. 2007), so their influence on fish communities in this study was not unexpected. A prior study in this area reported significant increases and decreases in fish abundance during the spring and fall, respectively, with summer having the highest abundances (Robards et al. 1999a). The results from my study exhibited similar seasonal trends (Figure 8) and had an overall similar trend in composition of fish collected (see Robards et al. 1999a). My study began collecting fish in the summer beginning late June and ended in mid-October. Although catches were quite variable, the average abundance for all fish groups was generally consistent through August (Figure 3). Fish catches became slightly more variable in September with some notably large catches of saffron cod and sand lance (Figure 2), and the catches for almost all fish declined dramatically in October. Some of the most abundant species included saffron cod, great sculpin, rock sole, Pacific sand lance, Pacific herring, Pacific cod, and Dolly Varden (Table 1).

Migrants and diadromous fish have seasonal occurrences in estuarine nearshore habitats (Elliott et al. 2007). In the Gulf of Alaska, migrants of marine, freshwater, and estuarine origin are likely taking advantage of increased coastal productivity as waters become warmer and the photoperiod increases. Many of Alaska's anadromous and semi-anadromous fish (e.g., Pacific salmon, eulachon, Pacific herring, saffron cod, Pacific cod) have recognized run times that place these populations in estuarine waters (ADFG 2019; Mecklenberg et al. 2002). Similarly, the young of the year (YOY) of these anadromous fish will utilize estuarine habitats seasonally as nursery grounds or migration corridors (Simenstad et al. 1982; Gillanders et al. 2003). The YOY fish reside in estuarine waters for varying periods of time, depending on their species life history (Quinn et al. 1992; Mecklenberg et al. 2002). This was evident in the early-season occurrence of juvenile Pacific salmon species giving way to returning adults (mostly pink salmon), as well as the mid-summer appearance of juvenile forage species (Figure 2).

Salmonids were caught in high abundances in the early months and then tapered off later in the season. This pattern is similar to prior studies in this (Moulton 1997; Armstrong et al. 2005) and other regions in the Pacific (Quinn et al. 1992) where juvenile Pacific salmon were found in high abundances at inshore habitats before they out-migrate to offshore systems. Typically, juvenile pink and chum salmon will bypass the estuary to rear in nearshore waters after spawning in early spring (Moulton 1997; Armstrong et al. 2005). In contract, juvenile chinook, coho, and sockeye salmon exhibit prolonged rearing in upstream habitats (Quinn et al. 2002), which can include heavy usage of estuarine habitats (Simenstad et al. 1992) as well as the nearshore (Hoem-Neher et al. 2013). The juvenile Pacific salmon collected in this study appear to have a shared rearing habitat in the estuarine nearshore, which was evident by concurrent catches of salmonids that exhibit different life histories (particularly at Seldovia Harbor, which contains a salmon run for each of the Pacific salmon species; Figure 2). My results showed juvenile Pacific salmon were abundant in June and July and were replaced by returning pink salmon adults during late July and August. Dolly Varden exhibited a more consistent seasonal abundance across sites and seasons compared to Pacific salmon species in this study, perhaps because juvenile Dolly Varden are common in the anadromous waters surrounding Kachemak Bay (this study; Johnson and Blossom 2018). Similar to chinook, coho, and sockeye salmon, Dolly Varden exhibit a life history with prolonged rearing in upstream habitats. It could be that Dolly Varden are out-competing other salmonids for food in the nearshore or are being out-competed in upstream habitats (e.g., protected estuarine channels). Another possibility is that Dolly Varden and Pacific salmon species are consuming different prey. Partitioning of estuarine habitat and food by salmonids has been reported in other regions, although the patterns of partitioning and the ecological reasons for such are unknown (Levings 2016). It would be informative to examine both the feeding habits and energetic requirements for these estuarine nearshore salmonids to better understand their interacting role in the community.

While forage fish, such as saffron cod, Pacific herring, capelin, and Pacific sand lance were some of the most abundant species in this study, they were seasonally variable. This variability may be due to their schooling nature, which is prone to being unevenly collected when seining and can confound spatial and seasonal trends in abundance. Still, there were noticeable increases in abundance for these species at different times (Figure 2). For example, Saffron cod were the most abundant cod caught in this project, with a general increasing trend throughout the sampling period. Saffron cod, more so than other cods (e.g., walleye pollock), are reported to enter brackish waters during spawning migrations (Mecklenburg et al. 2002). Herring are semi-anadromous fish that spawn in the intertidal and estuarine nearshore during the early spring (Mecklenburg et al. 2002; ADFG 2019). Pacific herring, primarily as schools of YOY, were caught in high abundances from late July to mid-September. Capelin exhibited similar trends as herring, with large schools of YOY caught beginning in early August and observed into September. Capelin are reported to spawn on inshore beaches (Mecklenburg et al. 2002) and may not favor brackish waters, which may explain their lower abundance compared to herring (Figure 2). Pacific sand lance abundance increased from June–October, with particularly high abundances in the fall months. This can be attributed to the fall spawning of sand lance populations, which has been previously reported in this area (Robards et al. 1999b). Overall, the seasonal occurrence and relative abundance of these forage fish reinforce the importance of estuarine nearshore habitats for spawning and rearing purposes.

Influence of Water Conditions on Fish Community

Each of temperature, salinity, dissolved oxygen, and turbidity were significant in their ability to account for variance in fish community structure; though the proportion of variability explained was relatively minimal (Table 2). The seasonal and spatial trends observed in water conditions could be explained by patterns of surface water quality in the bay reported during the study period (Holdereid et al. 2019; NOAA NERRS 2019). Water temperatures and dissolved oxygen were correlated with seasonality, which likely resulted in the significant relationship to the seasonal trends observed in species abundances. The relatively strong correlation between dissolved oxygen and seasonality is likely caused by the combined effect of increasing temperatures and growing freshwater content from melting snowpack and glacial sources in the summer. This effect was also expected, but not observed, in salinity levels (Holdereid et al. 2019), perhaps because sites were in close proximity to river outflows. Salinity did follow established patterns within the bay (NOAA NERRS 2019), with sites closer to the bay's mouth recording higher salinity levels (Figures 1 and 7). These 'outer bay' sites (Seldovia Harbor and Barabara) had higher catches of cod compared to the other locations (Figure 2). Turbidity was expected to increase with sites located more toward the head of the bay (Holdereid et al. 2019). Indeed, Glacier Spit was the innermost site in the bay and had the highest average turbidity levels (Figures 1 and 7). There was no obvious pattern in group or species abundances correlated to turbidity.

Variability within site-visits was too large to relate to variance in fish community structure. There were notably large fluctuations in the water conditions measured at some site visits (see Figure 7), which can be attributed to the estuarine mixing that occurs at river mouths. Another source of variability stems from sampling within two hours of the low tide, which kept the tidal stage similar amongst samples; however, the shifting of waters between ebb and flood could change the water body for each replicated seine. The strongest example of this was reflected in the temperature, salinity, and turbidity conditions at Glacier Spit during late August (Figure 7). Due to the relatively high glacial coverage in its watershed (S Baird, pers. comm.), the glacial signal during this site visit was particularly strong. As expected, the glacial signal was reflected in decreased temperature and salinity levels and increased turbidity; however, by the time a third replicate was conducted, the glacial signal was reduced due to the changing tide. Although variability at the site-visit level was too high to examine correlations to fish communities, the data provides a good representation of the natural variability of river mouth signals.

After accounting for other water conditions and seasonal variability, water flow appeared to have a strong influence on estuarine nearshore fish community structure (Tables 2 and 3). Although water flow was not analyzed as a continuous variable, the grouping categories of ‘flow class’ and ‘flow score’ accounted for much of the variance in fish community structure. It should be noted that these flow groupings were tied to site selection in the sampling design, so other site-specific factors (e.g., physical habitat) may be contributing to the significance of the results. However, mean water speed was found to be significantly different among sites when comparing by flow score or by flow class.

Water flow is widely reported as an environmental factor that influences fish distribution and community structure (Bunn and Arthington 2002; Simenstad et al. 2006; Elliott et al. 2007); however, few studies have directly examined natural flow conditions as an ecological mechanism in the estuarine nearshore (Maes et al. 2004; Selleslagh and Amara 2008; Ferguson et al. 2013). Water flow likely influences habitats and fish on multiple levels (e.g., coastal morphology, flushing rates, local conditions) such that directly attributing fish abundance to flow to fish is difficult. However, water flow conditions could be an influencer of community patterns that are more evident in different functional groups of fish. For example, the difference in fish community at high and low flow sites was particularly apparent by the abundance of active swimming (e.g., sand lance, smelt, herring) and benthic-dwelling species (e.g., gunnels and pricklebacks) (Figure 2; Tables 4 and 5). Fish have adapted to different flow regimes. Species morphology can be attributed different energy trade-off scenarios, which results in varied swimming ability (Langerhans 2008). Phenotypic variation in fish morphology relates to different flow conditions assuming a functional trade-off between swimming strategies. For example, stronger flow conditions result in phenotypes that optimize ‘active’ (or steady) locomotion, where fish tend to be more streamlined in morphology and with emphasized fins for swimming (e.g., anal fin) (Langerhans 2008; Videler 2010). In this study, the fish that fit this description include Pacific sand lance, Pacific herring, capelin, and various salmonids.

In contrast, weaker flow conditions result in phenotypes that promote ‘non-active’ swimming (e.g., sit-and-wait types), as seen in sculpins and gunnels. Considering swimming ability alone, forage species would be expected to be relatively more abundant in areas with higher flow conditions. This is somewhat supported by my results. Both capelin and Pacific sand lance, for example, were more abundant at high flow sites regardless of the season (Figure 8). ‘Non-active’ swimmers would be expected to be more abundant at low flow sites, but this did not hold for groups such as sculpin or gunnels, which did not contribute strongly to either high or low flow sites (Figures 2 and 8). Thus, swimming ability may only partially explain why certain fish are found in different flow conditions. For instance, certain salmonids exhibit estuarine habitat preferences linked to the maintenance of triglycerides (Hansen et al. 2012), which promotes ‘burst’ swimming in the ocean where they must avoid predators and fight stronger currents (Levings 2016). A better understanding of species’ behavioral traits would offer more insight into the role of water flow as an influencer of fish community.

To put the effects of water flow into context, it would be helpful to further consider the functional uses that certain fish guilds exhibit in the estuary. The proximity of an estuarine nearshore habitat to other highly utilized nearshore habitats may be influencing fish distribution. Take, for instance, the higher abundance of forage fish caught at high flow sites compared to low flow sites (no Pacific herring, capelin, or Pacific sand lance were caught at two of the low flow sites; Figure 2). Halibut Cove was the only low flow site where forage fish were caught, with high catches of YOY herring among the other forage species. Halibut Cove also had the highest average water speed of the three low flow sites (Figure 4). Historically, Halibut Cove is recognized as an important spawning location for herring (Rounsefell 1930). It may be that this site is still an important nursery habitat, but the degree to which water flow or other habitat conditions influences that role is not clear. The impact of the various estuarine conditions in relation to the functional services that these habitats provide deserves further attention.

Similar to the role of habitats for spawning and rearing, the role as refugia would be useful in examining differences in fish community in conjunction with water flow. Both crescent gunnels and pricklebacks are benthic-dwelling (Mecklenburg et al. 2002) and were found in particularly high abundances at low flow sites compared to high flow sites (Figure 2). The low flow sites chosen in this study were primarily mud and soft sediment, whereas the high flow sites were more sand and gravel in sediment. Soft sediments may offer more refugia opportunity (e.g., burrowing beneath rock crevices) than would gravel habitats. However, another benthic-dwelling fish, the snailfish (*Liparis spp.*), was primarily found at high flow sites. Although not reported here, many of the snailfish caught in this project were likely the kelp snailfish (*Liparis tunicatus*), which is reported to associate with kelp, as its name implies (Mecklenburg et al. 2002). A number of the seines collected large amounts of detached algal rafts, and these tended to occur more at the exposed high flow sites. It may be that snailfish, though expected to occur more at locations conducive to benthic-dwelling species (i.e., low flow sites), also highly associate with the dense algal rafts found at higher flow conditions.

Estuarine nearshore fish communities are shaped by a variety of natural and anthropogenic influences that are under constant flux. As evidenced here, water flow can be a contributing indicator of fish community structure. It is likely that water flow influences fish at multiple levels and through different ecological mechanisms. When examining the seasonal structure of fish communities in an area, water flow conditions should be considered a useful metric.

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Zane Chapman

Identifying Hatch Dates and Potential Hatch Location of Arctic Cod (*Boreogadus saida*) through Otolith Analysis

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Abstract

Arctic Cod (*Boreogadus saida*) are one of the most important pathways for transferring energy from primary producers to upper trophic level species throughout the Arctic, including the Chukchi and Beaufort Seas. In a rapidly changing climate, understanding when and where Arctic Cod spawn is becoming more important in order to inform the management of Arctic marine ecosystems and their natural resources. As the Arctic seas warm and sea ice continues to decline, these waters become more navigable to humans, allowing for an increase in exploration for potential oil, mineral, and fish resources. Arctic Cod are very sensitive to temperature increases, as well as anthropogenic disturbances, making a better understanding of their life history key for conserving this vital resource. The objectives of this study were to (1) use otolith aging to estimate and compare the hatch dates for young-of-year Arctic Cod collected throughout the Chukchi and Beaufort Seas and (2) test the use of otolith trace element composition to distinguish hatch locations for young-of-year Arctic Cod collected from the Chukchi and Beaufort Seas. Otoliths from Arctic Cod have previously been used and validated for measuring daily growth in larval and juvenile cod. Otoliths were extracted from age-0 cod, mounted on slides, and polished until daily growth rings could be counted and measured under a microscope. Hatch dates were then estimated by subtracting the estimated age from the known sampling date. Trace elemental analyses were performed using laser ablation to estimate the ratio of trace elements to calcium along a cross-section of the otolith. The analysis of the hatch date distribution showed that the samples obtained from eastern Beaufort Sea hatched a later time in the year then the western samples. The chemical analysis provided evidence that the eastern samples hatched in a water mass with a different chemical composition than the chemical composition of the western samples. My results were consistent with previous work and provide additional evidence for the existence of two distinct Arctic cod populations in the Chukchi Sea/western Beaufort Sea and the eastern Beaufort Sea, respectively. The findings from this study will provide a better understanding of the early life history of Arctic Cod and help inform a biophysical transport model for larval cod that is being developed concurrently.

Introduction

The environment in the Arctic is changing rapidly, and how this may affect the distribution, abundance, condition, and phenology of marine organisms is a topic of great interest. 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 (Richter-Menge et al. 2016; Mueter et al. 2017). These changes are reshaping the ecosystem, allowing southern, warmer-water species to move north and causing keystone species such as Arctic Cod (*Boreogadus saida*) to compete against species such as Saffron Cod (*Eleginus gracilis*), capelin (*Mallotus villosus*), and other gadids for resources (Hop and Gjøsæter 2013; Marsh 2019). Large concentrations of age-0 Arctic Cod have recently been documented in the northeast Chukchi Sea on and around Hanna Shoal, an area of potential interest to oil development (De Robertis et al. 2017). When and where these Arctic Cod spawn and hatch is currently unknown; however, it is crucial to determine the location and timing of Arctic Cod hatching in order to provide protective measures during their delicate life stage. Juvenile Arctic Cod have also been documented in the western Beaufort Sea, but it is unclear if these populations are connected in spite of much progress, 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).

This study estimated the hatch date distribution of Arctic Cod in the Chukchi and Beaufort Seas in order to better understand their early life history and population connections between the two seas and to inform modeling studies being developed to identify spawning locations. Based on laboratory and field studies, Arctic Cod eggs remain suspended at the surface of the water just under the sea ice and can tolerate sub-zero temperatures without an effect on survival (Laurel et al. 2015). However, Arctic Cod eggs are extremely vulnerable to high water temperatures and will not survive past a threshold of 3.8°C (Laurel et al. 2015). While there is some information on the time of Arctic Cod hatching from other areas, little is known about the timing and location of spawning or hatching off Alaska, nor about movements after spawning (Bouchard et al. 2011). Better knowledge of these hatch dates is important to accurately parameterize the biophysical transport model.

Arctic Cod are a relatively small fish belonging to the family Gadidae and adults occur throughout the circumpolar seas. Arctic Cod provide an important pathway for energy transfer from planktonic prey to larger animals throughout the circumpolar Arctic. With high abundances, high energy content, and an assimilation efficiency around 80%, Arctic Cod are ideal prey for many Arctic predators (Hop et al. 1997; Parker-Stetter et al. 2011; Bluhm and Gradinger 2008). Many larger predators such as seals, whales, and marine birds depend on Arctic Cod as a source of energy; in turn, polar bears rely on some of these marine mammals for their caloric intake (Welch et al. 1992). Given the importance of Arctic Cod in the ecosystem, it is crucial to determine where and when the eggs are hatching. These data can be used to better inform conservation and management measures in the face of cumulative impacts from climate change and human development.

The determination of Arctic Cod hatch dates is made possible by the deposition of daily growth rings on their otoliths, a pair of calcified structures used for balance and/or hearing in all teleost fishes. Otoliths are acellular and metabolically inert, which allows elements to be stored in the otolith in proportion to their concentration in the surrounding water. Otoliths cannot be reabsorbed; therefore, the otoliths provide a record of environmental conditions from the time a fish hatches until it dies (Campana 1999). The unique properties of otoliths allow researchers to estimate age through calcified growth increments and use Laser Ablated, Inductively Coupled Plasma, Mass Spectrometry (LA-ICP-MS) to identify trace elements within the otoliths. These data support inference of environmental conditions that a fish experienced at different life stages. Bouchard et al. (2015) used LA-ICP-MS to estimate isotope concentrations for five trace elements (^{7}Li, ^{25}Mg, ^{55}Mn, ^{88}Sr, and ^{138}Ba) and Calcium (^{48}Ca) in juvenile Arctic Cod otoliths. The elements were expressed as ratios and were used to determine relationships to the surrounding environment. Results of the Bouchard study (2015) suggest that the trace elemental composition of Arctic Cod otoliths can serve as a proxy for environmental salinities at different stages of their lives.

Field-based information on hatch timing is needed in part to validate the parameterization of growth in a biophysical transport model being developed for Arctic Cod (Vestfals et al. 2019). The model, in turn, will help identify potential spawning aggregations and can be used to simulate how sea ice reduction and changes in water temperature may affect early life survival. 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. Advancing understanding of the life history and hatching habitats of Arctic Cod will help inform conservation and management under the Fisheries Management Plan for the marine resources in the Arctic (NPFMC 2009).

Hypotheses

1. Hatch dates of samples from the Beaufort Sea will be distinct from those of samples collected in the Chukchi Sea.
2. Elemental composition of samples from the Beaufort Sea will be distinct from those of samples collected in the Chukchi Sea.

Objectives

1. Use otolith aging to estimate and compare the hatch dates for young-of-year Arctic Cod collected throughout the Chukchi and Beaufort Seas.
2. Test the use of otolith trace element composition to distinguish hatch locations for young-of-year Arctic Cod collected from the Chukchi and Beaufort Seas.

Methods

Study Region

Arctic Cod samples from the Chukchi and Beaufort Seas were assigned to four distinct regions based on major oceanographic characteristics and differences between the Chukchi Sea and Beaufort Sea shelves. Only three of the regions were used in this study, including the northern Chukchi (N.C), the western Beaufort (W.B), and the eastern Beaufort (E.B) (Figure 1). The majority of the Chukchi Sea is a shallow (40–60 m) and relatively flat continental shelf, which was split into two regions due to the difference in water masses. Water masses entering through the Bering Strait are the Alaska Coastal Water, the Bering Shelf Water, and the Anadyr Water. The latter two are warm saline waters that are high in nutrients, while the Alaska Coastal Water is fresher and nutrient-poor (Weingartner 1997). Winter Water, a distinct water mass generally present in the N.C, originates from previous winters and is characteristically very cold with a high salinity, making it very dense (Pickart et al. 2010). The Winter Water extends as far south as 70°N; therefore, 70°N was used as the dividing line between the northern and southern Chukchi Sea. In contrast to the Chukchi Sea, the Beaufort Sea has a narrow shelf that quickly drops into the Arctic Basin. The Beaufort Sea receives relatively nutrient-poor water as a continuation of the ACW, some of which enters the Beaufort Sea via Barrow Canyon. Other water masses in the Beaufort Sea include Mackenzie River runoff, Summer Shelf water, which is influenced by both Arctic and sub-Arctic currents, and deep Atlantic water, which enters from the west (Carmack and Macdonald 2002). For this analysis, the Beaufort Sea was separated into an eastern (E.B, samples east of 142.2°W) and western region (W.B, west of 154 °W) (Figure 1), with the eastern region influenced by the Mackenzie River drainage and the western region influenced by waters originating in the Chukchi Sea.

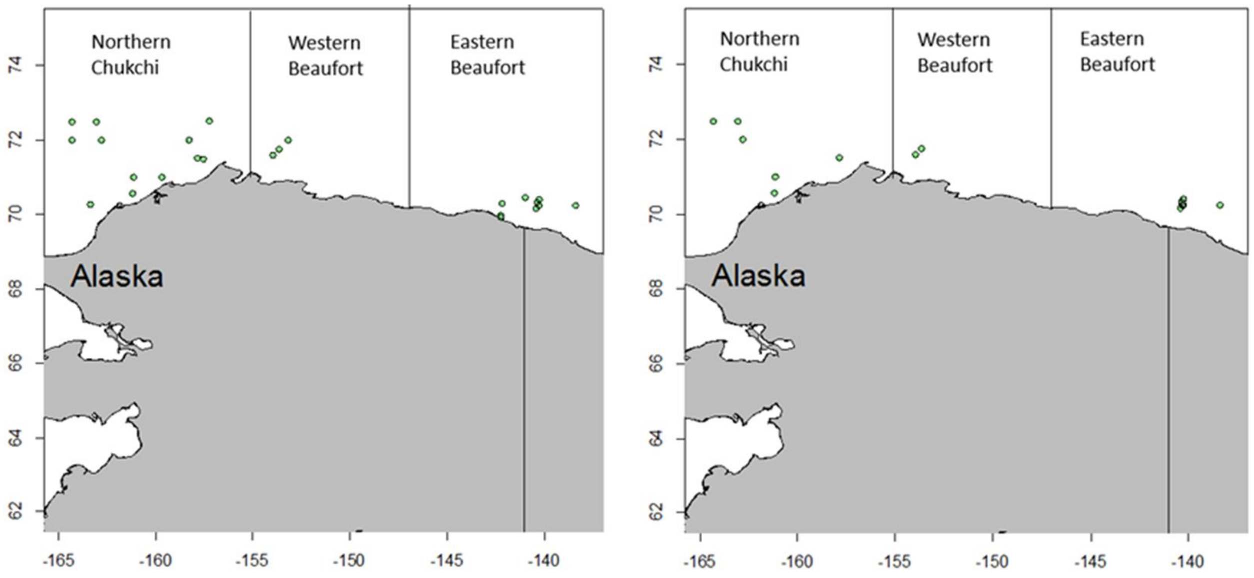

Figure 1: Locations of otolith samples used for aging (left) and for LA-ICP-MS analysis from the AMBON, Arctic IES, and Transboundary surveys (right).

Sample Collection

Juvenile Arctic Cod used for this analysis were collected from four Arctic surveys: Arctic Marine Biodiversity Observation Network (AMBON) survey in 2017, Arctic Integrated Ecosystem Survey (Arctic IES II) in 2017, and Transboundary Surveys in 2013 and 2014 (Table 1, Figure 1). The AMBON and Arctic IES II surveys sampled northern Chukchi and western

Beaufort Seas. The 2013 and 2014 Transboundary Surveys sampled on both sides of the US Canadian border in the eastern Beaufort Sea. Sampling methods included (1) an Isaacs-Kidd Midwater Trawl deployed to collect larval and juvenile pelagic fish within aggregations at different depths in the water column as identified by acoustic backscatter, and (2) a bongo net, primarily used to sample zooplankton. Larval Arctic Cod were removed opportunistically prior to sample preservation. All larval and juvenile gadids were saved and stored in 95% ethanol and shipped to Juneau, AK, where they were identified and processed for further analysis. Each gadid specimen was measured for standard length, fork length, and weight to assess length and weight at age. Additional samples were obtained by Dr. Brenda Norcross, who has archived otoliths from throughout much of the Beaufort Sea. Samples were analyzed to address both of the above objectives.

Table 1: A summary of the number of samples available, the cruise they came from, and how many have been aged and tested for trace elemental compositions (T.E.C.). Sampling goals shows targets for aging.

Cruise	Period	Region	Samples Available	Samples Aged	T.E.C	Sampling Goals
Arctic IES 17	Aug/Sept	S. Chukchi	22			22
Arctic IES 17	Aug/Sept	N. Chukchi	109	31	76	30
Arctic IES 17	Aug/Sept	W. Beaufort	30	11	18	30
Transboundary 13	Aug/Sept	E. Beaufort	56	27	29	15
Transboundary 14	Aug/Sept	E. Beaufort	16	3	10	15
ASGARD 17	June	S. Chukchi	367			30
ASGARD 17	June	N. Bering	29			29
Ambon 17	August	N. Chukchi	116			30
Ambon 17	August	S. Chukchi	45			30

Otolith Aging

To identify the hatch dates, the sagittal otoliths of juvenile Arctic Cod were examined for growth increments. The sagittal otoliths were removed under a dissecting microscope with fine-tipped forceps. The left otolith was extracted by removing the operculum and rinsed off with 95% Ethanol to remove any organic matter. The otolith was then embedded on a clean microscope slide using crystal bond. The right otolith was removed as well and was cleaned and stored to be used if the left otolith was unusable from damage. The mounted otoliths were then polished using a varying level of grit to expose the daily growth increments within the otolith. Due to the uneven shape of the otolith, some were required to be flipped over to polish both sides. To estimate hatch dates, daily growth increments were counted on the otoliths from a selected subset of specimens across the study region. In a previous study, the deposition of daily growth increments was confirmed using a tetracycline marking experiment and visual examination under light and scanning electron microscopy (Bouchard and Fortier 2011). Under the microscope (40x magnification), the growth increments were identified and read with Image Pro Plus©, where each visible ring was assumed to represent one day of growth (Figure 2). The growth rings were counted from a hatch mark which was determined visually and validated using lab-reared Arctic Cod (Figure 3). A similar method was used to determine the hatch mark by examining otoliths shortly after the fish hatched (Eckmann and Rey 1987). To determine the date of hatching, the number of growth increments from the hatch mark was subtracted from the known date when the specimen was caught (Bouchard et al. 2015). Hatch dates were estimated for samples from a variety of different catch sites to examine spatial patterns in hatch timing.

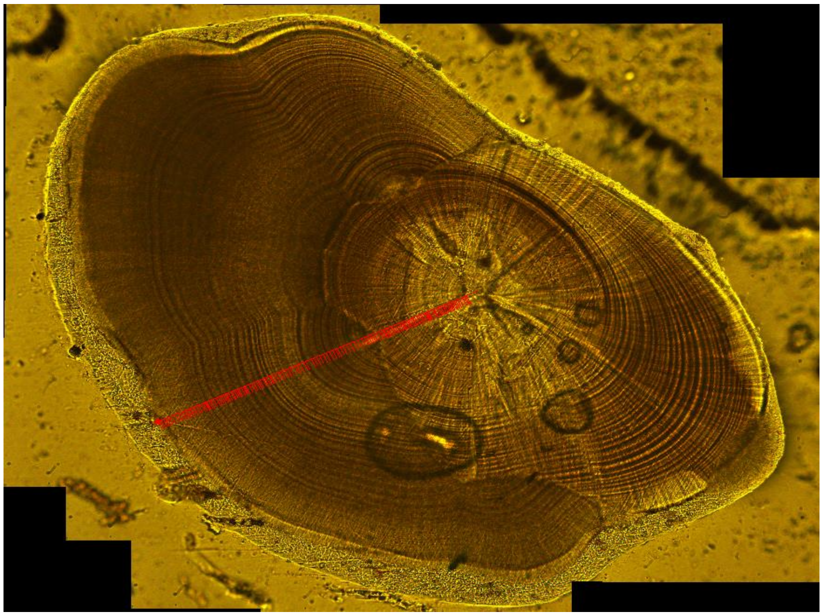

Figure 2: Polished otolith at 40X magnification with daily growth increments marked.

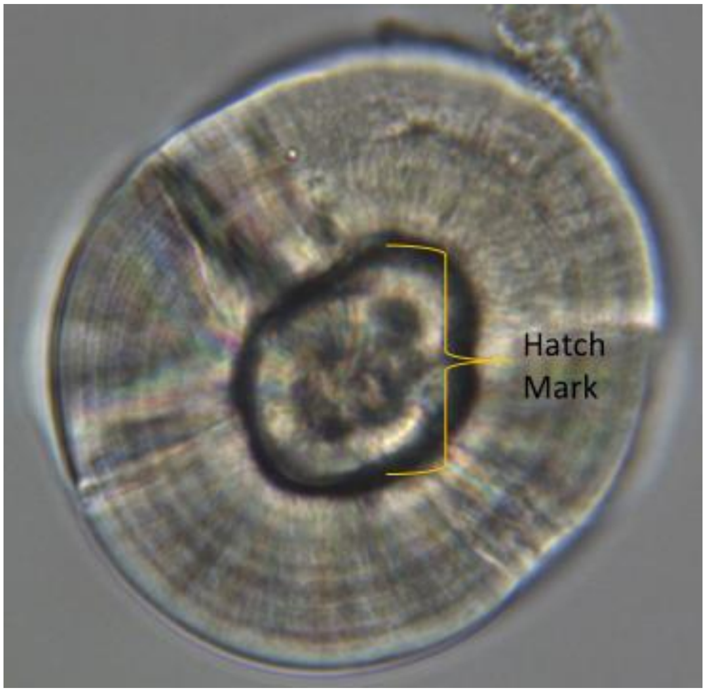

Figure 3: Arctic Cod (lab-reared) sagittal otolith hatch mark at 100X magnification.

Hatch Date Analysis

Statistical analysis was conducted to compare hatch dates among regions and compare laboratory and field derived growth rates. Hatch date distributions were plotted as histograms to compare means and ranges visually. Hatch dates were estimated separately for the three different regions in Figure 1. Differences in hatch dates may suggest the existence of separate hatching populations among those regions. A Tukey Honest Significant Difference (HSD) *post hoc* test was performed to statistically compare differences among regions and to compare hatch dates to previous field derived hatch dates for the E.B (Bouchard and Fortier 2011; Gallaway et al. 2017).

Additionally, age-length relationships for each region were estimated, and growth rates were compared among regions. Measured standard lengths were plotted against estimated ages, and the growth rates for each region were estimated as the slopes of simple linear regressions of length (response variable) as a function of age (explanatory variable). Growth rates will be used to validate hatch age estimates and considered as an indicator that Arctic Cod form two separate populations in the Beaufort and Chukchi Seas.

Otolith Chemistry

The chemical composition of the otoliths provided a record of environmental conditions. For this study, selected trace elements within the otoliths were analyzed using a New Wave UP213 Laser with an Agilent 7500ce Inductively Coupled Plasma, Mass Spectrometry (LA-ICP-MS). Nine otoliths were placed on a slide and prior to being run Nist610 and Febs pellets were ablated to ensure a consistent standard for each run. 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. The transect shows possible environment conditions of the specimen at different life stages. To help infer possible hatch locations, isotope abundances at the edge, and along a transect from the hatch mark to the edge of the juvenile Arctic Cod otoliths was examined (Figure 4). The transect was used to compare post-hatch chemical composition among the different regions to determine if the Arctic Cod hatched in chemically distinct waters. This analysis is considered a pilot study to assess the possibility of using the elemental signatures of the otolith cores to infer possible spawning locations. Previously aged otoliths were used for the chemical analysis. Using an LA-ICP-MS at University of Alaska Fairbanks Advanced Instrumentation Laboratory, the trace elemental composition near the hatch mark of each otolith (11 µm from otilith core) was examined as it reflects the growth that occurred around the time of hatching. Chemical data were extracted from the LA-ICP-MS and processed using the Igor Pro version 6.37 and the Iolite software package version 3.0.

Figure 4: Polished otolith with ablation scar from LA-ICP-MS.

Otolith Chemical Analysis

Multivariate statistical analyses were conducted to compare elemental compositions among regions. Prior to analysis, the trace elemental ratios were transformed and/or standardized as needed. Pairwise Euclidean distances among samples were calculated for the hatch mark data to compare the elemental composition among regions. Differences in the hatch mark's chemical composition among regions were visualized using a multivariate ordination based on Principle Component Analysis (PCA). Multivariate analyses of variance (MANOVA) were used to determine overall differences in chemical composition among regions. When overall differences were significant, these were followed by individual ANOVAs to determine which of the elemental ratios differ among regions. Significant ANOVA results were followed by Tukey HSD *post hoc* tests to test pairwise differences between regions for significance. All analyses were performed using the R statistical computing environment version 3.5.3 (R Core Team 2019).

Results

Hatch Dates

The hatch dates presented here are based on the first preliminary readings of the otoliths. Larvae and juveniles collected in the E.B had a mean hatch date of 4/28 with a range of 3/2–6/23, those from the W.B had a mean hatch date of 4/5 with a range of 1/3–5/9, and those from the N.C had a mean hatch date of 4/8 with a range of 1/13–6/3 (Figure 5, Table 2). There was a significant difference in hatch dates between the N.C and the E.B (Tukey's HSD test, $p = 0.0303$). There was also a noticeable difference between the W.B and the E.B, although it was not significant at the 95% significance level (Tukey's HSD test, $p = 0.0788$). In contrast, there was no evidence for a significant difference in hatch dates between the N.C and the W.B (Figure 6), suggesting that hatching occurred later in the E.B compared to the other two regions.

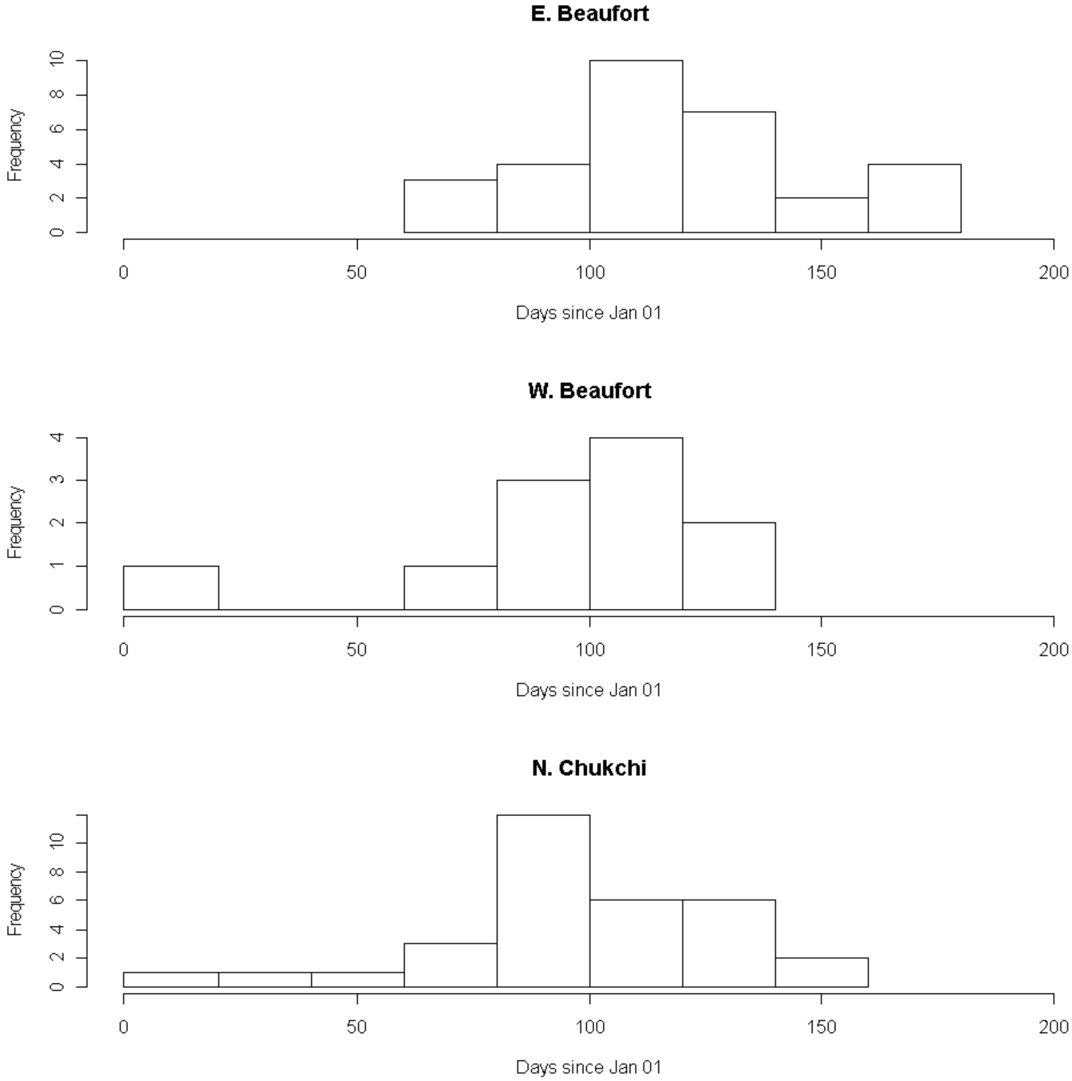

Figure 5: Frequency distribution of hatch dates for three regions (in days since January 1).

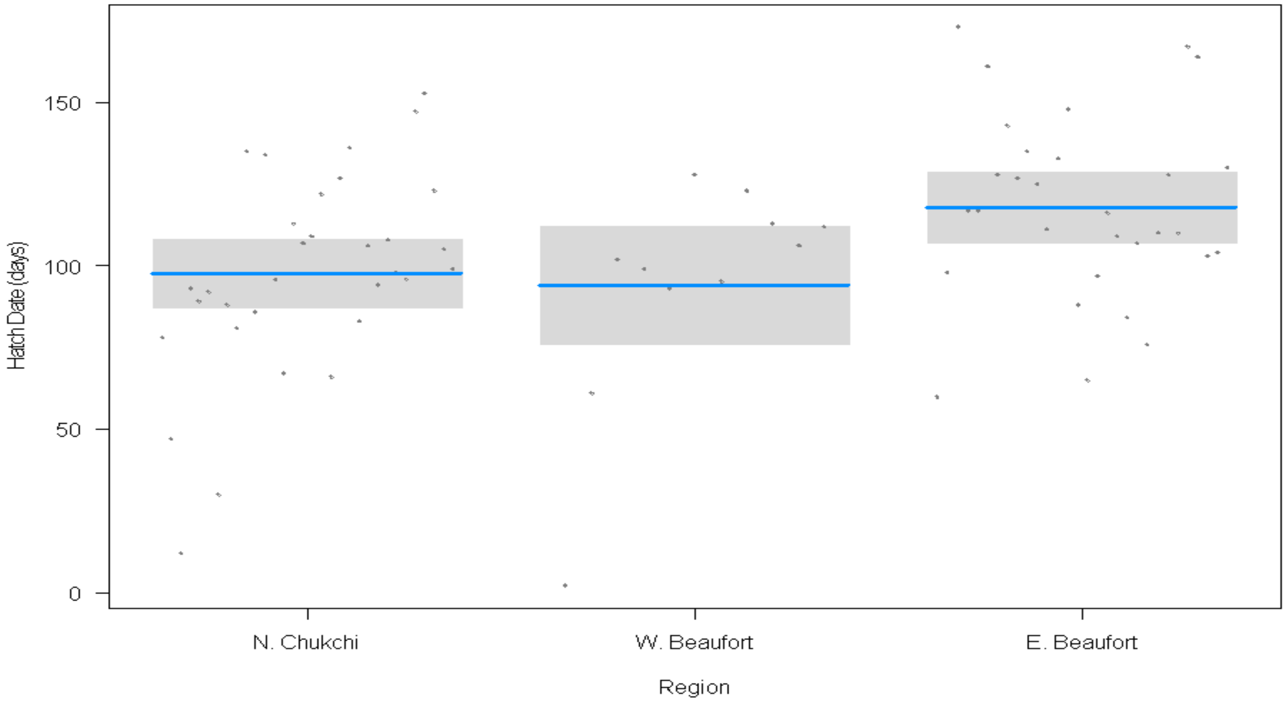

Figure 6: Boxplot of the mean hatch date (day of year) for the three regions. Gray boxes highlight 95% confidence intervals.

Table 2: Summary of the mean hatch date (HD) for different regions and sampling periods with their range and standard deviations (SD) based on this study and published studies.

Region	Year	Mean HD	Min HD	Max HD	SD	Source
E. Beaufort Sea	2005	20-Apr	26-Dec	13-Jul	35.2	Bouchard, Fortier 2011
E. Beaufort Sea	2006	11-Apr	12-Jan	22-Jul	30.6	Bouchard, Fortier 2011
E. Beaufort Sea	2011	9-Apr	10-Jan	13-Jun	32.37	Gallaway et al. 2017
E. Beaufort Sea	2013	28-Apr	2-Mar	23-Jun	31.95	This study
W. Beaufort Sea	2017	5-Apr	3-Jan	9-May	35.31	This study
N. Chukchi Sea	2017	8-Apr	13-Jan	3-Jun	31.18	This study

Age-Length Relationships and Growth Rates

Arctic Cod estimated growth rates were 0.1871 mm/day for the E.B (R^2 = 0.5025, Figure 7), 0.1343 mm/day for the W.B (R^2 = 0.3447, Figure 7), and 0.1115 mm/day (R^2 = 0.2928, Figure 7) for the N.C.

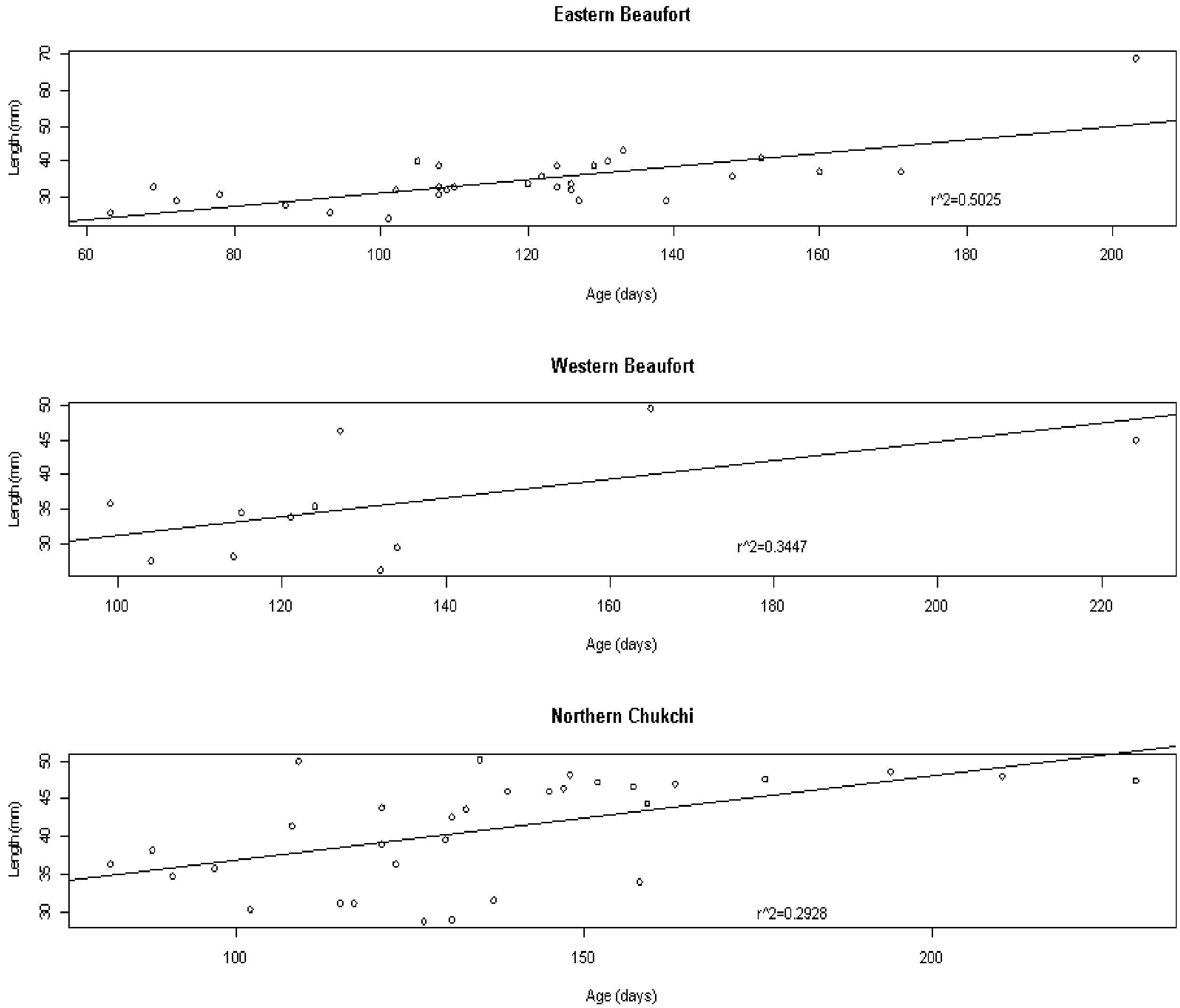

Figure 7: (Top) Length-at-age regression of Arctic Cod captured in the eastern Beaufort Sea. Length = 12.71 + 0.1871 * days, (R^2 = 0.5025, n = 30). (Middle) Length-at-age regression of Arctic Cod captured in the western Beaufort Sea. Length = 17.94 + 0.1343*days (R^2 = 0.3447, n = 11). (Bottom) Length-at-age regression of Arctic Cod captured in the northern Chukchi Sea. Length = 25.71 + 0.1115 * days (R^2 = 0.2928, n = 31).

Hatch Mark Chemical Signatures

The PCA ordination based on trace elemental compositions does not show distinct separation between the three regions (Figure 8). However, there was an overall statistical difference in the elemental composition among regions (MANOVA: Pillai's trace = 0.3196, p = 0.0056). Specifically, the elemental ratios of Mn/Ca, Zn/Ca, Sr86/Ca, Sr88/Ca and Ba/Ca differed significantly among regions (ANOVAs: p = 0.0122, 0.0028, 0.199, 0.0023 and 0.0347, respectively, Figure 9). The Mn/Ca ration was significantly higher in the E.B than the N.C (ANOVA: p = 0.0105). Zn/Ca was significantly lower in the E.B than in the N.C (ANOVA: p = 0.0079) and the W.B (ANOVA: p = 0.0133). Sr86/Ca was significantly lower in the E.B than the N.C (ANOVA: p = 0.36) and W.B (ANOVA: p=0.062). Sr88/Ca was significantly lower in the E.B than in the N.C (ANOVA: p = 0.0089) and W.B (ANOVA: p = 0.0083). Ba/Ca was significantly lower in the E.B than in the N.C (ANOVA: p = 0.074) and W.B (ANOVA: p = 0.07). The means, medians, and ranges of each elemental ratio for the three different regions are summarized in Table 3.

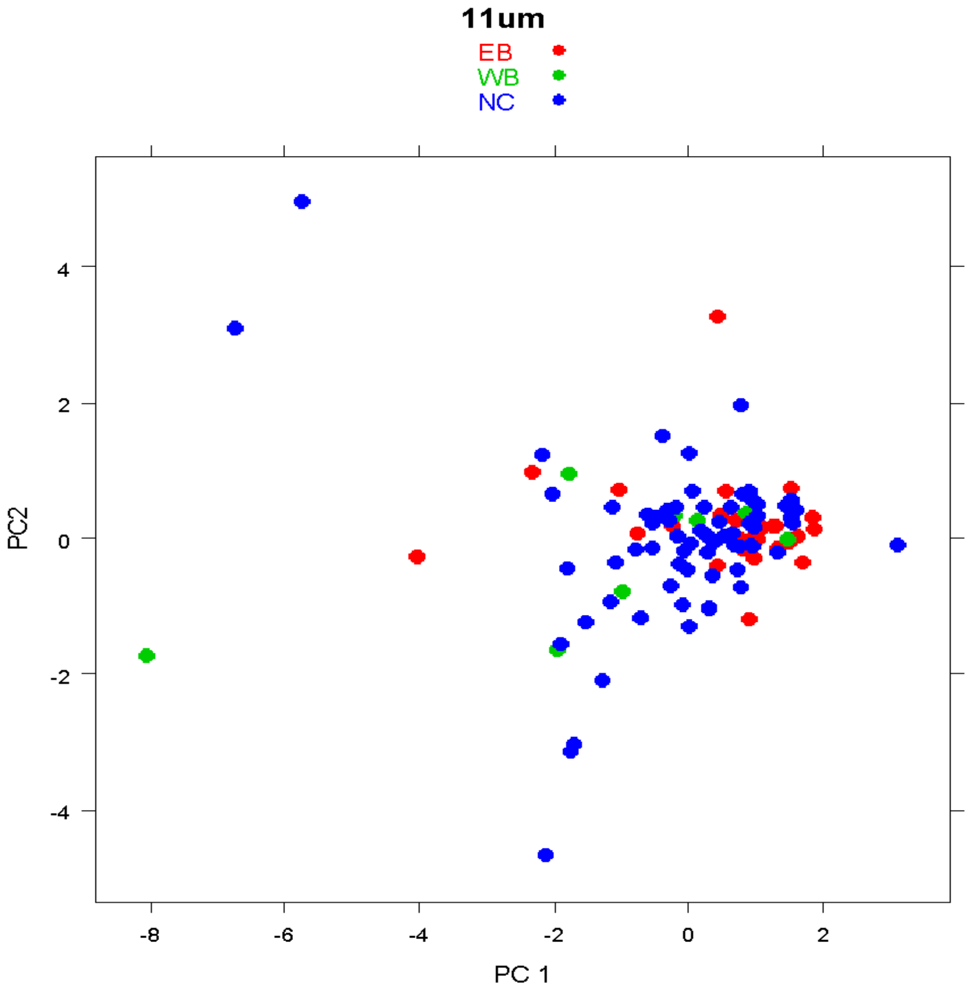

Figure 8: PCA ordination of the trace elemental ratios for the three regions.

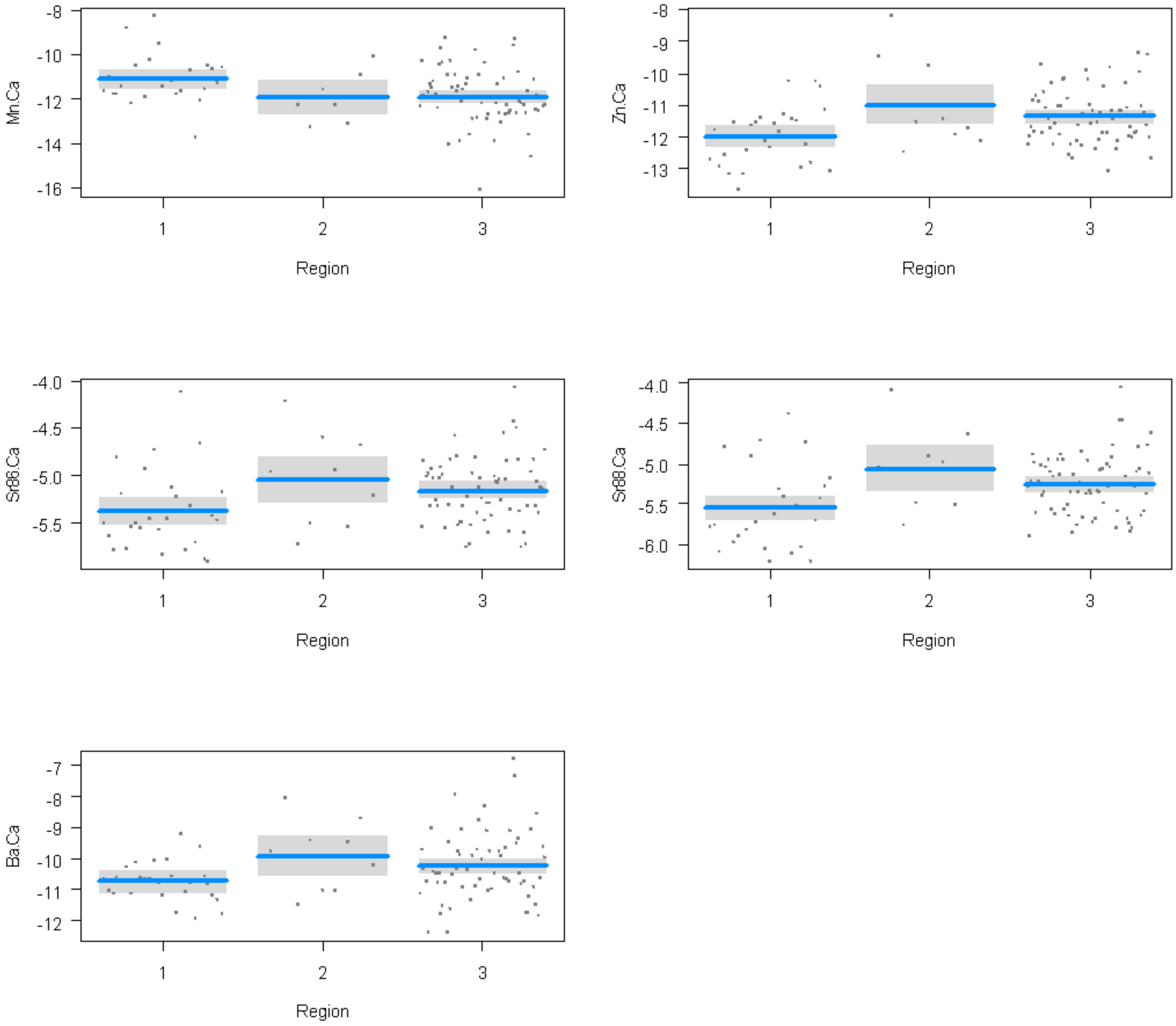

Figure 9: Box plots of mean log-transformed elemental ratios for the five significant ratios across the three different regions; E.B (1), W.B (2), and N.C (3). Gray boxes highlight 95% confidence intervals.

Table 3: Summary of the mean, standard deviation, median, and range of the log-transformed elemental ratios in otioliths from the three regions. All samples were taken at 11 μm from the otolith cores as this distance reflects the growth that occurred around the time of hatching.

Element	Region	Mean	SD	Median	Min	Max
Li/Ca	East Beaufort	-12.45303	1.053272	-12.36341	-14.992	-10.007
Mg/Ca	East Beaufort	-7.776513	0.507286	-7.816129	-8.9226	-6.7951
Mn/Ca	East Beaufort	-11.09312	1.085345	-11.17417	-13.783	-8.2317
Cu/Ca	East Beaufort	-11.37016	1.372388	-11.36914	-14.094	-8.7203
Zn/Ca	East Beaufort	-12.00374	0.9151935	-11.83856	-13.689	-10.249
Sr86/Ca	East Beaufort	-5.378867	0.4314032	-5.484937	-5.9162	-4.1165
Sr88/Ca	East Beaufort	-5.554262	0.5056059	-5.639383	-6.2286	-4.3842
Ba/Ca	East Beaufort	-10.75415	0.6209841	10.74037	-11.923	-9.2093
Li/Ca	West Beaufort	-12.44974	1.228329	-12.05994	-15.502	-11.458
Mg/Ca	West Beaufort	-7.538353	0.433846	-7.5173	-8.286	-6.8433
Mn/Ca	West Beaufort	-11.93747	1.008866	-12.00874	-13.287	-10.065
Cu/Ca	West Beaufort	-10.29502	1.202345	-9.883036	-12.29	-8.4061
Zn/Ca	West Beaufort	-10.98466	1.468151	-11.56824	-12.506	-8.1964
Sr86/Ca	West Beaufort	-5.045447	0.4953907	-4.956503	-5.7228	-4.2208
Sr88/Ca	West Beaufort	-5.059306	0.5040481	-5.039007	-5.7671	-4.0927
Ba/Ca	West Beaufort	-9.908846	1.152741	-9.789429	-11.489	-8.0549
Li/Ca	North Chukchi	-12.27904	0.8979886	-12.09142	-15.502	-10.544
Mg/Ca	North Chukchi	-7.590377	0.6863677	-7.570057	-8.9263	-5.9132
Mn/Ca	North Chukchi	-11.81151	1.226221	-11.90759	-16.114	-9.2139
Cu/Ca	North Chukchi	-10.90461	1.677191	-10.98679	-13.891	-7.3137
Zn/Ca	North Chukchi	-11.33818	0.9342283	-11.45189	-13.069	-8.1964
Sr86/Ca	North Chukchi	-5.130601	0.3669687	-5.133808	-5.7674	-4.0678
Sr88/Ca	North Chukchi	-5.211827	0.4022356	-5.1947	-5.8987	-4.0498
Ba/Ca	North Chukchi	-10.12908	1.069192	-10.29197	-12.423	-6.7738

Discussion

Hatch Dates

The results from the preliminary otolith reads revealed that, on average, the E.B samples hatched later in the year than samples from the other two regions. The greatest difference in hatch dates was between the E.B and the other regions, with a very small and non-significant difference between the N.C and the W.B samples. The significant differences in the hatch dates between the E.B and the other regions are consistent with the findings from Forster (2019), who found a gap in the distribution of juvenile Arctic Cod between the western and eastern Beaufort Seas. Moreover, there is evidence of genetic differences between Arctic Cod in the Chukchi Sea and in the eastern Beaufort (Wilson et al. 2019). Together, these results support the existence of two separate populations consisting of a western stock in the Chukchi Sea/western Beaufort Sea and an eastern stock in the eastern Beaufort Sea.

Growth Rates

Estimated growth rates from the E.B agreed well with the growth rates from Bouchard et al. (2015) (0.182 mm/day) and are similar to estimates by Gallaway et al. (2017) for samples from 2011 (0.1916 mm/day). The growth rates from the W.B and N.C samples were lower than the growth rates from the E.B samples. The fitted linear model for the E.B had the best fit with an R^2 value of 0.5 and is likely a good representation of the true growth rates. The similarity of age-length results to those of prior studies supports the validity of hatch age estimates found in this study; however, growth rate variability was too high to indicate a clear separation of the Arctic Cod populations of Beaufort and Chukchi Seas.With more aged otoliths and a second read on the otoliths, it is expected that the overall fit will improve. The much smaller R^2 values for the other two regions could indicate incorrect aging of otoliths and will be reassessed after additional readings. At present, the length-at-age relationships for the N.C and W.B are highly uncertain and possibly non-linear, but they may also improve after additional re-reads.

Otolith Chemistry

The chemical analyses suggest considerable overlap in the trace-elemental compositions of otoliths from different regions. Nevertheless, small but statistically significant differences in individual elemental ratios among regions was found. Similar to this study, Bouchard et al. (2015) found significant differences in several of the same trace elements (Mn/Ca, Sr/Ca, and Ba/Ca) across multiple circumpolar regions. Bouchard et al. (2015) found that Mn/Ca was negatively correlated with salinity, while Sr/Ca and Ba/Ca had positive correlations with salinity. The E.B samples had reduced Ba/Ca, Sr86/Ca, and Sr88/Ca ratios and a higher Mn/Ca ratio than the other two regions which suggests that the E.B samples hatched in waters with lower salinity than the W.B and N.C samples. The otoliths chemistry results support the existence of at least two unique spawning populations that hatch in chemically different water masses.

Conclusions

I found a significant difference in hatch timing between the E.B and the western regions (W.B and N.C) with a later mean hatch date in the E.B. The E.B samples displayed the largest growth rate of the three regions. There were also significant differences in otolith chemical signatures between the E.B and the other two regions. In contrast, hatch timing and chemical signatures did not differ significantly between samples from the W.B and N.C regions. These results suggest that hatching occurs later and in different water masses in the E.B than in the western regions. However, it is possible that the observed differences could also be explained by differences among years. The Transboundary survey in the E.B region occurred during 2013 and 2014, while the AMBON and Arctic IES surveys occurred in 2017. In order to compare regions directly, it would be ideal to obtain samples from all regions during the same year. While the time difference between the Transboundary survey and the AMBON and Arctic IES survey can create some complications, the samples between the N.C and the E.B are from the same year and are directly comparable. This study provides additional evidence and supports results from a previous study (Forster et al. 2019) that Arctic Cod in the eastern Beaufort Sea originate from a different spawning population than Arctic Cod in the other two regions. Whereas Arctic Cod captured in the two western areas hatched in chemically similar waters during similar time periods, suggesting that they originated from a single spawning population.

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