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Otolith chemistry discriminates water mass occupancy of Arctic fish in the Chukchi Sea

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Abstract. The microchemistry of otoliths has the potential to reconstruct fish movement patterns and habitat use between environmentally different habitats for individual age classes of Arctic marine fish. Herein, we tested the relationship between the bottom water mass from which a fish was collected and the microchemistry of the most recent growth edge of the fish's otolith using Mg, Sr, Ba and Ca, and then determined the physical and biological factors that affected the chemical signatures. A discriminant function post hoc analysis of fish occupying bottom water masses resulted in 76% correct classification of Arctic or Polar cod (*Boreogadus saida*) and 82% correct classification of Arctic staghorn sculpin (*Gymnocanthus tricuspis*) into bottom water masses of capture when ages were pooled. By separating age classes, correct classifications into water masses of capture were as high as 87% for Arctic cod (three water masses) and 90% for Arctic staghorn sculpin (two water masses). Otolith Ba : Ca, Mg : Ca and Sr : Ca ratios were most consistently affected by bottom water temperature; the latter two were also affected by fish age and fish length. The use of otolith microchemistry to determine occupancy of water masses over time is most promising for Arctic cod, which is widespread and occupies the most thermally diverse habitats in Arctic waters.

Additional keywords: *Boreogadus saida*, *Gymnocanthus tricuspis*.

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Introduction

In the Chukchi Sea, otolith chemistry can serve as a potential tool to reconstruct the occupation of bottom water masses by demersal fish if otoliths reflect physical characteristics (e.g. temperature and salinity) associated with water masses. Bottom water masses are characterised by specific ranges of temperature, salinity and density. Ambient environmental variables, such as temperature (Thresher 1999; Elsdon *et al.* 2008; DiMaria *et al.* 2010) and salinity (Elsdon and Gillanders 2002; Martin and Thorrold 2005; Panfili *et al.* 2015), can be correlated with Mg : Ca, Sr : Ca and Ba : Ca ratios in fish otoliths (Elsdon and Gillanders 2002; Avigliano *et al.* 2014). This suite of elements in otolith edge signatures has been used as a tracer in marine fish to discriminate frontal zones and water mass occupation in the Antarctic Circumpolar Current in the southwestern Atlantic Ocean (Ashford *et al.* 2007). In addition, these elements and the Mn : Ca ratio in otoliths and fish growth rate have demonstrated classifications of Atlantic cod (*Gadus morhua*) as high as 78% correct into nearshore and offshore spawning areas of capture in the north-western Atlantic Ocean (D'Avignon and Rose 2013). Thus, Mg, Sr and Ba in otoliths may serve as a multielement tool to discriminate residence of fish among water masses on a mesoscale in Arctic marine waters.

Barium in ambient seawater has the potential to affect Ba : Ca in otoliths of marine fish (Hamer and Jenkins 2007). In the Chukchi Sea, Ba is elevated in surface seawater near fluvial sources, although it is generally depleted in oceanic surface waters and enriched at depth because of exported particulates from high biological productivity in overlying surface waters (Guay and Falkner 1997; Abrahamsen *et al.* 2009). Benthic hot spots with high biomass, located in the eastern and north-eastern Chukchi Sea, have been persistent from the early 1970s to 2004 (Grebmeier *et al.* 2006), and these hot spots may be traceable with Ba measured in bottom seawater because of the tight benthic–pelagic coupling characteristic of the Chukchi Sea. The demersal fish otoliths may correlate with Ba concentrations in bottom seawater because these fish live near the sea floor and rest on sediment.

Arctic cod (*Boreogadus saida*, Gadidae) and Arctic staghorn sculpin (*Gymnocanthus tricuspis*, Cottidae) are both circumpolar fish species found in the Chukchi Sea and are important prey for apex predators in Arctic marine food webs (Lowry and Frost 1981; Welch *et al.* 1992). Arctic cod have the largest geographical distribution of these fish, spanning from river mouths to depths of 731 m offshore in Arctic basin waters (Mecklenburg *et al.* 2002). Arctic staghorn sculpin are found throughout the

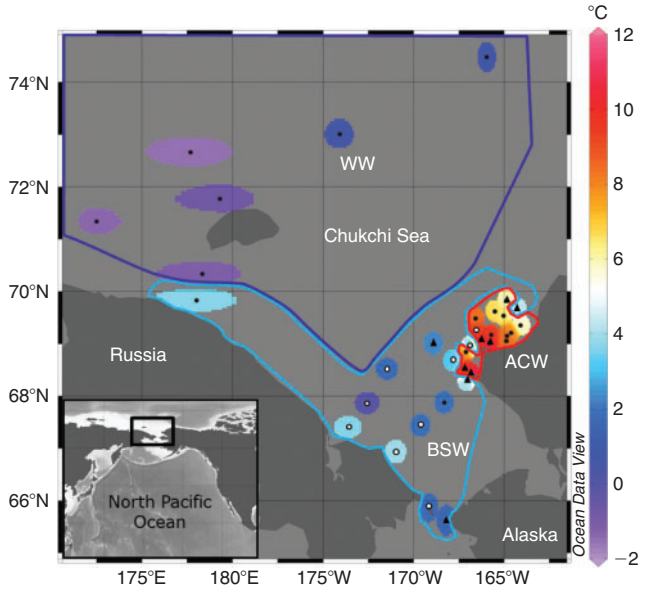

Fig. 1. Study area in the Chukchi Sea where demersal fish were collected from 3 to 30 September 2009 and from 21 August to 4 September 2010. Bottom water temperatures are indicated by colour at each collection site. Both Arctic cod and Arctic staghorn sculpin were collected from nine stations (outlined circles), whereas Arctic cod only were collected at 17 stations (solid circles) and Arctic staghorn sculpin only were collected at nine stations (triangles). Three major bottom water masses (outlined areas) were detected in the Chukchi Sea: Alaska coastal water (ACW; red; 2010), Bering Sea water (BSW; light blue; 2009 and 2010) and winter water (WW; dark blue; 2009).

Chukchi Sea at depths of 7–240 m (Mecklenburg *et al.* 2002). Both species have pelagic egg and larval stages and then settle to demersal environments (Wyllie-Echeverria *et al.* 1997; Norcross *et al.* 2010). Arctic cod can occupy demersal, pelagic and cryopelagic (ice-associated) zones in coastal and offshore marine habitats as juveniles and adults (Craig *et al.* 1982; Bluhm and Gradinger 2008; Norcross *et al.* 2013). Arctic staghorn sculpin are benthic dwellers (Mecklenburg *et al.* 2002) and lack swim bladders. Arctic cod spawn under the ice in winter, although nearshore and offshore spawning areas are largely unknown (Lowry and Frost 1981; Craig *et al.* 1982). Massive schools of Arctic cod have been observed entering lagoons and nearshore areas in the Beaufort Sea and Canadian Basin in late August to likely feed, but may return to lagoon waters during the winter (Craig *et al.* 1982; Welch *et al.* 1993). Although the reasons for the schooling behaviour are not known, such dense congregations of cod are thought to form before the arrival of predators and provide a feast for pinnipeds, seabirds and whales in autumn (Welch *et al.* 1993).

The Chukchi Sea is a marginal sea to the Arctic Ocean, typically consisting of several water masses found on the bottom (Fig. 1). This continental shelf sea is relatively shallow (~50 m) and flat bottomed, with a general northward flow of ocean currents through the Bering Strait towards the Arctic Ocean (Weingartner 1997; Weingartner *et al.* 1998). Bering Sea water (BSW) is a warm, saline and nutrient-rich water mass that is advected northwards through the Bering Strait and into the central Chukchi Sea (Weingartner 1997). Winter water (WW) is a very cold and saline water mass in the central and northern Chukchi Sea formed by remnant water from the previous winter (Pickart *et al.* 2010). Depending on the surface winds and freshwater input, two relatively fresh, nutrient-poor water masses, namely Alaska coastal water (ACW) and Siberian coastal current (SCC), can be present in the Chukchi Sea (Weingartner *et al.* 1999). ACW flows northward along the western coast of Alaska and receives freshwater inputs, creating the warm water mass (Weingartner 1997). The SCC flows southward along the Russian Chukotka Peninsula. From mid-July through to September, bottom water masses have relatively stable conditions compared with surface water masses, which are exposed to more solar radiation, riverine input, wind mixing and ice melting. With a reduction in sea ice in Arctic regions in recent years and further climate changes, it is expected that the water mass structure of the Chukchi Sea will shift, with more inflow from the Bering Sea carrying warmer water northwards (Woodgate *et al.* 2012) and an increase in freshwater discharge, potentially shifting Arctic marine fish distributions. The study area across the entire Chukchi Sea has the potential to have physically diverse habitats for Arctic marine fishes.

The objectives of the present study were to determine whether: (1) otolith edge chemistry could be used to correctly assign two Arctic marine fish into bottom water mass of capture in the Chukchi Sea; and (2) water temperature, salinity and Ba in bottom seawater affect chemical signatures. Arctic fish communities in the Chukchi Sea, which include the two fish species evaluated in the present study, are structured by bottom water masses (Norcross *et al.* 2010), which have distinct water temperature, salinity and density characteristics; therefore, these marine fish are living in a stable, yet unique set of environmental conditions. Over the short 1.5-month duration of the present study during autumn across 2 years, we assumed the bottom water mass physical variables did not shift drastically. It was also assumed that the area analysed on the otoliths was formed while the fish inhabited one water mass and movement between water masses was minimal. The marginal area sampled on the otolith analysed was formed during the summer growth period, likely representing between ~2 weeks and 1 month of otolith accretion in one water mass. In the present study we assumed there were no ontogenetic effects causing age differentiation in elemental incorporation within otoliths, but rather that dissimilarities are due to water mass occupancy.

Materials and methods

Sample collection

Demersal Arctic fish and physical seawater data were collected aboard the Russian American Long-term Census of Arctic (RUSALCA) research cruise from 3 to 30 September 2009 and the Alaska Monitoring and Assessment Program (AKMAP) coastal survey from 21 August to 4 September 2010 in the Chukchi Sea (Fig. 1). Sampling during the RUSALCA research cruise focussed on coverage across the entire Chukchi Sea to encounter multiple water masses, whereas the AKMAP coastal survey aimed to sample nearshore water masses, with potentially different physical characteristics, by the north-western coast of Alaska. Physical seawater variables (temperature, depth

Table 1. Sample sizes of age classes of Arctic cod and Arctic staghorn sculpin collected from bottom water masses in the Chukchi Sea
Data show the number of fish collected. Water masses were sampled on the bottom in the Chukchi Sea in 2009 and 2010. ACW, Alaska coastal water; BSW, Bering Sea water; WW, winter water (WW)

Fish species	Water mass	Fish age (years)							Total by water mass
		0	1	2	3	4	5	6	
Cod	ACW	24	19	6					49
	BSW	43	18	24	5				90
	WW	11	9	19	5				44
	Total by age	78	46	49	10				183
Sculpin	ACW	16	17	10	3				46
	BSW	38	7	34	28	19	2	1	129
	Total by age	54	24	44	31	19	2	1	175

and salinity) were measured at 35 stations (RUSALCA = 18, AKMAP = 17) and bottom seawater (1–3 m above the sea floor) was collected (RUSALCA = 12, AKMAP = 8) before fish trawls. Physical seawater data and bottom water samples were collected using a Sea-Bird model SBE911+ CTD (conductivity, temperature, depth) profiler (Sea-Bird Electronics, Inc., Bellevue, WA, USA) equipped with 10-L Niskin bottles on the RUSALCA research cruise and a Sea-Bird 33 and Sea-Bird 55 carousel on the AKMAP coastal survey. Water masses were unknown and determined with a post hoc analysis of CTD data. Arctic cod ($n = 183$) and Arctic staghorn sculpin ($n = 175$) were collected (Fig. 1; Table 1) with a plumb staff beam trawl with a 4-mm mesh codend liner. Details of bottom trawling methods can be found in Norcross *et al.* (2010). Fish were collected across a wide geographical scale encompassing the entire Chukchi Sea. At sea, fish were identified to species and frozen at −20°C until further laboratory analyses.

Bottom seawater samples were collected in acid-washed high-density polyethylene Nalgene bottles (125 mL, amber; Nalgene, Nalge Nunc International Corporation, Rochester, NY, USA) that were preloaded with nitric acid. A simple serial dilution:

$$C_1V_1 = C_2V_2 \quad (1)$$

was used to determine the concentration (C_n) and volume (V_n) of nitric acid to acidify samples. Clean bottles were preloaded with 12.5 mL of Omnitrace 20% nitric acid (EMD Millipore Corporation, Billerica, MA, USA) for a final acidified seawater sample concentration of 1.8% (Eqn 1). Blank samples consisted of acid-washed and preloaded bottles that were filled with MilliQ water at sea under the same atmospheric conditions at stations where bottom seawater was collected. Sampling bottles were filled to the brim and stored in coolers at sea (air temperature 0–10°C) and refrigerated at 4°C in the laboratory until analysis.

Otolith preparation and analyses

Sagittal otoliths were removed from fish and prepared for chemical analysis. After removal, otoliths were rinsed with MilliQ water and each was mounted onto a single coverslip with Crystalbond 509 thermoplastic cement (Aremco Products Inc., Valley Cottage, NY, USA). Each coverslip was mounted randomly onto 25 × 45 × 1.27-mm petrographic slides with wax to individually prepare the otoliths. Otoliths were then thin sectioned using a Buehler isomet low-speed saw (Buehler, Lake Bluff, IL, USA), reheated and rotated to cut edge down onto the coverslip and ground down to a thickness of ~200–400 μm with 5-, 9- and 15-μm lapping film on a Buehler rotating wheel until growth rings were visible under a compound microscope. The second otolith for each fish was prepared if the first otolith broke during processing. Polished otoliths on individual coverslips were then transferred (up to nine) onto a single slide for analysis. Both a transmitted and reflected light photograph of each sectioned otolith was taken with a camera-mounted dissecting microscope (M165C; Leica Microsystems Inc., Buffalo Grove, IL, USA). Otoliths were aged from photographs by C. M. Gleason and two independent readers (KW and PD) by counting annuli (transmitted light, light annuli; reflected light, dark annuli). When readers disagreed on an age, they re-aged the questionable otoliths concurrently to assign a final age. To assess the validity of assigned ages (Campana 2001), length–frequency histograms were constructed for each species and the histogram modes and length at age were then compared with Arctic cod data from Lowry and Frost (1981), Craig *et al.* (1982) and Gillespie *et al.* (1997), Smith *et al.* (1997) for Arctic staghorn sculpin, and B. Norcross (unpubl. data) for both species. Sample slides were rinsed with MilliQ water and air dried in a slide storage box before chemical analysis.

Otoliths were ablated with a 350–500-μm-long line (25 μm wide; ~2–4 weeks of accretion) parallel to the most recent growth ring on the ventral edge in the summer growth area (refers to the wide, white opaque zone when viewed with reflected light). In the University of Alaska Fairbanks (UAF) Advanced Instrumentation Laboratory (AIL), a New Wave UP-213 laser ablation (LA) system (Electro Scientific Industries, Inc., Portland, OR, USA) coupled with an Agilent Technologies (Palo Alto, CA, USA) 7500ce quadrupole inductively coupled plasma–mass spectrometer (ICP-MS) fitted with a cs lens stack was used to detect a suite of elements in otolith samples. A cs lens stack has larger apertures for ions to enter compared with the ce lens stack installed as a default on the Agilent 7500ce ICP-MS. For ICP-MS applications with comparably low levels of analytes, such as LA, using the cs lens stack helps increase the sensitivity and allows for lower limits of detection compared with the ce lens stack. In each sample, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{24}Mg, ^{88}Sr and ^{138}Ba were measured. This suite of elements and isotopes were chosen for their highest natural abundance with the least interference in the ICP-MS and were measured above the detection limits in a preliminary analysis of otoliths from both fish species. The LA-ICP-MS was cleaned and tuned at the start of each day of LA. Otolith samples were randomly arranged by species and age class for each collection year before chemical analysis to reduce the effects of ICP-MS instrument drift (Campana *et al.* 1995). The LA settings were set to 80% power, 30-s warm up, 5-s dwell time, a pulse rate of 10 Hz, a laser sampling rate of 10 μm s^{-1}, 5-μm sample depth and 25-μm line width.

The standard reference materials (SRM) and edge sampling procedure in the LA-ICP-MS were the same for both species and all ages of fish. Each time the sample chamber was opened, a set

of three SRMs was placed on the otolith slide and ablated with a 120 µm-long continuous line before otolith ablation. Background levels of the analytes were collected for 30 s before each SRM and otolith sample. National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) 610 glass material was used as the primary SRM in otolith chemical analysis to calibrate the otolith dataset. NIST612 (glass) and National Research Council (NRC) Canada (Ottawa, ON, Canada) FEBS-1 (otolith) SRMs were used as secondary SRMs to determine the accuracy and precision of ICP-MS measurements.

Elemental ratios, limits of detection (LOD) and relative errors were calculated for the otoliths. The element to Ca molar ratio from otoliths collected in 2009 was determined by a series of calculations (Longerich *et al.*1996). Elemental ratios from otoliths collected in 2010 were calculated using the Iolite software package (ver. 2.5; see http://www.iolite.org.au/Iolite.html, accessed 15 May 2014) in IGOR Pro software (ver. 6.2; WaveMetrics, see http://www.wavemetrics.com/index.html, accessed 15 May 2014; Paton *et al.* 2011). Averaged counts per second (c.p.s.) background levels were subtracted from averaged c.p.s. of each analyte measured in samples and standards. The internal standard (ISTD) ^{43}Ca was used to account for instrument drift (Campana *et al.*1997). Concentrations of analytes were calculated in micrograms per millilitre (Longerich *et al.*1996) and were converted to molar concentrations by dividing the concentration (µg mL^{-1}) by the molar weight of the analyte. Analytes were divided by the molar concentration of ^{42}Ca to normalise the ratios (Campana 1999). For 2009 samples, the LOD of each analyte were calculated for otolith samples (Longerich *et al.* 1996) and were as follows: ^{42}Ca = 97.03 mg kg^{-1}; ^{24}Mg = 0.08 mg kg^{-1}; ^{88}Sr = 0.30 mg kg^{-1}; and ^{138}Ba = 0.01 mg kg^{-1}. The LOD from the 2010 samples were similar: ^{42}Ca = 309.33 mg kg^{-1}; ^{24}Mg = 0.36 mg kg^{-1}; ^{88}Sr = 0.35 mg kg^{-1}; and ^{138}Ba = 0.13 mg kg $^{-1}$. The percentage relative error is a determination of how accurate instrument measurement values (v_{approx}) are compared with reference values (v) of the SRM using Eqn 2:

$$\text{Percentage relative error} = \left(\frac{|v - v_{approx}|}{|v|}\right) \times 100 \qquad (2)$$

The percentage relative errors of FEBS-1 for 2009 analyses were as follows: ^{42}Ca = 4.8%; ^{24}Mg = 1.7%; ^{88}Sr = 11.4%; ^{138}Ba = 19.2%. Those from 2010 analyses were: ^{42}Ca = 0.6%; ^{24}Mg = 33.6%; ^{88}Sr = 7.4%; and ^{138}Ba = 0.4%.

Seawater preparation and analyses

Ambient seawater concentrations of ^{137}Ba and ^{138}Ba were measured in the bottom seawater using standard addition methods (Danzer 2007) with an ICP-MS equipped with an Agilent high matrix sample introduction accessory at the UAF AIL. The ICP-MS components were cleaned and tubing was replaced after the completion of each analytical period. Cones were conditioned for ~20 min with BSW (28 µg L^{-1} ^{138}Ba) at the appropriate dilution levels before analysing Chukchi Sea water samples. Ultrarobust settings were used and the instrument was tuned at the beginning of each sampling day. The certified reference material consisted of NASS-5 (Leonhard *et al.* 2002), which is offshore seawater from the NRC of Canada, and was used to determine the accuracy and precision of the ICP-MS measurements. Seawater, blanks and standard samples were individually filtered in the laboratory with disposable 45-µm Teflon filters attached to Leur-Lock 10-mL syringes (VWR International, Radnor, PA, USA) Samples were refrigerated at 4°C until analysis. *ChemStation* software (ver. B04.00; Agilent Technologies, Tokyo) calculated the LOD for Ba, which was determined as three times the standard deviation at the zero concentration level. The LOD for ^{138}Ba for bottom seawater samples was 0.01 µg L^{-1}. The percentage relative error (Eqn 2) of NASS-5 for ^{138}Ba was 9.4% for bottom seawater analyses.

Calcium was calculated based on *in situ* salinity and was used to report Ba : Ca ratios for bottom seawater. Conservative elements in ambient seawater are at a constant ratio to salinity and can be calculated with Eqn 3:

$$\text{chlorinity} = \text{salinity}/1.80655 \qquad (3)$$

and Eqn 4 (Campana 1999; Libes 2009):

$$\text{Ca} = 0.02125/\text{chlorinity} \qquad (4)$$

Elemental ratios to chlorinity are 2.13×10^{-2} g kg^{-1} for Ca, 6.68×10^{-2} g kg^{-1} for Mg and 4.10×10^{-4} g kg^{-1} for Sr (Riley and Chester 1971; Libes 2009). The offshore marine environment Mg : Ca will always yield a molar ratio of 5.18 mol mol^{-1} and Sr : Ca yields 8.83 mmol mol^{-1} (Riley and Chester 1971; Libes 2009); therefore these elements were not measured in seawater for the present study.

Statistical analyses

Several statistical approaches were used to determine the usefulness of individual element and multielement otolith edge chemistry to distinguish and classify fish species into bottom water masses of capture. Bottom water masses were delineated with a potential temperature and salinity plot overlaid with potential density contours in *Ocean Data View* (ver. 4.3.5; R. Schlitzer, see http://odv.awi.de, accessed 1 October 2011) using 111 stations from the RUSALCA 2009 cruise and 27 stations from the AKMAP 2010 survey. Individual element to Ca ratio mean values were tested to determine differences among bottom water mass occupancy for both fish species (pooled ages) with one-way ANOVA ($P < 0.05$) and Tukey's honestly significant difference (HSD) tests ($P < 0.05$) in *Sigma Plot* software (ver. 11.0; Systat Software Inc., San Jose, CA, USA). Data assumptions of independence, multicollinearity, equal variance and normality were satisfied before parametric analysis. Outliers, defined as >3 s.d. of the mean value per element and fish species, were removed for otolith chemistry data (DiMaria *et al.* 2010). A $\log_{10}$ transformation was applied to otolith chemistry for Mg : Ca, Sr : Ca and Ba : Ca to satisfy normality assumptions for statistical analyses. Canonical discriminant analysis (CDA), based on the multielement otolith chemistry (Mg : Ca, Sr : Ca and Ba : Ca) of each fish species, was used to determine correct classification of fish by age classes

(individual and pooled) into bottom water masses (D'Avignon and Rose 2013) using *Systat* software (ver. 13; Systat Software Inc.). A jackknife resampling classification method (leave-one-out) was used to evaluate the predictive ability of multielement otolith signatures to reclassify a removed otolith based on the existing otolith dataset (D'Avignon and Rose 2013) in *Systat* software (ver. 13). A CDA plot was constructed for Arctic cod because this species met the minimum requirement of three groups (water masses) to construct the plot. Individual ages of fish with an adequate sample size and ability to meet parametric statistical assumptions were used in CDA classification procedures.

Regressions, correlations and ANOVAs were used to examine potential relationships between elemental ratios for each fish species and water masses, temperature, salinity, fish age and total body length. First, a forward stepwise regression was used for each elemental ratio and fish species to select a regression model with the fewest significant independent variables; temperature, salinity and fish age or fish length were analysed using *Sigma Plot* software. Second, selected variables were used in either multiple linear regressions (MLR) or linear regressions (LR) in *Sigma Plot* software. Partial correlations were calculated (*R* statistical program ver. 2.13.1; R Foundation for Statistical Computing, see http://www.R-project.org, accessed 10 December 2012) for variables in MLRs, whereas Pearson correlations were calculated for LRs in *Sigma Plot*. The resulting significant variables determined from the forward stepwise regression, MLR and LR were correlated with the elemental chemistries. The Sr : Ca ratio in the age-0 and age-2 classes Arctic cod did not meet the statistical assumptions for the model selection process with the given variables; therefore, only in this case, Spearman's rank correlation was conducted and resulting statistically significant variables reported. The strength of correlations (r) was considered weak (±0.1–0.3), moderate (±0.3–0.5) or strong (±0.5–1.0). Bottom seawater chemistry samples (Ba : Ca) were excluded from the MLRs because of smaller sample sizes; instead, LRs were used to determine whether a significant relationship existed between Ba : Ca in otolith and seawater. To determine whether there were differences in water temperature and fish length among water masses, one-way ANOVAs were performed except when assumptions were violated; then, non-parametric analysis of ANOVA on ranks was used.

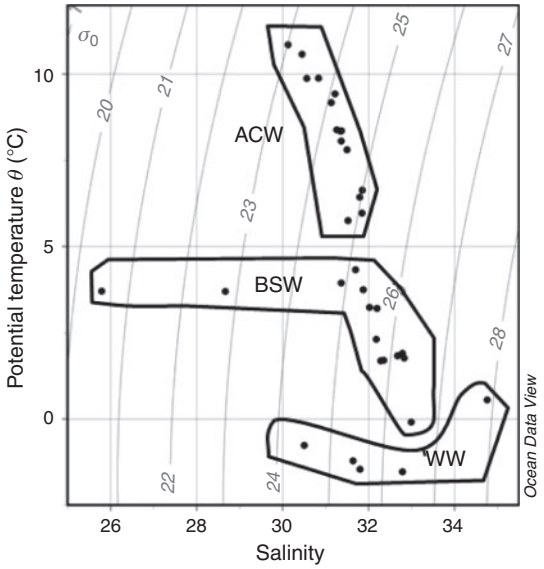

Fig. 2. Potential density in the Chukchi Sea in September 2009 and August 2010. Three bottom water masses (outlined areas) were detected: Alaska coastal water (ACW), Bering Sea water (BSW) and winter water (WW). Fish otoliths were analysed from 35 stations (solid circles) in bottom water temperature from approximately −2 to 11°C.

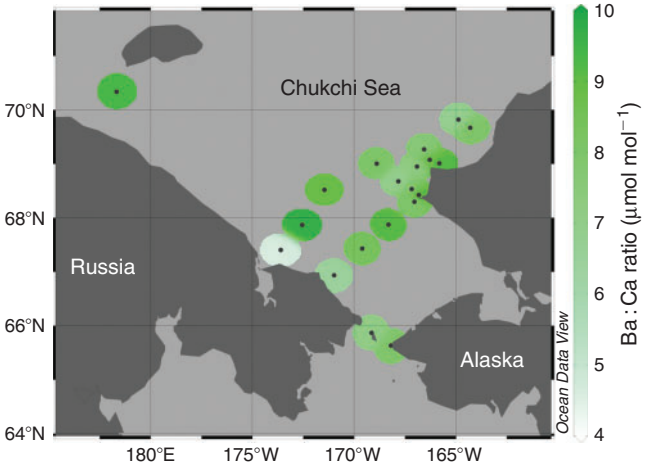

Fig. 3. Ba : Ca ratio measured in bottom seawater at fish collection stations in the Chukchi Sea in September 2009 and August 2010.

Results

Water masses and fish distribution

During September 2009 and August–September 2010, Arctic cod and Arctic staghorn sculpin were captured in three bottom water masses in the Chukchi Sea (Figs 1, 2). In 2009, the bottom water masses over a broad geographical scale of the Chukchi Sea were BSW and WW. In 2010, ACW and BSW water masses were encountered in the nearshore sampling area along northwestern Alaska. BSW was the only water mass sampled over both years (2009, $n = 2$; 2010, $n = 12$); therefore, only data for this water mass were pooled for the two sampling years and there were inadequate samples to test between years. Across 35 stations, temperatures separated water masses at ~0 and 5°C (Figs 1, 2). Across both years, Arctic cod (ages 0–3 years) occupied bottom waters ranging from 5.7 to 9.4°C in ACW, from −0.1 to 3.9°C in BSW and from −1.5 to 0.8°C in WW, whereas Arctic staghorn sculpin (ages 0–6 years) were only collected in ACW (6.0–10.9°C) and BSW (−0.1–4.3°C; Table 1). Arctic cod collected along the coast of Russia in 2009 were likely in the relatively fresh (salinity <29) SCC (Fig. 1); however, because there were only two stations, these stations were included with BSW, which had similar water temperatures (Fig. 2). In bottom seawater, Ba : Ca averaged about the same across Arctic cod stations ($n = 11$; mean (±s.d.) 7.7 ± 1.5 µmol mol^{-1}) and Arctic staghorn sculpin stations ($n = 18$; 7.8 ± 1.2 µmol mol^{-1}), with a range of 4.6–9.7 µmol mol^{-1} across the Chukchi Sea (Fig. 3).

Table 2. Significant multiple and simple linear regression models and associated parameters for Arctic cod and Arctic staghorn sculpin otolith edge chemistry with physical environmental and biological variables
MLR, multiple linear regression; LR, linear regression

Fish species	Regression	Element ratio	d.f.	R^2	F	P-value	Variable	r	P-value
Cod	MLR	Mg : Ca	2, 179	0.49	84.65	<0.001	Age	−0.58	<0.001
							Temperature	0.49	<0.001
		Sr : Ca	3, 173	0.25	18.73	<0.001	Age	0.32	<0.001
							Temperature	−0.31	<0.001
							Salinity	0.19	0.011
	LR	Ba : Ca	1, 177	0.18	38.78	<0.001	Temperature	−0.42	<0.001
Sculpin	MLR	Mg : Ca	2, 171	0.42	61.05	<0.001	Age	−0.49	<0.001
							Temperature	0.37	<0.001
	LR	Sr : Ca	1, 164	0.50	160.68	<0.001	Age	0.7	<0.001

Individual elemental ratios

Otolith chemistry of pooled age classes of Arctic fishes was distinct for Mg : Ca among most bottom water masses, whereas Sr : Ca and Ba : Ca ratios were only unique for Arctic cod between the warm and cold bottom water masses. Mg : Ca was significantly different among ACW, BSW and WW water masses for Arctic cod (ANOVA, Tukey; $F_{2,179} = 48.69$, $P < 0.001$) and between ACW and BSW for Arctic staghorn sculpin (ANOVA, Tukey; $F_{1,173} = 68.18$, $P < 0.001$). Fish age and water temperature affected otolith Mg : Ca in both Arctic fish, showed similar strengths and directions of correlations (Table 2). For Arctic cod, Sr : Ca (ANOVA, Tukey: $F_{2,174} = 70.13$, $P < 0.001$) and Ba : Ca (ANOVA, Tukey: $F_{2,176} = 20.79$, $P < 0.001$) otolith chemistry were significantly different in ACW and BSW (warmer water masses) compared with WW. Sr : Ca and Ba : Ca were inversely affected by water temperature, opposite to Mg : Ca trends (Table 2). Ba : Ca in bottom seawater was not related to Ba : Ca in the otoliths of either fish species for pooled ages.

Because fish age was the most influential variable to affect pooled ages of both Arctic cod and Arctic staghorn sculpin, size effects were investigated within each age class for each element. The age of the fish was related to Mg : Ca and Sr : Ca for Arctic cod (pooled ages 0–3 years) and Arctic staghorn sculpin (pooled ages 0–6 years), although fish length only affected these two elements and the relationship varied among individual age classes of Arctic cod based on the significant variables resulting from the model selection process (Table 3). Mean (±s.d.) body length for the age-0, -1, -2 and -3 classes of Arctic cod was 62 ± 10, 106 ± 18, 141 ± 23 and 169 ± 14 mm respectively; body length for the age-0, -1, -2, -3, -4, -5 and -6 classes in Arctic staghorn sculpin was 38 ± 4, 59 ± 9, 80 ± 7, 93 ± 8, 116 ± 19, 104 ± 15 and 156 mm respectively. The Mg : Ca in the otoliths decreased as body length increased for Arctic cod from the age-0 to age-1 classes (Table 3). The within-element trend between Sr : Ca and body length of Arctic cod varied in direction for age-0 ($r = -0.48$) compared with age-2 ($r = 0.47$; Table 3).

Mean body length of Arctic cod in the age-0 and age-1 but not age-2 classes differed among water masses. The length of age-0 Arctic cod was significantly larger in BSW compared with WW and ACW (non-parametric ANOVA, Dunn's $H = 42.94$, $P < 0.001$), but length was not correlated with bottom water temperature. Alternatively, the length of age-1 fish was larger in the colder water masses, namely BSW and WW compared with ACW (ANOVA, Tukey; $F_{2,43} = 6.67$, $P = 0.003$) and fish were longer in colder bottom water temperatures ($r = -0.46$, $P = 0.00125$). There were no differences in the length of age-2 Arctic cod among water masses.

Bottom water temperature was the variable that most consistently related to all three elemental ratios in the otoliths of individual age classes of Arctic cod and to Mg : Ca in Arctic staghorn sculpin (Table 3). For Arctic cod, bottom water temperature was significantly different among all three water masses for age-0 (non-parametric ANOVA, Dunn's $H = 62.82$, $P < 0.001$) and age-1 (non-parametric ANOVA, Dunn's $H = 39.53$, $P < 0.001$) fish, whereas WW was significantly different from BSW and ACW for age-2 fish (non-parametric ANOVA, Dunn's $H = 39.89$, $P < 0.001$). In pooled (ages 0–3) and individual (age-0, -1 and -2) age classes of Arctic cod, Mg : Ca increased whereas Sr : Ca and Ba : Ca decreased with increasing temperatures (Table 3). For Arctic staghorn sculpin, temperature had a similar effect on Mg : Ca in pooled (ages 0–6), age-0 and age-2 classes (Table 3).

Multielemental ratios

For both pooled and individual age classes of Arctic cod, multielement otolith chemistry discriminated occupancy among bottom water masses in the Chukchi Sea and was analogous to individual element trends. The multielement signature (Mg : Ca, Sr : Ca and Ba : Ca) in otoliths separated ACW, BSW and WW (Fig. 4) in Arctic cod pooled age classes (ages 0–3). Discriminant function (DF) 1 (Fig. 4) accounted for 83% of the variation in the CDA and was positively affected by Mg : Ca and inversely affected by Sr : Ca and Ba : Ca, based on their respective CDA standardised within variances of 0.674, −0.629 and −0.668. A similar pattern of all elements influenced the single dominant DF for each age class of Arctic cod, accounting for 45% of the variation in age-0 (DF 2: Fig. 5*a*), 91% of the variation in age-1 and 86% of the variation in age-2 (DF 1: Fig. 5*b*).

The CDA, based on Mg : Ca, Sr : Ca and Ba : Ca in otoliths, correctly classified pooled and individual age classes of Arctic cod into the three bottom water masses of capture in the Chukchi Sea. Otolith chemistry of Arctic cod pooled ages 0–3 had an

Table 3. Significant variables correlated with Mg : Ca, Sr : Ca and Ba : Ca in Arctic cod and Arctic staghorn sculpin otolith edge chemistry

The bold variables indicate that most often the direction of the correlation for an elemental ratio is the same within an age class between fish species. The italic variables indicate that most often the direction of the correlation for an elemental ratio is the same between age classes within a fish species

Element ratio	Age class	Variable	Arctic cod		Arctic staghorn sculpin	
			r	*P*-value	*r*	*P*-value
Mg : Ca	Pooled	**Age**	**−0.58**	**<0.001**	**−0.49**	**<0.001**
		Temperature	***0.49***	***<0.001***	***0.37***	***<0.001***
	Age-0	*Fish length*	*−0.41*	*0.0002*	n.s.	
		Temperature	***0.39***	***0.0005***	***0.33***	***0.015***
	Age-1	*Temperature*	*0.42*	*0.0042*	n.s.	
		Fish length	*−0.31*	*0.041*	n.s.	
	Age-2	***Temperature***	***0.60***	***<0.001***	***0.54***	***0.002***
		Salinity	n.s.		0.38	0.01
Sr : Ca	Pooled	**Age**	**0.32**	**<0.001**	**0.7**	**<0.001**
		Temperature	*−0.31*	*<0.001*	n.s.	
		Salinity	0.19	0.011	n.s.	
	Age-0	Fish length	−0.48	<0.001	n.s.	
		Salinity	**−0.31**	**<0.001**	**−0.39**	**0.004**
		Temperature	*−0.23*	*0.013*	n.s.	
	Age-1	*Temperature*	*−0.31*	*0.035*	n.s.	
	Age-2	*Temperature*	*−0.50*	*<0.001*	n.s.	
		Fish length	0.47	<0.001	n.s.	
		Salinity	0.42	0.005	n.s.	
Ba : Ca	Pooled	*Temperature*	*−0.42*	*<0.001*	n.s.	
	Age-0	Salinity	−0.28	0.016	n.s.	
		Temperature	***−0.25***	***0.033***	**−0.46**	**0.0005**
	Age-1	*Temperature*	*−0.68*	*<0.001*	n.s.	
	Age-2	*Temperature*	*−0.41*	*0.004*	0.30	0.05

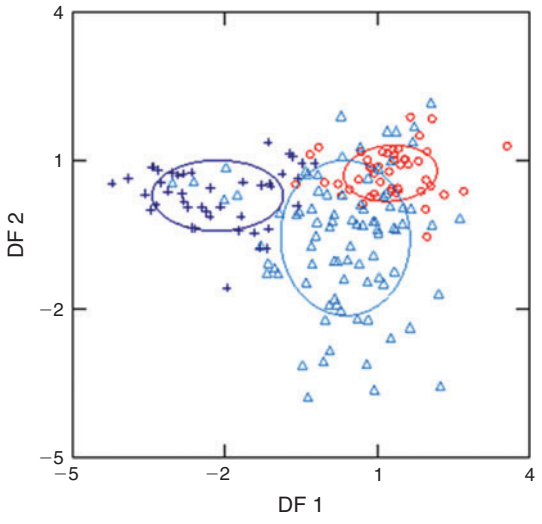

Fig. 4. Discriminant functions (DF) of multielement otolith chemistry of Arctic cod age classes 0–3. Data points are individual Arctic cod otolith ($n = 177$) chemical signatures based on Mg : Ca, Sr : Ca and Ba : Ca. Bottom water masses from which the fish were captured are as follows: Alaska coastal water (circles, red); Bering Sea water (triangles; light blue) and winter water (crosses; dark blue). Ellipses are 50% confidence bounds surrounding the mean chemical signatures in otoliths.

overall correct classification of 76% for ACW, BSW and WW water masses, with each water mass performing equally well (75–77%; Table 4). Resampling classifications using a jackknife method (leave-one-out) had comparable water mass assignment success of 75% (Table 4). Otolith chemistry signatures performed well to discriminate and classify individual ages of Arctic cod into each water mass of capture (Table 5). Resampling classifications (Table 5) were only slightly lower than the overall classification of pooled ages and the age-1 class and matched the overall classification for age-0 (81%) and age-2 (85%) classes. The CDA plots and classifications results for Arctic cod mirror the ANOVA and regression results of the three elemental ratios and are chemically different among most water masses based on correct classification. The dominant variables of both pooled and individual age class analyses of Arctic cod contributed to the opposing correlations of Mg : Ca to Sr : Ca and Ba : Ca in the primary DF of the CDAs. Pooled age CDA classifications were oppositely affected by fish age and bottom water temperature for Mg : Ca and Sr : Ca, whereas temperature only affected Ba : Ca and Sr : Ca in the same direction (Table 3).

Mg : Ca, Sr : Ca and Ba : Ca classified Arctic staghorn sculpin into the two bottom water masses of capture in the Chukchi Sea. Overall correct classification of pooled ages (0–6) was 82%, with the BSW water mass having higher (90%) correct assignment of otoliths compared with ACW (Table 6).

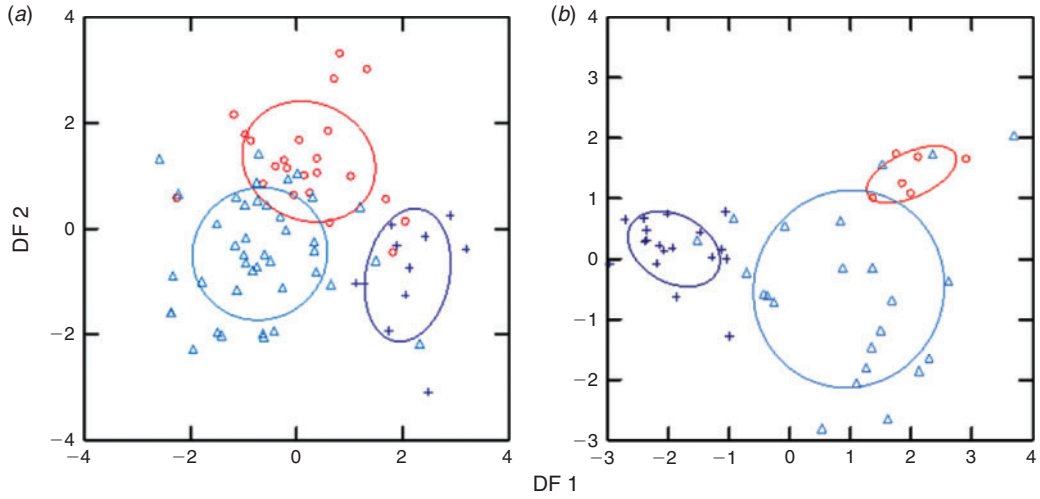

Fig. 5. Discriminant functions (DF) of multielement otolith chemistry of Arctic cod (*a*) age-0 and (*b*) age-2 classes. Data points are individual Arctic cod otolith age-0 ($n = 75$) and age-2 ($n = 47$) chemical signatures based on Mg : Ca, Sr : Ca and Ba : Ca. Bottom water masses from which the fish were captured are as follows: Alaska coastal water (circles, red); Bering Sea water (triangles; light blue) and winter water (crosses; dark blue). Ellipses are 50% confidence bounds surrounding the mean chemical signatures in otoliths.

Table 4. Classification matrices of Arctic cod pooled age classes (0–3) assigned correctly into bottom water masses

Alaska coastal water (ACW), Bering Sea water (BSW) and winter water (WW) were the bottom water masses sampled for cod in the Chukchi Sea in 2009 and 2010. Overall classifications and reclassifications into bottom water masses are based on Mg : Ca, Sr : Ca and Ba : Ca otolith chemistry using a discriminant function analysis. Correct classification of the number and overall percentage of otoliths correctly assigned to the bottom water mass of capture are underlined

	ACW	BSW	WW	Percentage correct
Overall classification matrix				
ACW	36	12	0	75
BSW	15	66	5	77
WW	0	10	33	77
Total	51	88	38	76
Resampling classification matrix				
ACW	36	12	0	75
BSW	17	64	5	74
WW	0	10	33	77
Total	53	86	38	75

Table 5. Total classifications of Arctic cod age classes correctly assigned into three bottom water masses

Overall classifications and reclassifications into bottom water masses are based on Mg : Ca, Sr : Ca and Ba : Ca otolith chemistry using a discriminant function analysis

Fish age (years)	Sample size (*n*)	Overall classification	Resampling classification
Pooled (0–3)	177	76%	75%
0	75	81%	81%
1	45	87%	73%
2	47	85%	85%

Table 6. Classification matrices of Arctic staghorn sculpin pooled age classes (0–6) assigned correctly into bottom water masses

Alaska coastal water (ACW) and Bering Sea water (BSW) were the bottom water masses sampled for sculpin in the Chukchi Sea in 2009 and 2010. Overall classifications and reclassifications into bottom water masses are based on Mg : Ca, Sr : Ca and Ba : Ca otolith chemistry using a discriminant function analysis. Correct classification of the number and overall percentage of otoliths correctly assigned to the bottom water mass of capture are underlined

	ACW	BSW	% correct
Overall classification matrix			
ACW	26	19	58
BSW	13	115	90
Total	39	134	82
Resampling classification matrix			
ACW	26	19	58
BSW	14	114	89
Total	40	133	81

Differences between overall and resampling classifications were minimal (Table 6). Overall classifications of individual ages 0–2 were relatively high and reduced slightly or remained the same after resampling into both water masses (Table 7). Age-0 and age-2 classes had higher sample sizes than the other ages and the otolith chemistry classified these age classes (80 and 90% respectively) into water masses of capture, even after resampling (74 and 90% respectively; Table 7). The age-1 class had the lowest classification success, although this was likely attributed to the lowest sample size of all age classes (Table 7). It was more difficult to determine which variables influenced otolith chemistry of Arctic staghorn sculpin to a greater degree than Arctic cod. In pooled ages, Mg : Ca and Sr : Ca related to age in opposite directional trends, similar to Arctic cod (Table 3).

Table 7. Total classifications of Arctic staghorn sculpin age classes correctly assigned into two bottom water masses

Overall classifications and reclassifications into bottom water masses are based on Mg : Ca, Sr : Ca and Ba : Ca otolith chemistry using a discriminant function analysis

Fish age (years)	Sample size (*n*)	Overall classification	Resampling classification
Pooled (0–6)	173	82%	81%
0	54	80%	74%
1	23	57%	52%
2	41	90%	90%

The effect of water temperature on Mg : Ca and Ba : Ca in individual age classes was mostly consistent with the trends observed in Arctic cod (Table 3). Fewer variables were significant among the Arctic staghorn sculpin age classes examined.

Discussion

This study demonstrates the utility of otolith chemistry, both individual and multielement chemical signatures, to discriminate bottom water mass occupation of two Arctic fish in the Chukchi Sea bordering the Arctic Ocean. The combination of Mg : Ca, Sr : Ca and Ba : Ca otolith signatures successfully classified Arctic cod into three bottom water masses across the entire continental shelf sea, as well as Arctic staghorn sculpin into ACW and BSW in the southern and nearshore areas of the Chukchi Sea. Individual and pooled age classes for both Arctic fish species classified relatively well and consistently into water masses of capture. Both environmental and biological factors affecting individual age classes should be examined before drawing conclusions about movement between habitats. Bottom water temperature, not salinity or Ba : Ca water chemistry, was the primary environmental variable influencing the otolith chemistry of Arctic fish captured in demersal habitats with a range of approximately −2–11°C. Otolith chemistry has the potential to be a powerful tool in fishery science to gain information about fish movement between habitats during life history events. The present study highlights the importance of sampling over a wide range of bottom temperature and salinity to make otolith chemistry an effective tool, whereas a relatively homogeneous marine environment will likely result in poor ability to discriminate fish habitats.

Individual elemental ratios

In general for both Arctic fish species examined, Mg : Ca decreases as fish get older and their bodies grow longer and the bottom water temperature gets colder. Concentrations of Mg, Sr and Ca in otoliths of marine fish are more likely to be affected by physical environmental and biological and physiological variables, and not directly by salinity and ambient chemical concentrations in the seawater, because these elements are conservative in seawater (Campana 1999; Brown and Severin 2009). Conservative elements in seawater are in constant ratios to salinity (Libes 2009) and will not vary enough in the offshore environment among water masses, as seen in other marine otolith chemistry studies of demersal fish (Hamer and Jenkins 2007). It is not surprising that salinity is not the primary or most consistent variable affecting otolith chemistry of Mg : Ca in these two Arctic fish, despite collecting fish from both nearshore and offshore areas, because there is less variation in salinity in this study area compared with temperature.

Sr : Ca and Ba : Ca also help discriminate Arctic cod among nearshore and offshore bottom water masses of capture and are likewise influenced consistently by temperature, but both in the opposite direction to Mg : Ca. Similar to the present study of an Arctic gadid, Sr : Ca and Ba : Ca on the otolith edge of cold-adapted (2, 5 and 8°C) larval Pacific cod (*Gadus macrocephalus*) reared under laboratory conditions are inversely correlated with water temperature (DiMaria *et al.* 2010). Typically, during the open water season in the western Arctic (mid-June to October), ACW is seen as a small water mass along the coast of Alaska that flows from the south, making it relatively warm, and receives freshwater discharge, making it less saline. The BSW also flows northwards, although it is slightly cooler than the ACW and more saline because it entrains a mixture of marine waters from the Bering Sea and receives some freshwater discharge from Siberian Rivers in the western Chukchi Sea (Weingartner *et al.* 1999). WW is located offshore and receives the least freshwater discharge, making it the coldest and most saline of the three bottom water masses. Although both Arctic fish species experienced a range of 11°C in demersal habitats, Arctic cod was captured from three water masses at slightly colder temperatures and across a larger geographical area than Arctic staghorn sculpin, contributing to the strength of the temperature relationship of Sr : Ca and Ba : Ca in Arctic cod otoliths.

There should be higher concentrations of Ba in marine waters with lower salinity because of freshwater discharge (Cooper *et al.* 2008); however, that was not reflected in the otolith chemistry of demersal Arctic fish in the present study. Mostly the salinity range encountered was relatively small (30–34.8). Ba, a non-conservative element, is elevated in riverine input in the Chukchi Sea and near the sea floor beneath biologically productive surface marine waters (Guay and Falkner 1997; Abrahamsen *et al.* 2009). In the present study, Ba : Ca in bottom seawater of the Chukchi Sea was elevated offshore in the BSW and at a few stations in the ACW near the north-western coast of Alaska. Ba : Ca in ambient bottom seawater had no relationship with Ba : Ca otolith chemistry in either fish species, despite the 5.1 μmol mol^{-1} range of Ba : Ca in bottom seawater within the study area. In contrast, snapper (*Pagrus auratus*) and sand flathead (*Platycephalus bassensis*) are demersal marine species in which Ba : Ca in otoliths correlates to a similar range of Ba : Ca in ambient bottom seawater (Hamer and Jenkins 2007) as in the present study. Otolith chemical composition can also be influenced by diet (Gallahar and Kingsford 1996; Buckel *et al.* 2004) and water temperature (DiMaria *et al.* 2010; Woodson *et al.* 2013); therefore, relationships of otolith chemistry and ambient seawater may not be transferable between biomes and species because Ba : Ca in otoliths may not be solely correlated with seawater concentrations. Ba : Ca otolith chemical signatures are lower in the warmer water masses (ACW and BSW) compared with WW, primarily relating to water temperature in both Arctic fish in the present study. Similarly, Ba : Ca in otoliths is elevated in cold upwelling water off the coast of

California in the eastern Pacific Ocean, likely due to the cold water temperatures and regeneration of Ba at depth (Woodson *et al.* 2013).

Interestingly, Sr : Ca was inversely correlated with salinity in age-0 fish of both fish species examined, indicating a possible physiological or diet-specific influence. Sr : Ca variability is associated with the rate of protein synthesis in relation to the crystallisation rate of the otolith (Campana 1999), the drivers of which are not well understood. In this case, the driver of the rate of protein synthesis could be because the smaller fish have less of an osmoregulation capability. Smaller bodied age-0 Arctic cod and Arctic staghorn sculpin incorporated higher concentrations of Sr compared with larger age-0 fish, possibly as a result of less osmoregulation capability in small fish (Kalish 1991) and diet-specific differences of Arctic cod less than 70 mm in length and Arctic staghorn sculpin less than 30 mm in length (Gray 2015; Gray *et al.*, in press). Age-0 Arctic cod were larger in BSW, a colder, more saline water mass than ACW, yet otoliths of larger fish had less Sr. Larvae and small juveniles have immature osmoregulation capabilities, allowing more ions to enter the body that, in turn, are available for otolith accretion (Kalish 1991; Fowler *et al.* 1995); as fish increase in size, the discriminant ability of the fish improves (Kalish 1991). In addition, stress (low condition factor, fin erosion etc.) can affect the binding, movement and quantity of ions entrained in blood and endolymph fluid, which can then result in elevated Sr concentrations in Australian salmon (*Arripis trutta*) otoliths (Kalish 1989) and in larval Atlantic herring (*Clupea harengus*) reared in a laboratory (Townsend *et al.* 1989).

Sampling closer to shore in estuarine areas and earlier in the spring season (during peak freshwater run-off) may provide a greater contrast of Ba in seawater, because the major freshwater source flows from the south into the eastern Chukchi Sea and is elevated in Ba during this time compared with summer, autumn and winter (Cooper *et al.* 2008). Sampling during the warmest months (June and July) or closer to shore where the water is warmer would maximise differences between coastal and offshore habitats and, as a result, have more clarity in otolith chemical comparisons for these habitats. Comparing riverine with nearshore fish habitats would likely result in differences in Sr : Ca and Ba : Ca in otoliths. Otolith chemistry studies can differentiate freshwater *v.* marine habitat use (Hamer *et al.* 2006; Walther *et al.* 2011).

Fish age and size effects

In this field study, Mg : Ca and Sr : Ca were affected by fish age in pooled ages of both fish species and fish length influenced some individual age classes of Arctic cod. Ontogenetic and size effects can influence elemental concentration in the otoliths of fish (Campana 1999) and need to be considered when interpreting otolith chemistry. In a laboratory study of larval Pacific cod reared in temperatures comparable to those of our field-collected fish, temperature affected Sr : Ca and Ba : Ca in a similar manner as seen in the present study and there was no evidence of somatic growth rate or otolith precipitation on Mg : Ca, Sr : Ca and Ba : Ca (DiMaria *et al.* 2010). In Atlantic cod, fish length affects Mg : Ca, Sr : Ca and Ba : Ca otolith chemistry, but the nature of the effect on Mg : Ca and Ba : Ca can vary in different environments and could be influenced by prey choice and growth conditions (D'Avignon and Rose 2013). Arctic cod can have different average lengths in separate habitats; for example, in the Bering Sea, 101 mm, age-1; and Chukchi Sea, 71 mm, age-1 (Lowry and Frost 1981). Although the aim of the present study was not to determine the effect of fish size on otolith chemistry, there was an effect of fish length, similar to the effect of fish age class. Arctic cod had a wide variation in length at age, which may explain why fish length affected otolith chemistry in that species, but not in the smaller Arctic staghorn sculpin.

The effects of fish length on the classification success of the otolith chemical signature into water masses was likely different for age-0, age-1 and age-2 Arctic cod, whereas thermal difference among water masses was more consistent among individual age classes. In age-0 Arctic cod, fish were larger in BSW than ACW and WW. Age-0 Arctic cod may prefer that particular water mass, because this fish species has been known to be most abundant in the BSW in the middle of August (Gillespie *et al.* 1997; Norcross *et al.* 2010); temperature was not related to age-0 fish length in the present study. Age-1 Arctic cod fish were larger in the BSW and WW (colder water masses) than ACW (warmest water mass), which could relate to the distribution preference of this fish species because BSW and WW have a higher abundance of Arctic cod than ACW (Norcross *et al.* 2010) and temperature was inversely related to fish length for age-1 fish in the present study. As in age-1 Arctic cod, growth rate can be the primary factor affecting Sr : Ca in otoliths of fish from different locations and temperature regimes (Sadovy and Severin 1992, 1994). Age-2 Arctic cod length did not differ among water masses; therefore, fish length likely did not play a role in assigning otolith chemical signatures into water masses for this age class.

Application

Arctic cod and Arctic staghorn sculpin otolith chemistry was classified by bottom water masses in the Chukchi Sea, as opposed to a spatially isolated location for a portion of the fish's life history as in other studies (Hamer and Jenkins 2007; D'Avignon and Rose 2013). Otolith chemistry is influenced by the water temperature and sometimes salinity in which these fish species live; therefore, sampling among relatively stable water masses resulted in successful classification of otolith chemical signatures into these water masses. Both fish species were captured in the demersal environment, similar to previous collection efforts in the Chukchi Sea, and fish community assemblages and distribution have been documented for these species in the Chukchi Sea in relation to water masses (Gillespie *et al.* 1997) and the physical properties (sediment type, temperature and salinity) of the demersal habitat (Smith *et al.* 1997; Norcross *et al.* 2010). Water masses do not remain constant in the Chukchi Sea interannually, because the physical characteristics (temperature and salinity) and three-dimensional space (Norcross *et al.* 2010) of water masses will shift due to seasonal Bering Sea inflow, freshwater influx, sea-ice processes and wind forcing (Weingartner *et al.* 2005). During the present study, sampling occurred during the late summer to early autumn, and bottom water masses were relatively isolated from atmospheric effects compared with surface waters, resulting in a relatively stable sampling area and time period. Otolith edge signatures of

Mg : Ca, Sr : Ca and Ba : Ca in oceanic fish have been used to discriminate frontal zones and water mass occupation of fish in the Antarctic Circumpolar Current in the south-western Atlantic Ocean (Ashford *et al.* 2007). The present study has no way to determine fish movement between water masses; we assume minimal movement occurred between water masses of capture during the brief sampling period. Both physical alternations of water masses and fish movement between water masses could contribute to the misclassifications of the CDA analysis in the present study.

Otolith chemistry could be a useful tool in fishery science to help understand life history events and movement of individual age classes of fishes among physically different water masses in Arctic marine environments. Arctic cod and Arctic staghorn sculpin have interesting distributions within the water column; they hatch as pelagic larvae and settle to the bottom (Wyllie-Echeverria *et al.* 1997; Norcross *et al.* 2010). Once settled in the demersal environment, Arctic staghorn sculpin remain near the sea floor for the duration of their lives, whereas Arctic cod can occupy demersal, pelagic and cryopelagic (ice-associated) zones in coastal and offshore marine habitats (Craig *et al.* 1982; Bluhm and Gradinger 2008; Norcross *et al.* 2013). Otolith chemistry has the potential to distinguish spawning areas and migrations between physically unique habitats of Arctic fish. Arctic cod is thought to make mass spawning migrations from offshore waters to estuarine areas in the Chukchi and Beaufort Seas in autumn season, but little information is known about the overwinter habitat (Craig *et al.* 1982). Arctic staghorn sculpin is also thought to spawn in nearshore environments during winter (Smith *et al.* 1997). A variety of ages of both species have been captured in coastal lagoons during the winter and summer months, as well as in offshore areas (Craig *et al.* 1982). Because these fish use a variety of physically unique habitats year round, the application of otolith chemistry could be developed further by analysing core-to-edge transects to help reconstruct migrations between habitats in winter and summer growth annuli. In nearshore and offshore marine environments, discrete spawning areas can have correct classifications ranging from 66 to 78% based on otolith edge chemistry (Mg, Mn, Sr and Ba ratios to Ca) and the growth rate of Atlantic cod *G. morhua* (D'Avignon and Rose 2013). Similarly, Sr and Ba otolith chemistry measured in the larval period of capelin in the north-west Atlantic Ocean correctly classified (71–77%) fish into beach and deep water spawning habitats (Davoren and Halden 2014).

Arctic cod can shift their geographical distribution within a sea, likely due to temperature and food availability associated with sea ice. In warmer waters in the southern Bering Sea, Arctic cod distribution is limited to the 'cold pool' (<2°C) during warm years, whereas their distribution expands during cold years (Wyllie-Echeverria and Wooster 1998). Similarly, in the Chukchi Sea, Arctic cod exhibit different seasonal distributions between offshore and nearshore waters in warm and cold years because this species prefers cooler water masses and is known to follow the annual sea ice retreat (Thedinga *et al.* 2013). Chemical examination of an otolith transect of core to edge could be used to learn about the timing and preferred water mass of these life history transitions between distinct thermal habitats. In addition, growth rates and fish age should be examined when interpreting Mg : Ca and Sr : Ca otolith chemistry in Arctic cod.

Some additional considerations could make the use of otolith chemistry as a tool to discriminate water mass occupancy of Arctic fish more effective. A balanced sampling design would make the CDA model robust. That would require collecting equal sample sizes of each fish species and age class, and selecting habitats that have a wide range of magnitudes of environmental variables. For example, in the Chukchi Sea, Arctic cod was the most abundant and geographically distributed fish species, which contributed to the higher success of using otolith chemistry to classify fish into water masses compared with Arctic staghorn sculpin. Otolith chemistry is most useful in detecting differences among habitats with contrasting environmental parameters (e.g. cold and warm, fresh and saline). Sr : Ca and Ba : Ca ratios (Campana 1999) are reliable and useful in studies in coastal and estuarine environments because these elements vary in magnitudes in otoliths more between marine and freshwater environments of varying salinities (Hamer *et al.* 2015) and may be more useful in nearshore surface waters, which receive more freshwater discharge than bottom waters. In the present study we found Sr : Ca and Ba : Ca have consistently similar patterns that are the reverse of Mg : Ca patterns, possibly making Mg to Sr and Ba ratios useful analyses in future investigations of water mass occupancy and life history transitions between these environments.

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