

# Spatial segregation, dispersion and migration in early stages of polar cod *Boreogadus saida* revealed by otolith chemistry

Caroline Bouchard · Simon R. Thorrold · Louis Fortier

Received: 22 September 2014 / Accepted: 4 February 2015 / Published online: 13 February 2015  
© Springer-Verlag Berlin Heidelberg 2015

**Abstract** Arctic marine food webs are centered on polar cod (*Boreogadus saida*), a small, largely pelagic gadid, which movement and migration remain unclear, especially for the early life stages. The present study examined the otolith chemistry of juvenile polar cod from six oceanographic regions of the Arctic Ocean in order to document patterns of spatial segregation, dispersion and migration during the species early life. The freshwater winter refuge hypothesis, suggesting that polar cod larvae start to hatch in winter in freshwater-influenced regions but only later in the season in purely marine regions, was also tested. Five elemental ratios (Li/Ca, Mg/Ca, Mn/Ca, Sr/Ca and Ba/Ca) were analyzed by laser ablation inductively coupled plasma mass spectrometry in three otolith zones representing the egg, larval and juvenile stages. The concentration of each of the five elements at the edge of the otoliths, corresponding to incorporation shortly before capture, was significantly correlated with surface salinity and temperature at capture site and date. Otolith chemistry differed between juveniles from freshwater-influenced regions (Laptev Sea, Hudson Bay Amundsen Gulf) and those from purely marine regions (Lancaster Sound, Baffin Bay, Frobisher Bay), in agreement with dissolved concentrations of at least some of the target elements in the Arctic Ocean. Discriminant function analyses including all five elements

provided valuable information on the species population structure and dispersion of early stages. The correspondence between otolith Mn/Ca, Ba/Ca and vertical profiles of dissolved Mn and Ba in the water column may reflect the ontogenetic vertical migration of juvenile polar cod in late-summer and fall.

## Introduction

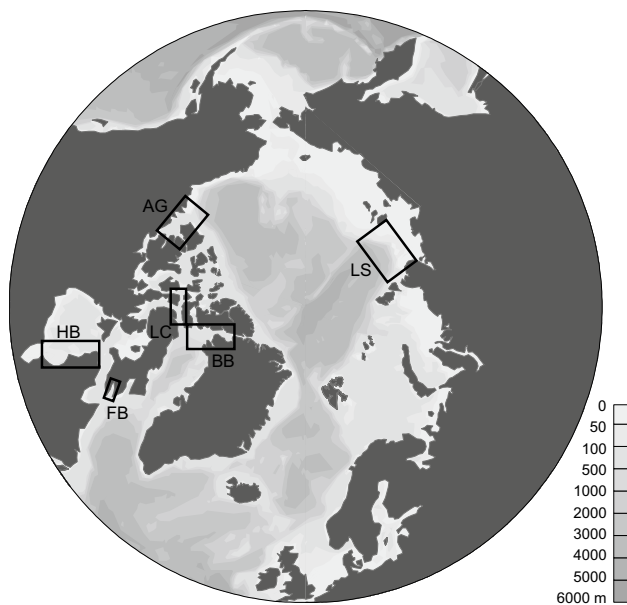
The polar cod *Boreogadus saida* is extremely important in Arctic food webs (Bradstreet et al. 1986), and its response to ongoing climatic changes holds the potential to profoundly affect the entire Arctic ecosystem (Tynan and DeMaster 1997; Smetacek and Nicol 2005). Despite the recognition of the crucial role that polar cod plays in the Arctic, and increasing efforts to understand different aspects of its biology, many key aspects of polar cod ecology remain uncertain. Notably, information on population structure and spatial segregation is very scarce. While no genetic structuring was detected by studies conducted with allozymes, RAPD and scnDNA (Fevolden and Christiansen 1997; Fevolden et al. 1999), more sensitive DNA markers have yet to be used. Furthermore, important questions remain unanswered in terms of spawning behavior and habitat use. Spawning occurs from November to March (Baranenkova et al. 1966) and, at least in the Amundsen Gulf region, adults spend this period aggregated near the bottom at depth greater than 140 m (Benoit et al. 2008; Geoffroy et al. 2011). In addition to these deep spawning aggregations, a number of discrete nearshore, shallow spawning grounds have been suggested in the North American (Craig et al. 1982; Thanassekos and Fortier 2012) and Siberian Arctic (see Fig. 1 of Fevolden and Christiansen 1997).

---

Communicated by D. Righton.

C. Bouchard (✉) · L. Fortier  
Québec-Océan, Département de Biologie, Université Laval,  
Québec, QC G1V 0A6, Canada  
e-mail: caroline.bouchard@qo.ulaval.ca

S. R. Thorrold  
Biology Department MS 50, Woods Hole Oceanographic  
Institution, Woods Hole, MA 02543, USA



**Fig. 1** Bathymetric map of the Arctic Ocean indicating sampling locations of juvenile polar cod in 2005–2006. *AG* Amundsen Gulf, *BB* Baffin Bay, *FB* Frobisher Bay, *HB* Hudson Bay, *LC* Lancaster Sound, *LS* Laptev Sea

Progress has been made in our understanding of the ecology of polar cod early life history stages with case studies undertaken in many regions of the Arctic Ocean, including Hudson Bay (Drolet et al. 1991; Gilbert et al. 1992; Fortier et al. 1996) and the Northeast Water Polynya (Michaud et al. 1996; Fortier et al. 2006). Recently, it has been hypothesized that the main process dictating the hatching season of polar cod is not the match between first-feeding larvae and maximum abundance of their prey, but rather the maximization of pre-winter size to reduce predation by seabirds at the surface in late-summer and then by adult polar cod on their common overwintering grounds at depth (Fortier et al. 2006; Bouchard and Fortier 2008). Since the chance of surviving to the first winter is higher for individuals with large pre-winter size, selection pressure pushes hatching to occur as early as possible to allow individuals to reach a larger size before their ontogenetic vertical migration toward deep overwintering grounds (Bouchard and Fortier 2008, 2011). The freshwater winter refuge hypothesis suggests that early hatching (January–March) occurs only near estuaries, where the temperature of under-ice river plumes remains relatively high in winter (just below 0 °C), allowing successful embryonic development and first feeding of the larvae. In purely marine regions, surface temperature during the winter (near −1.8 °C) restrains larval survival and the hatching is delayed until the surface waters warm up in April–May (Bouchard and Fortier 2008, 2011).

Movements during each life stage of polar cod also require more systematic investigations. Seasonal

vertical migrations, during which individuals gradually swim toward the bottom prior to winter, are observed in juveniles and adults (Ponomarenko 2000; Benoit et al. 2008; Geoffroy et al. 2011). Adult polar cod have been shown to perform diel vertical migrations (DVM) under the ice during the polar night (Benoit et al. 2010; Geoffroy et al. 2011) while the possibility of small-amplitude DVM, undertaken by the larvae during the summer, have received supportive observations (Bouchard et al. in press). The interaction between ocean circulation and polar cod depth-keeping behavior in winter can lead to passive transport of adults over significant distances (Benoit et al. 2008), but it is unclear if polar cod perform long distance active horizontal migration.

Otolith chemistry has become a powerful tool to study fish movements including migration, natal homing and connectivity among sub-populations (Elsdon et al. 2008). The calcium carbonate matrix of fish otoliths incorporates some elements in proportion to their concentrations in the ambient environment. When matched with specific growth increments in otoliths, these data provide information on specific environments that individual fish have inhabited throughout their lives. For instance, Sr/Ca and Ba/Ca ratios in otoliths have been used to trace movements of anadromous fishes from spawning locations in rivers to ocean environments (e.g., McCulloch et al. 2005). Multi-element analyses of otolith cores have also revealed spatial structure in marine fish populations at spatial scale ranging from a few kilometers (e.g., Standish et al. 2008; Clarke et al. 2009) to thousands of kilometers (e.g., Ashford et al. 2008; Macdonald et al. 2013).

In the present study, we test the freshwater winter refuge hypothesis by contrasting the otolith elemental signatures of polar cod collected in regions strongly influenced by riverine freshwater to those of fish captured in regions weakly influenced by freshwater. A second hypothesis, that an initial segregation of the eggs and a subsequent dispersion of the larvae and juveniles may be reflected in the otolith chemistry, is tested by analyzing the elemental signatures in three zones of the otolith representing different early life stages. Finally, the possibility that the ontogenetic vertical migration of polar cod juveniles is reflected in their otolith chemistry is investigated.

## Materials and methods

### Study areas

We collected samples from six regions of the Arctic Ocean characterized by differences in their physical and biological dynamics. The Laptev Sea, Hudson Bay and Amundsen Gulf are strongly influenced by freshwater, with river

**Table 1** Range of long-term annual mean surface salinity and temperature in six regions characterized by strong or weak river discharge

Region	Coordinates	River discharge (km <sup>3</sup> /year)	Salinity		Temperature (°C)	
			Min	Max	Min	Max
Laptev Sea	71–81°N; 100–155°E	738	9.88	32.67	−1.66	1.11
Hudson Bay	54–63°N; 75–85°W	714	22.77	32.03	−0.07	4.63
Amundsen Gulf	69–72°N; 118–135°W	330	23.00	31.11	−1.17	1.57
Lancaster Sound	73–75°N; 80–103°W	Weak	26.20	32.54	−1.51	−0.22
Baffin Bay	75–80°N; 65–80°W	Weak	29.61	32.91	−1.24	0.14
Frobisher Bay	62–64°N; 65–69°W	Weak	31.24	33.01	−0.65	1.19

Surface salinities and temperatures were extracted from the World Ocean Atlas 2013 on 0.25° grids for areas bounded by the geographical coordinates indicated. Salinity and temperature values are slightly biased as nearshore data are generally missing from the database

**Table 2** Region, year, sampling code, size range (standard length, SL), capture and hatch date range, age range, number of stations (stn) and number of otolith core (C), middle (M) and edge (E) zones successfully analyzed by laser ablation ICP-MS

Region	Year	Code	SL (mm)	Capture dates	Hatch dates	Age (d)	n stn	n otoliths		
								C	M	E
Laptev Sea	2005	LS	19–57	14–21 Sep	4 Jan–9 Jul	70–260	14	36	18	36
Hudson Bay	2005	HB	26–50	26 Sep–2 Oct	25 Feb–5 Jun	113–217	3	14	4	14
Amundsen Gulf	2005	AG05	26–58	2–14 Sep	26 Dec–26 May	99–262	6	20	6	20
	2006	AG06	29–55	29 Sep–17 Oct	12 Jan–24 May	131–278	7	22	21	31
Lancaster Sound	2005	LC05	22–40	22 Aug–19 Sep	9 Apr–24 May	102–163	2	19	0	18
	2006	LC06	18–39	20–21 Sep	29 Mar–10 Jul	73–176	2	22	11	21
Baffin Bay	2006	BB	29–47	15–18 Sep	27 Feb–30 May	108–200	3	26	15	32
Frobisher Bay	2006	FB	40–50	29 Oct	6 Mar–26 May	156–237	1	14	13	14

discharge of 738, 714 and 330 km<sup>3</sup> year<sup>−1</sup>, respectively (Déry et al. 2005; Gordeev 2006). Alternatively, Lancaster Sound, Baffin Bay and Frobisher Bay are located far from any large river and can be considered weakly influenced by riverine freshwater. These differences in freshwater input among the regions are reflected in their annual mean surface salinities (Table 1), as shown by climatological data extracted from the latest version of the World Ocean Atlas (Zweng et al. 2013). Annual mean surface temperatures (Locarnini et al. 2013) depend on several factors (e.g., salinity, latitude, oceanic circulation, sea ice dynamics) and are generally higher in the regions highly influenced by freshwater (Table 1). Long-term mean surface salinity during the months corresponding to polar cod hatching season in five of the six regions studied are mapped in Bouchard and Fortier (2011). Other important oceanographic features of the study areas, including the Laptev Sea polynyas, the Beaufort Sea stamukhi and the North Water Polynya, are detailed in Bouchard and Fortier (2008), (2011).

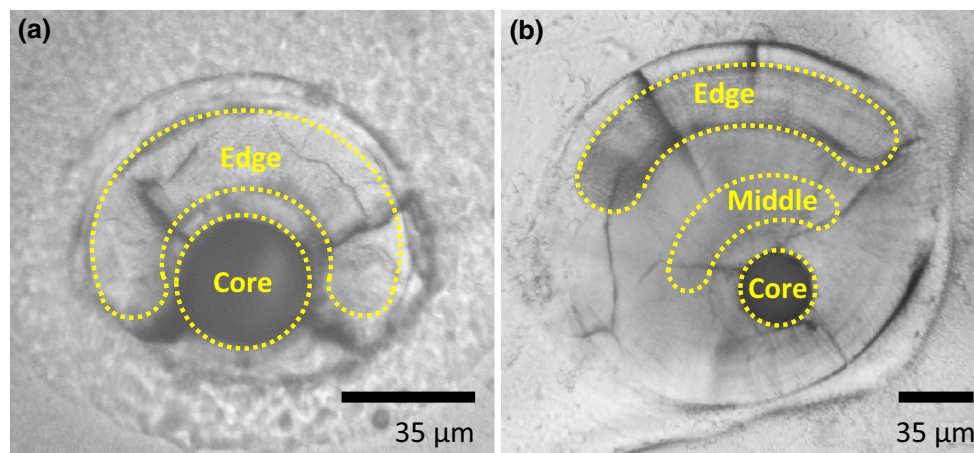
### Sampling

*Boreogadus saida* juveniles were collected from August 22 to October 2, 2005 and from September 15 to October 29, 2006 during expeditions of the icebreakers CCGS *Amundsen* in the Canadian Arctic and *Kapitan Dranitsyn* in the

Laptev Sea. Detailed sampling methodology is given in Bouchard and Fortier (2008), (2011). In summary, juveniles collected at 38 stations (Fig. 1, Table 2) with four different samplers at depths of 0–200 m were preserved individually in 95 % ethanol after being sorted from the zooplankton samples and measured fresh (standard length, SL, ranging 18–58 mm) on board. A CTD was used to obtain vertical profiles of salinity and temperature. As the vast majority of polar cod larvae and juveniles generally occur within 30 m of the surface (Bouchard et al. in press), but that a large fraction of the CTD data from 0 to 10 m were considered unreliable (due to mixing created by sea conditions or ship repositioning for example), sub-surface (10–30 m) salinity and temperature data were used in the present study.

### Otolith preparation

The validation of daily increments deposition in the otoliths of young polar cod has been performed previously by a tetracycline marking experiment and the examination of otolith microstructure under both light and scanning electron microscopy (Bouchard and Fortier 2011). For the present study, lapillar otoliths were removed, cleaned of adhering tissue and mounted separately on microscope slides in Crystal Bound<sup>®</sup> thermoplastic glue. One lapillus from each fish (preferentially the left) was used to estimate



**Fig. 2** Photographs of polar cod otoliths viewed under light microscopy (taken at 200 $\times$ ) after laser ablation. Yellow areas delineate ablation areas. **a** Small otoliths were analyzed in two zones (*core* and

*edge*). **b** Larger otoliths were analyzed in three zones (*core*, *middle* and *edge*). The *middle* zone of the otolith shown in **b** has not been ablated yet

age by counting the increments under a light microscope ( $\times 1000$  magnification) coupled to a camera and image analyzer system (Image Pro Plus<sup>®</sup>). Individual hatch dates were determined by subtracting a fish age (in days) from its date of capture. Aging precision (Campana 2001), estimated by comparing the first reading with a second independent reading made by the same reader (10 otoliths) or a different reader (10 otoliths), yielded a mean coefficient of variation of 3.6 and 2.9 %, respectively. The first readings were retained in subsequent analyses.

The matching otolith from each pair was ground on its medial side using fine-grain lapping paper in preparation for laser ablation inductively coupled plasma mass spectrometry (ICP-MS) analysis. The remaining preparation stages were conducted in a class 100 (ISO class 5) clean room. Otoliths were triple rinsed with Milli-Q water, sonicated for 2 min., rinsed with Milli-Q again and allowed to dry for 24 h under the laminar flow hood. Otoliths were then transferred on clean petrographic slides using double-sided tape, insert in plastic Petri dishes and clean resealable zipper storage bag, and transported to the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Facility.

#### Otolith analysis

We determined isotope abundances ( $^7\text{Li}$ ,  $^{25}\text{Mg}$ ,  $^{48}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{88}\text{Sr}$  and  $^{138}\text{Ba}$ ) in the core, middle and edge zones of otoliths using a 193 nm excimer laser ablation system (New Wave Research) coupled to a Thermo Finnigan Element 2 ICP-MS (Thermo Electron Corporation). Using an image analysis software, we selected ablation zones that included a 35- $\mu\text{m}$ -diameter spot centered on the otolith core, a 150–200  $\mu\text{m}$  long by 35- $\mu\text{m}$ -wide curved line following growth

increments close to the core (middle zone) and close to the otolith edge (Fig. 2). As daily growth increments of polar cod lapillar otoliths are ca. 1  $\mu\text{m}$  wide on average (e.g., Bouchard and Fortier 2008, 2011), ablation of the middle and edge zones correspond to ca. 35 days of growth. For all analyses, laser scan speed was 5  $\mu\text{m s}^{-1}$ , repetition rate was 5 Hz and dwell time was 40 s (30 s for middle zones). Ablated otolith material was carried to the ICP-MS with a carrier gas (He) and 2 %  $\text{HNO}_3$  nebulized wet aerosol. Instrument blanks of 2 %  $\text{HNO}_3$  were run at the beginning of each analytical session and then periodically after every 10 otolith samples. Dissolved standard reference materials (FEBS 1, Sturgeon et al. 2005) were used to correct for instrument mass bias and to determine instrument precision. Both standards, digested in 2 %  $\text{HNO}_3$  and diluted to a Ca concentration of 40  $\mu\text{g g}^{-1}$ , were analyzed after every blank. A total of 448 otolith samples were analyzed over 11 analytical sessions. Detection limits were calculated by dividing three times the standard deviation of the blanks that were run throughout the analyses ( $n = 63$ ) by the mean value of each isotope in all otolith samples and were (in %): Li (8.6), Mg (28.5), Ca (0.12), Mn (20.7), Sr (0.07) and Ba (11.4). Estimates of precision were determined by averaging within-run ( $n = 11$ ) relative standard deviation of the standard (Japanese Reference) measurements and were: Li/Ca: 4.1 %, Mg/Ca: 1.9 %, Mn/Ca: 10.3 %, Sr/Ca: 0.82 % and Ba/Ca: 2.8 %. A total of 18 core and nine edge samples were eliminated from subsequent analyses due either to inaccurate positioning of the laser during sampling, or because the ablation process caused significant damage to the otolith. Finally, a test for outliers found 13 core samples with elemental ratios showing either anomalously high or low values, and these samples were also subsequently removed from the data set.



## Data analysis

Elemental compositions were expressed as ratios to Ca, to account for fluctuations in the amount of material ablated (Sinclair et al. 1998). Following the results of a Box–Cox test, all elemental ratios were log<sub>10</sub>-transformed to better meet statistical assumptions of the tests used (e.g., normality of errors and homogeneity of variances between groups for ANOVA). Because some cases of non-normality of errors or heterogeneity of variances remained after transformation, parametric tests and equivalent nonparametric tests were performed. Statistical analyses were performed with the software SAS© 9.2, and a significance level of  $\alpha < 0.05$  used for all tests except for the Shapiro–Wilk test where data were considered non-normal when  $p < W < 0.01$ .

Relationships between elemental ratios, salinity, temperature, hatch date and capture date were assessed by linear regressions. Student *t* tests (exact Wilcoxon when normality of scaled residuals was not met) were used to detect differences in elemental ratios between regions highly influenced by freshwater and those with weak freshwater input, and to assess interannual differences in elemental ratios of otolith core, middle and edge zones in Amundsen Gulf and Lancaster Sound.

Among-region differences in the multi-elemental ratios were compared with one-factor multivariate analysis of variance (MANOVA). Mardia's skewness and kurtosis tests showed multi-dimensional non-normality; hence, exact permutation statistics (Legendre and Legendre 1998) were used with 10,000 permutations. Discriminant function analyses with leave-one-out cross-validation were used to graphically visualize the ability of otolith elemental ratios to classify juveniles to their region of origin. Analyses were performed separately for both years. Because the assumption of homogeneity of variances-covariances matrices was not met (verified with chisquare test), quadratic discriminant function analysis were used.

Differences in elemental ratios between core, middle and edge zones of otolith were tested by repeated-measures ANOVA (PROC MIXED). In the case of non-normality, a nonparametric test for longitudinal data was performed (Brunner et al. 2002).

## Results

Relationships between elemental concentrations, salinity, temperature, hatch date and capture date

Elemental ratios in otolith edges, incorporated into the calcium carbonate matrix shortly before capture, were regressed against sub-surface (10–30 m) salinity and temperature at the station (and date) where fish were captured.

The concentration of all five elements showed a significant relationship with salinity (Fig. 3). Four of the elemental ratios (Li/Ca, Mg/Ca, Sr/Ca and Ba/Ca) were positively correlated with salinity, while Mn/Ca showed a negative correlation with this variable (Fig. 3). Concentrations of two elements, Li/Ca and Mn/Ca, were negatively correlated with temperature; no significant relationship with temperature was found for the other elements (Fig. 3).

Elemental ratios in the core, incorporated into the otolith shortly after hatching, were plotted against hatch date in order to detect temporal changes over the hatching season from December to July (Fig. 4). Similarly, elemental ratios in otolith edges were plotted against capture date to document potential changes in otolith chemistry composition from August to October (Fig. 4). Core Li/Ca was negatively correlated with hatch date while core Mg/Ca was positively correlated with hatch date (Fig. 4). Edge elemental ratios and capture date showed significant correlations with Mg/Ca, Mn/Ca, Sr/Ca and Ba/Ca, but these relationships disappear when FB (with a single, late capture date) was removed (Fig. 4). Removing FB, however, led to a significant positive regression between edge Li/Ca and capture date.

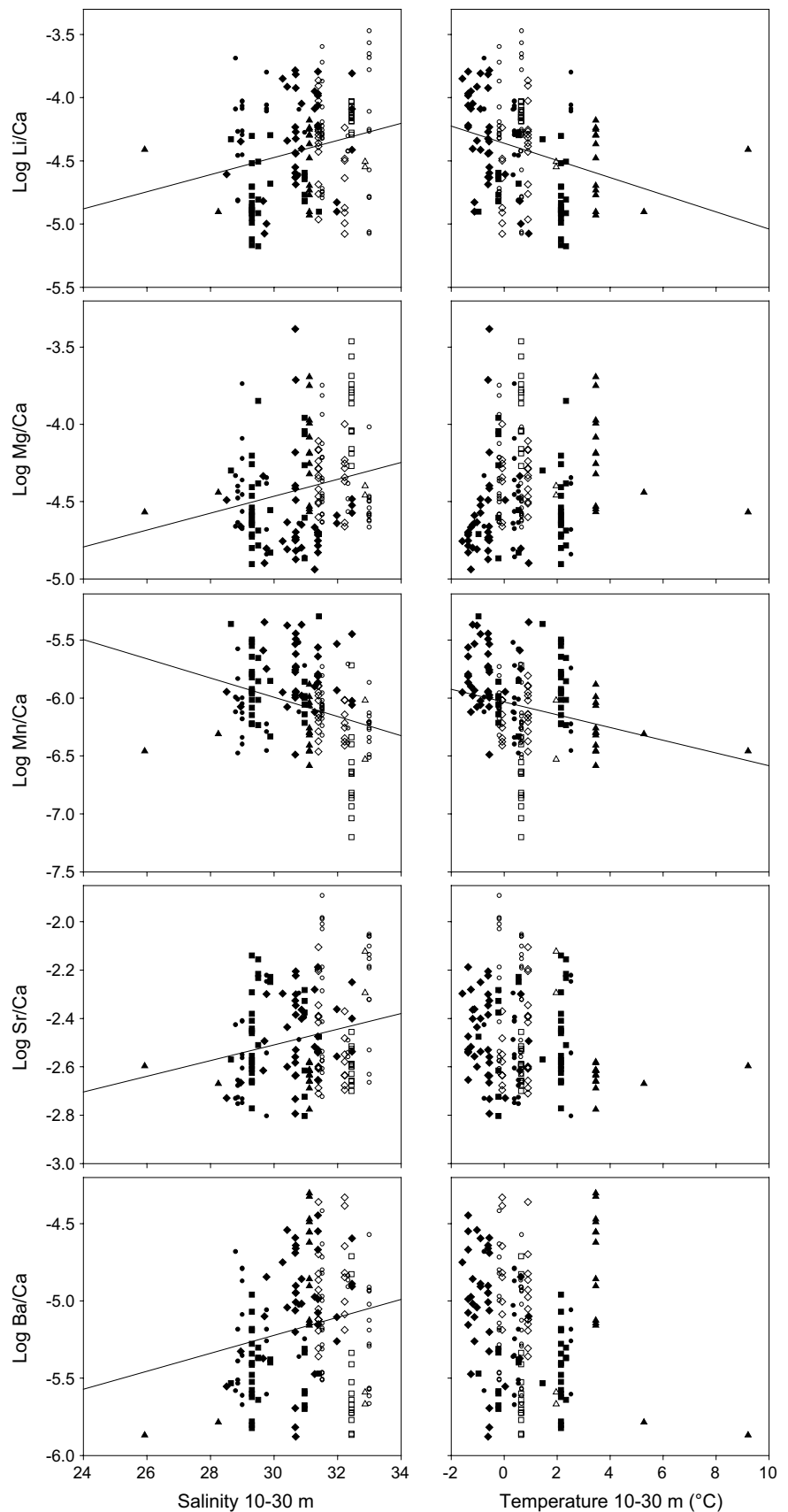
Differences in otolith chemistry between regions with high and low freshwater input

Differences in elemental ratios between regions highly influenced by freshwater from nearby rivers (Laptev Sea, Hudson Bay and Amundsen Gulf) and weakly freshwater-influenced regions (Lancaster Sound, Baffin Bay and Frobisher Bay) were tested for the three otolith zones (Fig. 5). Both Li/Ca and Mg/Ca were higher in regions with low freshwater input for middle and edge zones and core, middle and edge zones, respectively. Edge Mn/Ca values were significantly higher in freshwater-influenced regions, while Sr/Ca values were higher in freshwater-influenced regions for the otolith core but higher in purely marine regions for the edge zones (*t* tests or exact Wilcoxon,  $p < 0.0334$ ). Finally, Ba/Ca values were higher in freshwater-influenced regions for the otolith core but higher in purely marine regions for the middle zones (exact Wilcoxon,  $p < 0.0112$ ).

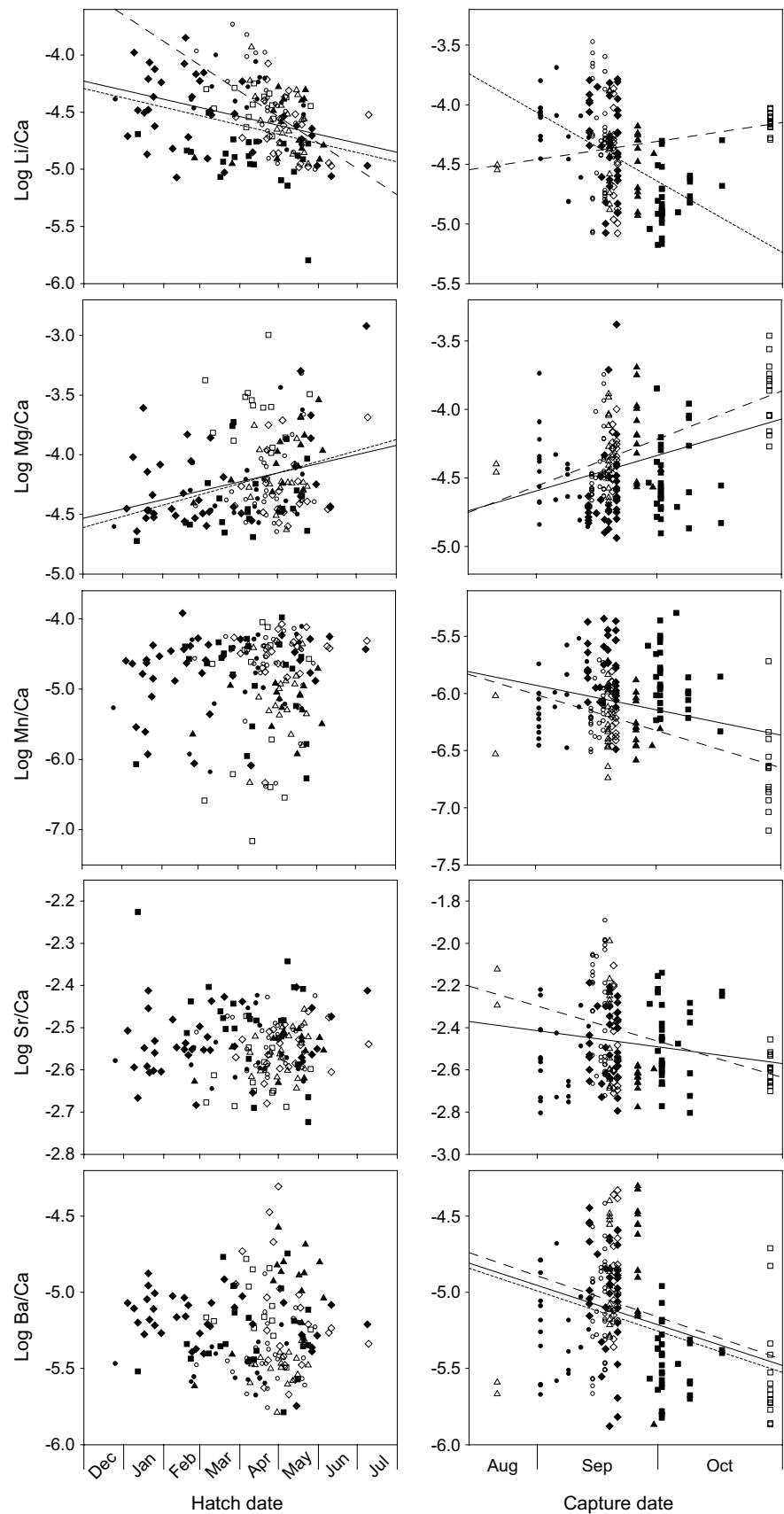
Differences in otolith chemistry among regions and years

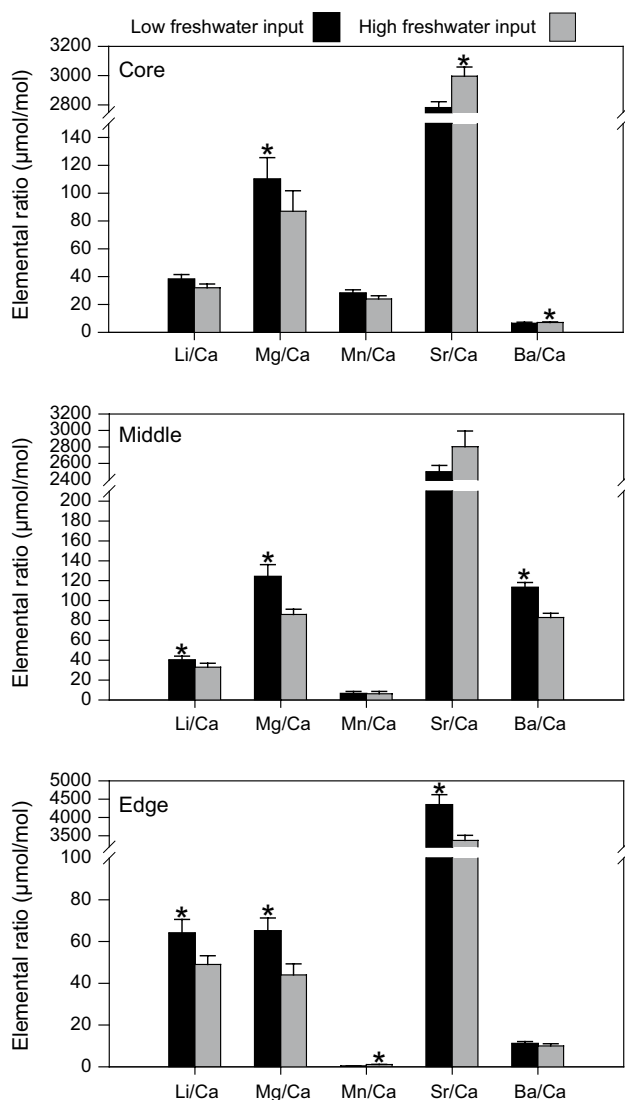
Interannual differences in otolith chemistry were found for Amundsen Gulf and Lancaster Sound (*t* tests or exact Wilcoxon,  $0.0001 < p < 0.0411$ ); hence, each year was treated separately. For both years, multi-elemental compositions differed significantly among regions at the otolith core, middle and edge zones (MANOVA,  $p < 0.0008$ ; exact permutation statistics  $< 0.0001$ ), except for the middle zone in 2005 (MANOVA,  $p = 0.2110$ ; exact

**Fig. 3** Elemental ratios in otolith edges in relation to salinity and temperature. *Solid lines* indicate significant regressions. *Open symbols* represent regions with weak freshwater input of Baffin Bay (*circles*), Frobisher Bay (*squares*) and Lancaster Sound for sampling year 2005 (*triangles*) and 2006 (*diamonds*). *Closed symbols* represents regions with high freshwater input of Amundsen Gulf for sampling year 2005 (*circles*) and 2006 (*squares*), Hudson Bay (*triangles*) and Laptev Sea (*diamonds*)



**Fig. 4** Elemental ratios in otolith core in relation to hatch date (*left panels*) and elemental ratios in otolith edge in relation to capture date (*right panels*). *Open symbols* represent regions with weak freshwater input of Baffin Bay (*circles*), Frobisher Bay (*squares*) and Lancaster Sound for sampling year 2005 (*triangles*) and 2006 (*diamonds*). *Closed symbols* represents regions with high freshwater input of Amundsen Gulf for sampling year 2005 (*circles*) and 2006 (*squares*), Hudson Bay (*triangles*) and Laptev Sea (*diamonds*). *Lines* indicate significant regressions for regions with weak freshwater input (*long dash*), regions with high freshwater input (*short dash*), and all regions grouped (*solid lines*)





**Fig. 5** Mean  $\pm$  SE elemental ratio ( $\mu\text{mol/mol}$ ) in *core*, *middle* and *edge* zones of otoliths from regions with low and high freshwater input. Asterisk above significantly higher values

permutation statistics = 0.2116), which had a low sample size (Table 2). Quadratic discriminant function analyses were used to visualize multivariate differences in elemental ratios among regions in the otolith core, middle and edge zones (Fig. 6). In 2005, overall jackknife reclassification success from the quadratic discriminant model was 54, 30 and 56 % for core, middle and edge, respectively (Table 3). These numbers are significantly higher than the 25 % expected by chance, with the exception of the middle zone which had a low sample size. In 2006, reclassification success was 56, 65 and 71 % for the core, middle and edge, respectively (Table 3). The discriminant power of individual elements differed greatly between years and otolith zones (Fig. 6).

#### Differences in otolith chemistry among otolith zones

Repeated-measures ANOVAs and the equivalent nonparametric tests showed significant differences among otolith zones for all elements ( $p < 0.0001$ ), and multiple comparisons showed that all pairs were significantly different (Fig. 7). The trends observed in the grouped regions were generally consistent among regions, with a few exceptions (Fig. 8). For each region, Li/Ca tended to increase and Mn/Ca to decrease from core to edge (Fig. 8). Mg/Ca generally decreased from highest in the middle, intermediate in the core, and lowest in the edge. For Sr/Ca, the general pattern was the highest ratio in the edge, followed by core and the lowest values in the middle zone. However, this was not the case for Frobisher Bay, Lancaster Sound in 2006 (no significant differences between zones) and Hudson Bay (higher ratio in the core than in the middle). Ba/Ca ratios were much higher in the middle zones for all regions and generally higher in the edge than in the core, except for Amundsen Gulf in 2006 and Frobisher Bay where the ratios were significantly higher in the core than in the edge (Fig. 8).

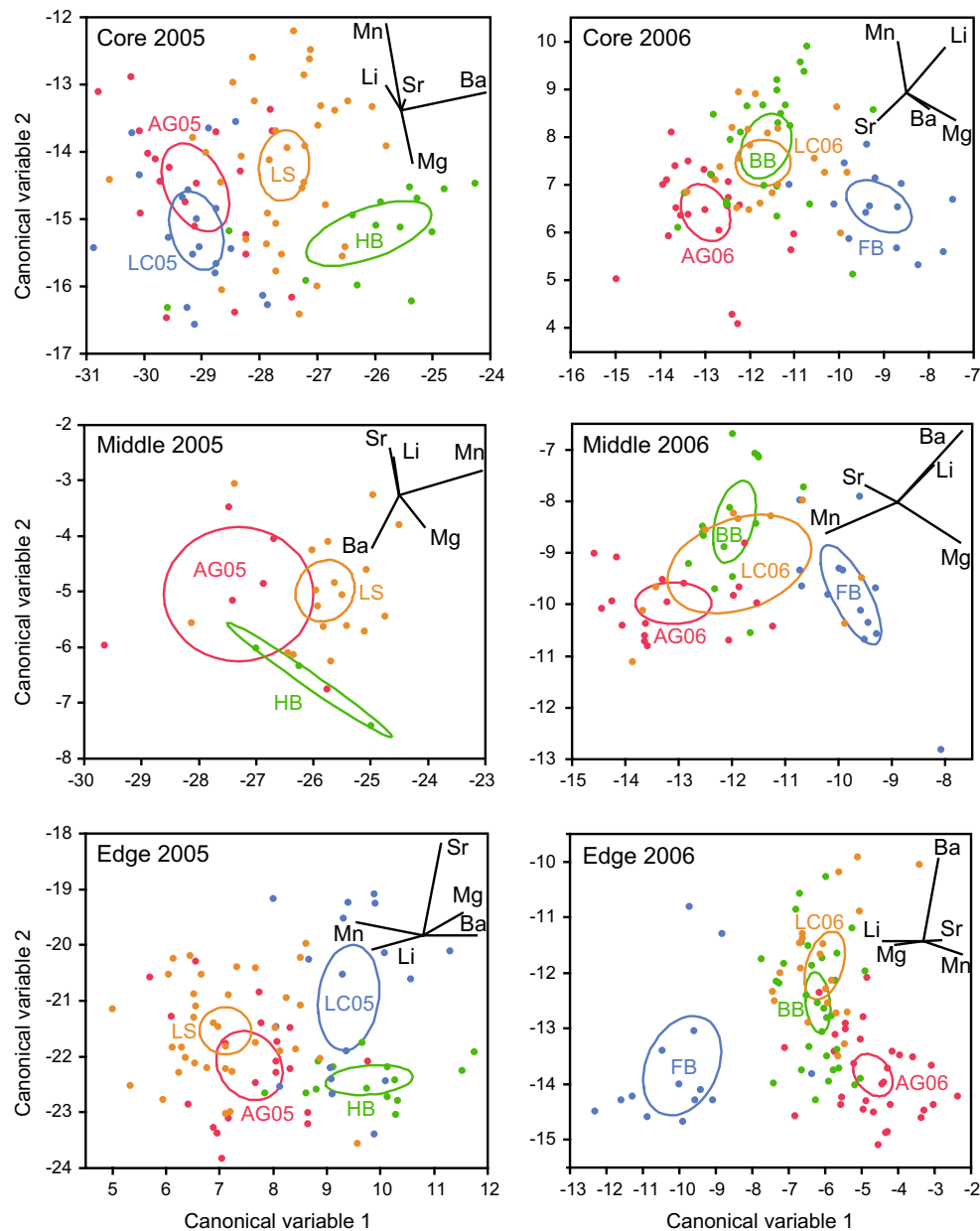
#### Discussion

##### Effects of salinity and temperature on element incorporation in polar cod otoliths

Dissolved concentrations of Li, Mg and Sr are in general higher in saltwater than in freshwater environments (Campana 1999). It can hence be hypothesized that the concentration of these elements in otolith edges (where elements incorporation occurred shortly before capture) should be positively correlated with Sr–Ca and salinity in ambient water at the time of capture. The significant positive regressions between Li/Ca, Mg/Ca and Sr/Ca and salinity in the otoliths of polar cod juveniles that we sampled supported this hypothesis. Conversely, environmental concentrations of Mn and Ba are generally higher in freshwater than in saltwater (Campana 1999), and negative relationships should be expected between Mn/Ca and Ba/Ca in otolith edge and salinity. Mn/Ca in polar cod otolith edges was indeed negatively correlated with salinity (Fig. 3). However, contrary to our expectations, Ba/Ca showed a significant positive relationship with salinity (Fig. 3), a pattern also observed in waters characterized by high salinities (Gillanders and Munro 2012).

Temperature has been shown to influence elemental incorporation in otoliths of larval and juvenile marine fishes (Bath et al. 2000; Martin et al. 2004; Martin and Thorrold 2005). In the present study, we found significant negative regressions between Li/Ca and Mn/Ca in otolith edges and sub-surface temperature at capture site and date. No temperature effect was detected for the other elements. Salinity and temperature





**Fig. 6** Quadratic discriminant function analyses based on multi-elemental ratios (Li/Ca, Mg/Ca, Mn/Ca, Sr/Ca, Ba/Ca) in otolith *core* (top panels), *middle* (middle panels) and *edge* (bottom panels) zones of juvenile polar cod collected in 2005 (left panels) and 2006 (right

panels) in six regions (see Table 2 for sampling codes). *Ellipses* indicate 95 % confidence intervals. Element loadings onto the canonical variables are shown in the *upper right* of each panel

effects on otolith chemistry may, however, interact (e.g., Elsdon and Gillanders 2002), but we would have been unable to detect this type of interactive effects with our experimental design.

**Polar cod larval ecology: a further test of the freshwater winter refuge hypothesis**

The freshwater winter refuge hypothesis suggests that polar cod larvae start to hatch in winter in freshwater-influenced

regions but only later (in spring) in purely marine regions (Bouchard and Fortier 2008, 2011). We tested this hypothesis by comparing otolith chemistry of juvenile polar cod collected in six regions with contrasting freshwater inputs. Since Li, Mg and Sr concentrations generally increase with ambient salinity (Campana 1999), we formulated the hypothesis that otoliths from purely marine regions should have higher concentrations of these elements than otoliths from regions highly influenced by freshwater. With the

**Table 3** Percentage of juveniles classified to each region by quadratic discriminant function analyses based on multi-elemental composition (Li, Mg, Mn, Sr, Ba) of otolith core, middle and edge zones for collection years 2005 and 2006 using jackknife leave-one-out cross-validation

Year	Position	Actual region	Predicted region			
			AG05	HB	LC05	LS
2005	core	AG05	<b>35</b>	5	35	25
		HB	0	<b>57</b>	14	29
		LC05	42	0	<b>47</b>	11
		LS	17	5	0	<b>78</b>
	middle	AG05	<b>0</b>	0	nd	100
		HB	0	<b>0</b>	nd	100
		LS	11	0	nd	<b>89</b>
	edge	AG05	<b>50</b>	15	0	35
		HB	7	<b>64</b>	22	7
		LC05	11	33	<b>50</b>	6
		LS	36	3	3	<b>58</b>
	2006	core	AG06	<b>50</b>	23	0
BB			27	<b>42</b>	0	31
IB			0	7	<b>72</b>	21
LC06			14	27	0	<b>59</b>
middle		AG06	<b>83</b>	6	0	11
		BB	13	<b>67</b>	0	20
		IB	0	8	<b>69</b>	23
		LC06	0	30	30	<b>40</b>
edge		AG06	<b>74</b>	16	0	10
		BB	19	<b>53</b>	6	22
		IB	7	0	<b>93</b>	0
		LC06	10	28	0	<b>62</b>

Correct classification percentage is in bold. See Table 2 for sampling codes

exception of Sr/Ca in otolith cores, all significant differences observed in Li/Ca, Mg/Ca and Sr/Ca between purely marine and freshwater-influenced regions were in accordance with the hypothesis. On the other hand, Mn and Ba are known to decrease with salinity (Campana 1999); hence, higher values of Mn/Ca and Ba/Ca should be found in otoliths from regions highly influenced by freshwater. Significant differences between region types in Ba/Ca from otolith cores and in Mn/Ca from otolith edges support this hypothesis. However, contrary to the prediction, the middle zone of otoliths from purely marine regions had significantly higher Ba/Ca than those from freshwater-influenced regions.

One particular pattern in polar cod otolith chemistry led to the hypothesis that egg survival may be favored by the lower salinities characterizing the regions highly influenced by freshwater. The positive regression found between otolith core Mg/Ca and hatch date in regions highly influenced by freshwater (Fig. 4) may be related to the trend of otolith Mg/Ca to increase with salinity if egg survival was differently affected by salinity over the hatching season. The juveniles used in our study were in the environment for several months prior to capture and can be considered as

survivors of the egg and larval cohort. Elemental signatures in the otolith cores hence represent those eggs that survived until the juvenile stage. We suggest that egg survival during the winter is enhanced by the lower salinities and concomitant higher temperatures associated with river plumes, as shown for the larvae (Bouchard and Fortier 2008, 2011). Survival of the eggs arriving in the environment later in the season would be less sensitive to freshwater input, and hence, a larger range of salinities and Mg/Ca would be represented in otolith cores, as seen in Fig. 4. This hypothesis could be tested by analyzing the effect of salinity and temperature on polar cod egg survival in the laboratory.

Spatial segregation, dispersion and migration in polar cod early stages

Significant regional variability was found in the elemental composition of juvenile polar cod otoliths. Reclassification success from the discriminant model, although low in some cases, indicates that a certain level of spatial segregation exists in polar cod early life stages and that this can be detected by otolith chemistry analyses. However, some reference groups were likely missing in our study and the

**Fig. 7** Elemental ratio in core, middle and edge zones of all otoliths analyzed. Horizontal line within box median value; top and bottom edges of box 25th and 75th percentiles; whiskers 10th and 90th percentiles; open circles outliers beyond 90th percentile. Lettering in each panel indicates multiple comparisons results obtained from repeated-measures ANOVA or an equivalent nonparametric test for longitudinal data. Groups with different letters are significantly different

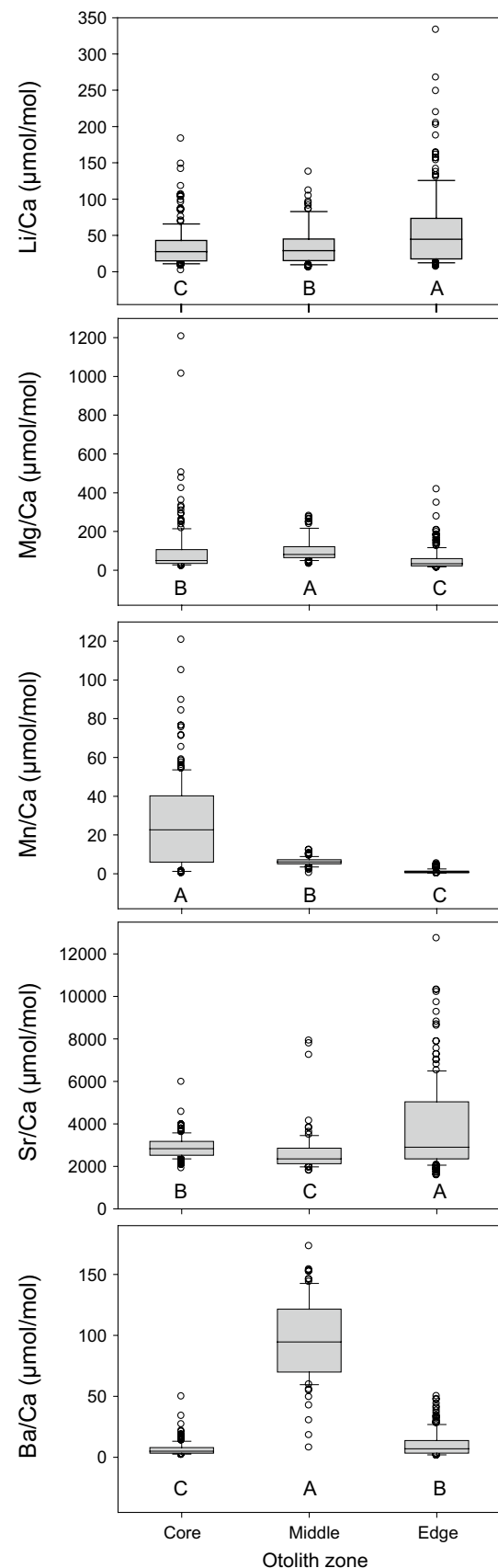
inclusion of other hatching regions in our analyses may have further increased classification success.

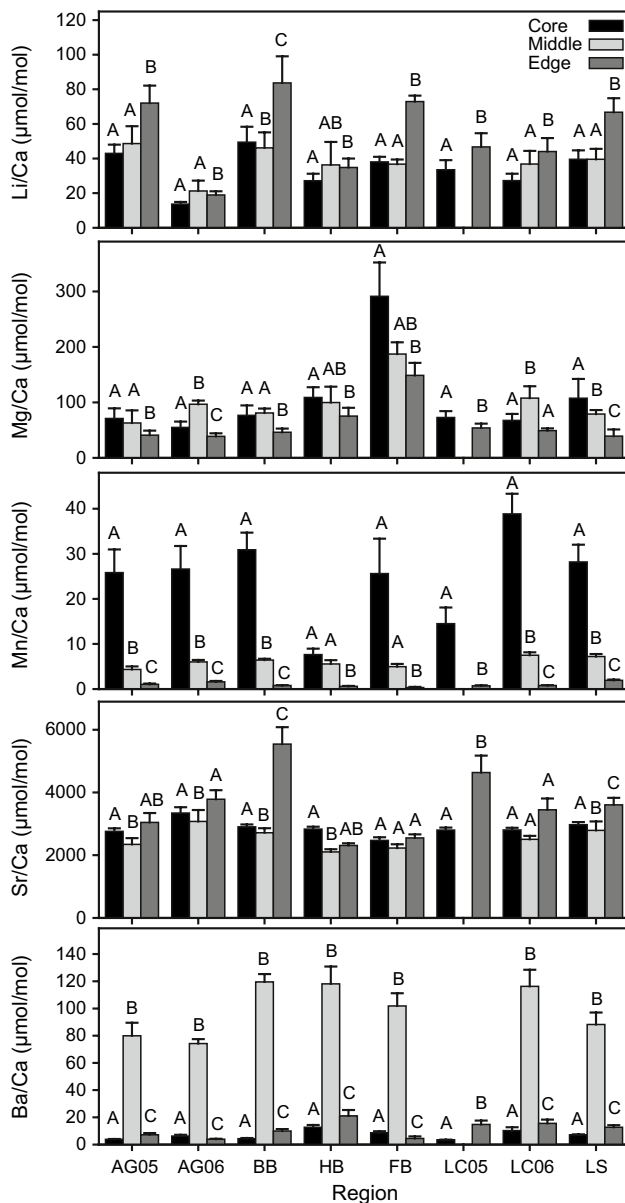
Otolith chemistry provided indirect evidence of significant displacement of eggs and early stage larvae from spawning locations. In 2006, classification success increased with the use of more recent otolith zones (56, 65 and 71 % for the core, middle and edge zone, respectively), suggesting that a significant number of larvae or early juveniles had moved into a different water mass at some stage before capture. Elemental ratios at the otolith edge represent the time shortly prior to capture (when individuals were in the same habitat), and higher classification success was observed in the discriminant models. These movements could correspond to larval dispersion, active migration or more likely a combination of both. In any case, the lower classification success at the core suggests significant mixing of eggs and newly hatched larvae.

Is ontogenetic vertical migration of polar cod juveniles reflected in otolith chemistry?

Some patterns found in polar cod otoliths may reflect the ontogenetic vertical migration of the juveniles which descend progressively to their deep overwintering grounds in late-summer and early-fall (e.g., Ponomarenko 2000).

We found a large ontogenetic effect on otolith Mn/Ca that was consistent across all locations, with core values 5–10 times higher than middle or edge zones (Fig. 8). A number of studies have also found elevated Mn/Ca in otolith cores that was apparently unrelated to dissolved Mn concentrations in the environment (Brophy et al. 2004; Ruttenberg et al. 2005; DiMaria et al. 2010). The exact mechanism generating high Mn/Ca values in otolith cores remains obscure. However, outside the core zone, otolith Mn/Ca ratios are generally reflecting ambient Mn concentrations (Campana 1999). Rivers are the main source of dissolved manganese for the oceans, and Mn/Ca ratios are typically higher in freshwater than in seawater (Campana 1999). In the Arctic, the vertical profile of dissolved manganese is characterized by high concentrations in surface water and decreasing concentrations with depth (Middag et al. 2011). Lower Mn/Ca in polar cod otolith edge zones than in middle zones (Fig. 8) suggests that juveniles were





**Fig. 8** Mean + SE elemental ratio in core, middle and edge zones of otoliths from different sampling locations (see Table 2 for sampling codes). Lettering above each panel indicates multiple comparisons results obtained from repeated-measures ANOVA or an equivalent nonparametric test for longitudinal data. For each region, values with different letters are significantly different

in waters relatively depleted in Mn compared to the larvae. This pattern may result from vertical migration of the juveniles, which leave surface waters before winter to reach their overwintering grounds. Alternatively, the observed differences may be only or partly due to ontogenetic effect, but disentangling the relative effect of the environment and the ontogeny is impossible at this point.

Barium concentrations are higher in freshwater than in seawater and otolith Ba/Ca tends to accurately reflect

ambient Ba/Ca (Campana 1999; Martin and Thorrold 2005). As in other oceanic regions, Ba in the Canadian Arctic displays a nutrient-type behavior with the highest surface concentrations observed at river mouths and typical profiles showing maximum concentration at the base of the surface layer decreasing above and below (Guay and Kenison Falkner 1997; Thomas et al. 2011). The lowest otolith edge Ba/Ca ratios found among polar cod juveniles from Frobisher Bay may result from their ontogenetic vertical migration. Juveniles from this region were sampled between 0 and 200 m late in fall (29 October) and were more likely to be in deep water than in the surface layer, both depleted in Ba. The negative correlation found between otolith edges Ba/Ca and capture date may also reflect juvenile polar cod migration toward deeper waters in fall.

Strontium is the most widely used element in otolith chemistry studies, and otolith Sr/Ca is generally positively correlated with salinity (Secor and Rooker 2000). Lower otolith edge Sr/Ca in marine regions than in freshwater-influenced regions would be consistent with the ontogenetic migration of juveniles prior to winter considering different age at capture between groups. Juveniles from freshwater-influenced regions were on average 175 days old at capture (40.2 mm) compared to 150 days old (35.5 mm) for those from purely marine regions. Since vertical migration begins at 30–35 mm (Baranenkov et al. 1966; Ponomarenko 2000), the first group was more likely to be found in deeper, saltier and Sr-enriched waters at the time of capture in fall. It is, however, impossible to determine the vertical position of the juveniles collected since sampling was not stratified but integrated over the water column.

## Conclusion

With the recent reduction in its ice cover, the Arctic Ocean is becoming more accessible to humans and resource exploitation projects are multiplying. Fisheries are declining worldwide, and commercial exploitation of polar cod in the foreseeable future is conceivable. Being a small pelagic fish highly connected in its food web and representing a high proportion of the ecosystem biomass (Welch et al. 1992), the polar cod will likely be very sensitive to harvesting (Cury et al. 2000; Shannon et al. 2000; Smith et al. 2011). This vulnerability would be superimposed on the direct effects of climate change on the species, which alone can strongly modify the ecosystem. A better understanding of polar cod population structure, reproductive strategy and migratory behavior would help in the prediction of the response of the Arctic marine ecosystems to climate change and in the management of natural resources of the Arctic Ocean. Our study of the otolith chemistry of juvenile polar

cod from six regions around the Arctic clearly show some level of spatial segregation in the species, a step toward a finer description of population structuring. Salinity had an effect on otolith incorporation of the five elements analyzed, and the results were generally consistent with the freshwater winter refuge hypothesis. Considering vertical profiles of some elements in the Arctic Ocean, notably Mn and Ba, we found that otolith chemistry may reflect the ontogenic vertical migration of juvenile polar cod. We think other migration and dispersion patterns in this species could be studied with the otolith chemistry procedure used here, especially if coupled with water mass characterization for the analyzed elements and experimental quantification of the effects of environmental and physiological parameters on elemental incorporation.

**Acknowledgments** We thank the numerous people who help with the sampling including officers and crews of the CCGS *Amundsen* and the icebreaker *Kapitan Dranitsyn*. Special thanks to H. Cloutier and S. Birdwhistell for support with otolith preparation and analyses and to G. Daigle for statistical advices. Support from the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT) and the Northern Scientific Training Program (Department of Indian and Northern Affairs Canada) to CB is acknowledged. This is a contribution to Québec-Océan (Université Laval), the Canadian Network of Centres of Excellence ArcticNet, the Nansen and Amundsen Basins Observational System (NABOS), and the Canada Research Chair on the response of marine arctic ecosystems to climate warming.

## References

- Ashford JR, Jones CM, Hofmann EE, Everson I, Moreno CA, Duhamel G, Williams R (2008) Otolith chemistry indicates population structuring by the Antarctic Circumpolar Current. *Can J Fish Aquat Sci* 65:135–146
- Baranenkova AS, Ponomarenko VP, Khokhlina NS (1966) The distribution, size and growth of the larvae and fry of *Boreogadus saida* (Lep.) in the Barents Sea. *Fish Mar Serv Transl Ser* 4025:498–518
- Bath GE, Thorrold SR, Jones CM, Campana SE, McLaren JW, Lam JWH (2000) Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochim Cosmochim Acta* 64:1705–1714
- Benoit D, Simard Y, Fortier L (2008) Hydroacoustic detection of large winter aggregations of Arctic cod (*Boreogadus saida*) at depth in ice-covered Franklin Bay (Beaufort Sea). *J Geophys Res (C Oceans)* 113:C06S90. doi:10.1029/2007JC004276
- Benoit D, Simard Y, Gagné J, Geoffroy M, Fortier L (2010) From polar night to midnight sun: photoperiod, seal predation, and the diel vertical migrations of polar cod (*Boreogadus saida*) under landfast ice in the Arctic Ocean. *Polar Biol* 33:1505–1520. doi:10.1007/s00300-010-0840-x
- Bouchard C, Mollard S, Suzuki K, Robert D, Fortier L Contrasting the early life histories of sympatric Arctic gadids *Boreogadus saida* and *Arctogadus glacialis* in the Canadian Beaufort Sea. *Polar Biol*. doi: 10.1007/s00300-014-1617-4 (in press)
- Bouchard C, Fortier L (2008) Effects of polynyas on the hatching season, early growth and survival of polar cod *Boreogadus saida* in the Laptev Sea. *Mar Ecol Prog Ser* 355:247–256. doi:10.3354/meps07335
- Bouchard C, Fortier L (2011) Circum-arctic comparison of the hatching season of polar cod *Boreogadus saida*: a test of the freshwater winter refuge hypothesis. *Prog Oceanogr* 90:105–116. doi:10.1016/j.pocean.2011.02.008
- Bradstreet MSW, Finley KJ, Sekerak AD, Griffiths WD, Evans CR, Fabijan MF, Stallard HE (1986) Aspects of the biology of Arctic cod *Boreogadus saida* and its importance in Arctic marine food chains. *Can Tech Rep Fish Aquat Sci* 1491:193
- Brophy D, Jeffries TE, Danilowicz BS (2004) Elevated manganese concentrations at the cores of clupeid otoliths: possible environmental, physiological, or structural origins. *Mar Biol* 144:779–786
- Brunner E, Domhof S, Langer F (2002) Nonparametric analysis of longitudinal data in factorial experiments. Wiley, New York
- Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar Ecol Prog Ser* 188:263–297
- Campana SE (2001) Accuracy, precision, and quality control in age determination, including a review of the use and abuse of age validation methods. *J Fish Biol* 59:197–242. doi:10.1006/jfbi.2001.1668
- Clarke LM, Walther BD, Munch SB, Thorrold SR, Conover DO (2009) Chemical signatures in the otoliths of a coastal marine fish, *Menidia menidia*, from the northeastern United States: spatial and temporal differences. *Mar Ecol Prog Ser* 384:261–271
- Craig PC, Griffiths WB, Haldorson L, McElderry H (1982) Ecological studies of Arctic cod (*Boreogadus saida*) in Beaufort Sea coastal water. *Can J Fish Aquat Sci* 39:395–406
- Cury P, Bakun A, Crawford RJM, Jarre A, Quiñones RA, Shannon LJ, Verheye HM (2000) Small pelagics in upwelling systems: patterns of interaction and structural changes in “wasp-waist” ecosystems. *ICES J Mar Sci* 57:603–618. doi:10.1006/jmsc.2000.0712
- Déry SJ, Stieglitz M, McKenna EC, Wood EF (2005) Characteristics and trends of river discharge into Hudson, James, and Ungava Bays, 1964–2000. *J Climate* 18:2540–2557
- DiMaria R, Miller J, Hurst T (2010) Temperature and growth effects on otolith elemental chemistry of larval Pacific cod, *Gadus macrocephalus*. *Environ Biol Fish* 89:453–462. doi:10.1007/s10641-010-9665-2
- Drolet R, Fortier L, Ponton D, Gilbert M (1991) Production of fish larvae and their prey in subarctic southeastern Hudson Bay. *Mar Ecol Prog Ser* 77:105–118. doi:10.3354/meps077105
- Elsdon TS, Gillanders BM (2002) Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. *Can J Fish Aquat Sci* 59:1796
- Elsdon TS, Wells BK, Campana SE, Gillanders BM, Jones CM, Limburg KE, Secor DH, Thorrold SR, Walther BD (2008) Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations, and inferences. *Oceanogr Mar Biol Ann Rev* 46:297–330
- Fevolden SE, Christiansen JS (1997) Allozymic and scnDNA homogeneity in Polar cod (*Boreogadus saida*) (Gadiformes: Gadidae). *Cybiurn* 21:411–414
- Fevolden SE, Martinez I, Christiansen JS (1999) RAPD and scnDNA analyses of polar cod, *Boreogadus saida* (Pisces, Gadidae), in the North Atlantic. *Sarsia* 84:99–103
- Fortier L, Gilbert M, Ponton D, Ingram RG, Robineau B, Legendre L (1996) Impact of freshwater on a subarctic coastal ecosystem under seasonal sea ice (southeastern Hudson Bay, Canada). III. Feeding success of marine fish larvae. *J Mar Syst* 7:251–265. doi:10.1016/0924-7963(95)00005-4
- Fortier L, Sirois P, Michaud J, Barber D (2006) Survival of Arctic cod larvae (*Boreogadus saida*) in relation to sea ice and temperature in the Northeast Water Polynya (Greenland Sea). *Can J Fish Aquat Sci* 63:1608–1616. doi:10.1139/F06-064



- Geoffroy M, Robert D, Darnis G, Fortier L (2011) The aggregation of polar cod (*Boreogadus saida*) in the deep Atlantic layer of ice-covered Amundsen Gulf (Beaufort Sea) in winter. *Polar Biol* 34:1959–1971. doi:10.1007/s00300-011-1019-9
- Gilbert M, Fortier L, Ponton D, Drolet R (1992) Feeding ecology of marine fish larvae across the Great Whale River plume in seasonally ice-covered southeastern Hudson Bay. *Mar Ecol Prog Ser* 84:19–30
- Gillanders BM, Munro AR (2012) Hypersaline waters pose new challenges for reconstructing environmental histories of fish based on otolith chemistry. *Limnol Oceanogr* 57:1136–1148
- Gordeev VV (2006) Fluvial sediment flux to the Arctic Ocean. *Geomorphology* 80:94–104
- Guay CK, Falkner KK (1997) Barium as a tracer of Arctic halocline and river waters. *Deep-Sea Res Part II Top Stud Oceanogr* 44:1543–1569. doi:10.1016/s0967-0645(97)00066-0
- Legendre P, Legendre L (1998) Numerical ecology, 2nd edn. Elsevier Science BV, Amsterdam
- Locarnini RA, Mishonov AV, Antonov JI, Boyer TP, Garcia HE, Baranova OK, Zweng MM, Paver CR, Reagan JR, Johnson DR, Hamilton M, Seidov D (2013) World Ocean Atlas 2013, volume 1: temperature. In: Levitus S, Mishonov A (eds.), NOAA Atlas NESDIS 73, p 40
- Macdonald JI, Farley JH, Clear NP, Williams AJ, Carter TI, Davies CR, Nicol SJ (2013) Insights into mixing and movement of South Pacific albacore *Thunnus alalunga* derived from trace elements in otoliths. *Fish Res* 148:56–63. doi:10.1016/j.fishres.2013.08.004
- Martin GB, Thorrold SR (2005) Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile spot *Leiostomus xanthurus*. *Mar Ecol Prog Ser* 293:223–232. doi:10.3354/meps293223
- Martin GB, Thorrold SR, Jones CM (2004) Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). *Can J Fish Aquat Sci* 61:34–42
- McCulloch M, Cappo M, Aumend J, Müller W (2005) Tracing the life history of individual barramundi using laser ablation MC-ICP-MS Sr-isotopic and Sr/Ba ratios in otoliths. *Mar Freshw Res* 56:637–644. doi:10.1071/MF04184
- Michaud J, Fortier L, Rowe P, Ramseier R (1996) Feeding success and survivorship of Arctic cod larvae, *Boreogadus saida*, in the Northeast Water Polynya (Greenland Sea). *Fish Oceanogr* 5:120–135. doi:10.1111/j.1365-2419.1996.tb00111.x
- Middag R, de Baar HJW, Laan P, Klunder MB (2011) Fluvial and hydrothermal input of manganese into the Arctic Ocean. *Geochim Cosmochim Acta* 75:2393–2408. doi:10.1016/j.gca.2011.02.011
- Ponomarenko VP (2000) Eggs, larvae, and juveniles of polar cod *Boreogadus saida* in the Barents, Kara, and White Seas. *J Ichthyol* 40:165–173
- Ruttenberg BI, Hamilton SL, Hickford MJH, Paradis GL, Sheehy MS, Standish JD, Ben-Tzvi O, Warner RR (2005) Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. *Mar Ecol Prog Ser* 297:273–281. doi:10.3354/meps297273
- Secor DH, Rooker JR (2000) Is otolith strontium a useful scalar of life cycles in estuarine fishes? *Fish Res* 46:359–371
- Shannon LJ, Cury PM, Jarre A (2000) Modelling effects of fishing in the Southern Benguela ecosystem. *ICES J Mar Sci* 57:720–722. doi:10.1006/jmsc.2000.0716
- Sinclair DJ, Kinsley LPJ, McCulloch MT (1998) High resolution analysis of trace elements in corals by laser ablation ICP-MS. *Geochim Cosmochim Acta* 62:1889–1901. doi:10.1016/s0016-7037(98)00112-4
- Smetacek V, Nicol S (2005) Polar ocean ecosystems in a changing world. *Nature* 437:362–368
- Smith ADM et al (2011) Impacts of fishing low-trophic level species on marine ecosystems. *Science* 333:1147–1150. doi:10.1126/science.1209395
- Standish JD, Sheehy M, Warner RR (2008) Use of otolith natal elemental signatures as natural tags to evaluate connectivity among open-coast fish populations. *Mar Ecol Prog Ser* 356:259–268. doi:10.3354/meps07244
- Sturgeon RE, Willie SN, Yang L, Greenberg R, Spatz RO, Chen Z, Scriver C, Clancy V, Lam JW, Thorrold S (2005) Certification of a fish otolith reference material in support of quality assurance for trace element analysis. *J Anal At Spectrom* 20:1067–1071
- Thanassekos S, Fortier L (2012) An Individual Based Model of Arctic cod (*Boreogadus saida*) early life in Arctic polynyas: I. Simulated growth in relation to hatch date in the Northeast Water (Greenland Sea) and the North Water (Baffin Bay). *J Mar Syst* 93:25–38. doi:10.1016/j.jmarsys.2011.08.003
- Thomas H, Shadwick E, Dehairs F, Lansard B, Mucci A, Navez J, Gratton Y, Prowe F, Chierici M, Fransson A, Papakyriakou TN, Sternberg E, Miller LA, Tremblay J-É, Monnin C (2011) Barium and carbon fluxes in the Canadian Arctic Archipelago. *J Geophys Res Oceans* 116:C00G08. doi:10.1029/2011jc007120
- Tynan CT, DeMaster DP (1997) Observations and predictions of Arctic climatic change potential effects on marine mammals. *Arctic* 50:308–322
- Welch HE, Bergmann MA, Siferd TD, Martin KA, Curtis MF, Crawford RE, Conover RJ, Hop H (1992) Energy flow through the marine ecosystem of the Lancaster Sound region, Arctic Canada. *Arctic* 45:343–357
- Zweng MM, Reagan JR, Antonov JI, Locarnini RA, Mishonov AV, Boyer TP, Garcia HE, Baranova OK, Johnson DR, Seidov D, Bidle MM (2013) World Ocean Atlas 2013, volume 2: salinity. In: Levitus S, Mishonov A (eds.), NOAA Atlas NESDIS 74, p 39