Polar Biol (2013) 36:1537–1542
DOI 10.1007/s00300-013-1371-z

SHORT NOTE

The nucleus of the lapillar otolith discriminates the early life stages of *Boreogadus saida* and *Arctogadus glacialis*

Caroline Bouchard · Dominique Robert · R. John Nelson · Louis Fortier

Received: 22 April 2013 / Revised: 14 June 2013 / Accepted: 27 June 2013 / Published online: 12 July 2013

Abstract Polar cod (*Boreogadus saida*) and ice cod (*Arctogadus glacialis*) are sympatric on continental shelves of the Arctic Ocean. The larvae and early juveniles of the two species are similar, and discrimination based on morphology and pigmentation is uncertain. We present a discrimination criterion based on the difference in lapillus nucleus size between genetically identified *B. saida* ($n = 441$, 4.2–55.0 mm standard length) and *A. glacialis* ($n = 82$, 8.6–45.5 mm). The product of the shortest and longest diameters of the nucleus (SN × LN) was 58 % larger in *A. glacialis* than in *B. saida*. The logistic regression ($\text{Ln}[p/(1-p)] = 0.02687\text{SN} \times \text{LN} - 17.5466$), where p is the probability that the fish is *A. glacialis*, correctly reassigned 501 of the 523 fishes (96 %) used to build the model to their genetically determined species (99 % of *B. saida* and 80 % of *A. glacialis*). The same regression correctly classified 97 % of 189 fish sampled in 2002 and 2003 and not used in building the model (99 % of *B. saida* and 89 % of *A. glacialis*).

Electronic supplementary material The online version of this article (doi:10.1007/s00300-013-1371-z) contains supplementary material, which is available to authorized users.

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

Present Address:
D. Robert
Centre for Fisheries Ecosystems Research, Fisheries and Marine Institute, Memorial University, St. John's, NL A1C 5R3, Canada

R. J. Nelson
Department of Biology, University of Victoria, Victoria, BC V8P 5C2, Canada

Keywords Arctic gadids · Ice cod · Polar cod · Otolith microstructure · Species identification

Introduction

The polar cod (*Boreogadus saida*) is a pivotal species in the food web of Arctic seas (e.g. Welch et al. 1992). A number of studies have recently reported on the hatch date frequency distribution (Bouchard and Fortier 2008, 2011), trophodynamics (Michaud et al. 1996; Walkusz et al. 2011), growth (Thanassekos and Fortier 2012), and survival (Fortier et al. 2006; Thanassekos et al. 2012) of the larvae and juveniles of polar cod. Field studies generally assume that the vast majority of young gadids sampled in arctic waters are polar cod *B. saida*. However, the post-yolk-sac larvae and juveniles of *B. saida* collected in Arctic seas are impossible to distinguish from those of the sympatric ice cod *Arctogadus glacialis* based on morphological criteria such as shape, myomere counts, or fin ray counts (Madsen et al. 2009; Bouchard and Fortier 2011). From ca. 10 to 20 mm in length, pigments on the ventrolateral surface of the gut tend to be larger and more ramose in *A. glacialis* than in *B. saida*, which may provide some indication of the species, with the caveat that intermediary pigment patterns are found in both species (Suzuki 2013). Hence, the larvae and juveniles of the two species are almost indistinguishable except through molecular genetics. Genetic markers have been developed to discriminate the two species (Madsen et al. 2009; Nelson et al. 2013). Based on these, *A. glacialis* may represent from 0 to 11 % of the larval and juvenile fish collected in the field and identified as *B. saida* depending on region and season (Bouchard and Fortier 2011). Hence, misidentification may represent a source of bias when assessing the hatch date

frequency distribution, early growth, and survival of the two species. Molecular genetics to discriminate the young stages of the two cod species are time-consuming and expensive. Here, we present a method to discriminate the early life stages of the two species based on interspecific differences in the size of the nucleus of the lapillar otolith.

Materials and methods

Larval fish sampling

Fish larvae and juveniles were collected during the annual expedition of the research icebreaker *Amundsen* to south-east Beaufort Sea (Arctic Ocean). The young gadids used in the present study were sampled under the ice and in open water in 2002, 2003, 2004, and 2008, using different plankton nets. The standard length (SL) of fish larvae and juveniles was measured fresh on board before preservation in 95 % ethanol. A stratified subsample of gadid larvae was assembled for species identification by randomly selecting individuals from predetermined length classes in each of the 4 years (Table 1).

Species determination based on genetic analyses

All selected fish from all years ($n = 712$) were assigned to species by amplifying locus *Gmo8* following Madsen et al. (2009). In their analysis, *Gmo8* alleles ranged in size from 122 to 134 base pairs (bp) in *B. saida* and from 146 to 342 bp in *A. glacialis* to the exception of two specimens out of 136 that presented 130-bp alleles (Madsen et al. 2009). In the present study, *Gmo8* alleles in the range 109–142 were assigned to *B. saida* and from 161 to 321 bp to *A. glacialis*.

As part of a separate study of genetic variability in arctic gadids, 295 of the 523 fish selected in 2004 and 2008 were genotyped at 19 microsatellite loci (including *Gmo8*), following Nelson et al. (2013). The microsatellite analyses provided an opportunity to compare identification based on the 18 microsatellites (excluding *Gmo8*) with that based on *Gmo8* only. The GENETIX software was used to cluster the 295 fish based on the factorial correspondence analysis of their microsatellite data (Belkhir et al. 2004).

In cases where the two methods assigned a fish to different species, a segment of the cytochrome *b* gene (*Cytb*) was sequenced to elucidate the ambiguity. *Cytb* was amplified with the forward primer *Cytb*F GGCTGATTCG GAATATGCAYGCNAAYGG and the reverse primer *Cytb*R GGGAATGGATCGTAGAATTGCRTA NGC RAA under PCR conditions of 95 °C for 3 min, followed by 30 cycles of 95 °C for 20 s, 50 °C for 20 s, 72 °C for 45 s, followed by 5 min at 72 °C.

Otolith analysis

The two lapillar otoliths of each fish were dissected, mounted with their flat-concave side down on a microscope slide in Crystalbound® thermoplastic polymer, and polished in the sagittal plane with 0.5-µm metallurgic lapping film until the hatch mark delineating the nucleus appeared clearly. Measurements were taken under a light microscope (1,000× magnification) coupled to a camera and image analysing system (Image Pro Plus®). The shortest (SN) and the longest (LN) diameters of the nucleus were measured on the left lapillus of each fish (Fig. 1). The product SN × LN was used as an index of otolith nucleus area.

The SN × LN index for the 523 fish sampled in 2004 and 2008 and identified by genetics was used to build a logistic regression of the form $\mathrm{Ln}[p/(1-p)] = a + b$ (SN × LN) that estimates the probability p of a fish belonging to *A. glacialis* (or the probability $1 - p$ of belonging to *B. saida*) for a given value of SN × LN (e.g. Scherrer 2009). Such a model should be validated with data other than those on which it is based. Thus, the logistic regression was used to classify 189 fish sampled in 2002

Table 1 Number, sampling period, mean standard length ± SD (range), and mean lapillus nucleus area index ± SD (range) of *B. saida* and *A. glacialis* collected from 2002 to 2008 in the Beaufort Sea and identified by molecular genetics

Species/year	Number	Capture dates	Standard length (mm) Mean ± SD (range)	SN × LN (µm^2) Mean ± SD (range)
B. saida				
2002	131	22 Sep–14 Oct	29.0 ± 7.4 (15.8–45.0)	454 ± 104 (237–723)
2003	30	30 Sep–28 Dec	39.2 ± 8.2 (25.1–51.5)	494 ± 105 (281–712)
2004	213	29 Apr–12 Sep	22.8 ± 13.9 (4.9–55.0)	470 ± 91 (197–711)
2008	228	10 May–03 Aug	13.2 ± 6.3 (4.2–29.4)	416 ± 96 (213–685)
A. glacialis				
2002	14	23 Sep–14 Oct	33.7 ± 4.2 (26.4–40.9)	740 ± 151 (396–943)
2003	14	30 Sep–23 Dec	37.8 ± 3.9 (33.3–45.5)	805 ± 122 (593–1,119)
2004	52	19 Apr–12 Sep	25.4 ± 8.2 (8.6–38.5)	797 ± 125 (442–1,097)
2008	30	11 May–02 Aug	18.6 ± 5.9 (8.7–31.2)	720 ± 136 (406–1,016)

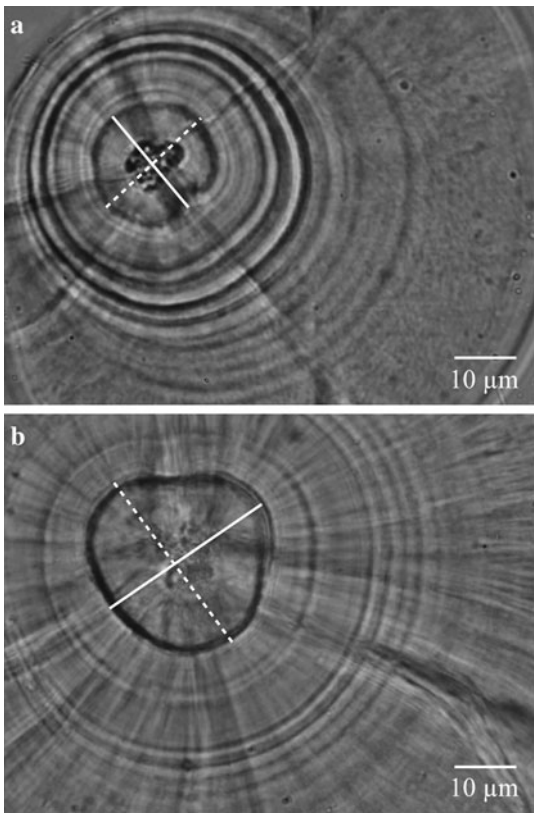

Fig. 1 Light micrograph (×1,000 magnification) of the nucleus region of the lapillus of *B. saida* (**a**) and *A. glacialis* (**b**) showing the shortest nucleus diameter (SN *full line*) and the longest nucleus diameter (LN *dashed line*) measurements used to calculate the index of nucleus area (SN × LN)

and 2003 and identified by *Gmo8*, which were not used in building the logistic regression.

Results

Molecular identification of the two species

The factorial correspondence analysis of the 18 microsatellites (excluding *Gmo8*) grouped the 295 fish into a tight cluster of 250 *B. saida* and a looser cluster of 45 *A. glacialis* (Fig. 2). Seventeen fish identified as *B. saida* by *Gmo8* associated with the *A. glacialis* cluster (Fig. 2). *Cytb* was successfully amplified and sequenced for 14 of these ambiguous fish, all of which corresponded to the reference sequence for *A. glacialis* found in GenBank. Given that 14 out of 14 amplified *Cytb* corresponded to *A. glacialis*, the non-amplified three were most likely *A. glacialis* as well, and the 17 ambiguous fish were reclassified as *A. glacialis*. Similarly, 5 fish assigned to *A. glacialis* by *Gmo8* grouped with the *B. saida* cluster. *Cytb* was successfully amplified in 4 of these 5 ambiguous fish and corresponded to *B. saida* in GenBank. All 5 fish were classified as *B. saida*.

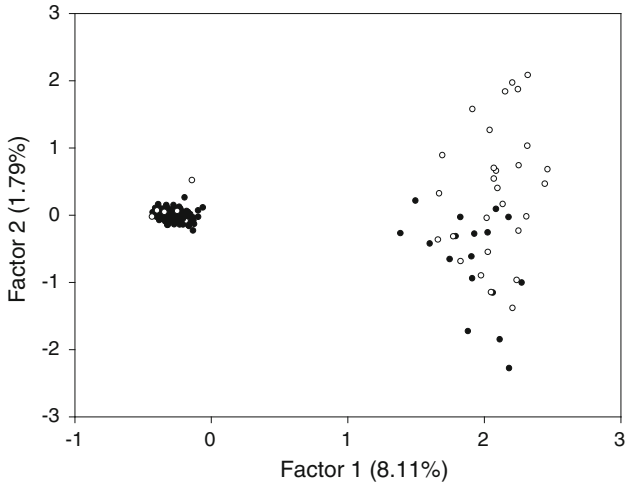

Fig. 2 Projection of 250 *B. saida* (*left cluster*) and 45 *A. glacialis* (*right cluster*) in the first factorial plane of the correspondence analysis of 18 microsatellites (excluding *Gmo8*). *Closed* and *open symbols* are fish identified as *B. saida* and *A. glacialis*, respectively, by *Gmo8* analysis

Pooling the 4 years, molecular analyses identified 602 (85 %) of the 712 young gadids as *B. saida* and 110 (15 %) as *A. glacialis* (Table 1). The length range of both species over the 4 years spanned from the newly hatched larval stage to the late juvenile stage.

Classification of genetically identified fish using the area of the lapillus nucleus

In each of the 4 years, the average index of lapillus nucleus area (SN × LN) was larger in *A. glacialis* than in *B. saida* (*t* tests, $p < 0.0001$, Table 1). Pooling all 4 years, SN × LN was 58 % larger on average in *A. glacialis* (771 ± 136 µm^2) than in *B. saida* (447 ± 100 µm^2). The statistical distribution of nucleus area overlapped slightly between the two species (Fig. 3).

Average nucleus area varied among years in both *B. saida* (ANOVA, $p < 0.0001$) and *A. glacialis* ($p = 0.048$) and tended to increase with the mean standard length of fish, which in turn increased as captures took place later in the year (Table 1). At the individual level, nucleus area increased slowly but significantly with fish length in both species (Fig. 3). The rate of increase with length did not differ significantly (ANCOVA, $p = 0.868$) between *B. saida* (2.7 µm^2 mm^{-1}) and *A. glacialis* (2.9 µm^2 mm^{-1}).

To provide a sufficient number of the relatively rare *A. glacialis*, the 523 fish sampled in 2004 and 2008 (441 *B. saida* and 82 *A. glacialis* according to genetics) were pooled to build the logistic regression relating otolith nucleus area to species (online resource 1). The model and parameter estimates of the regression $\mathrm{Ln}[p/(1-p)] =$

$0.02687SN \times LN - 17.5466$, where p is the probability that a specimen is *A. glacialis*, were highly significant ($p < 0.0001$). When the value $SN \times LN = 653\ \mu m^2$ corresponding to $p = 0.5$ was used as a threshold to reassign specimens to species, otolith nucleus area correctly classified 501 (96 %) of the 523 fish identified by prior genetic analysis for 2004 and 2008, i.e. the years used to build the model (Table 2). Identification based on otolith area was more accurate for *B. saida* (99 %) than for *A. glacialis* (80 %).

Otolith nucleus area correctly assigned 97 % (99 % of *B. saida* and 89 % of *A. glacialis*) of the 189 fish collected in 2002 and 2003 (pooled data), which were not used in building the logistic regression. Reclassification success and errors were similar between the 2 years (Table 2). Combining all years, otolith nucleus area correctly assigned 96 % (99 % of *B. saida* and 83 % of *A. glacialis*) of the 712 fish.

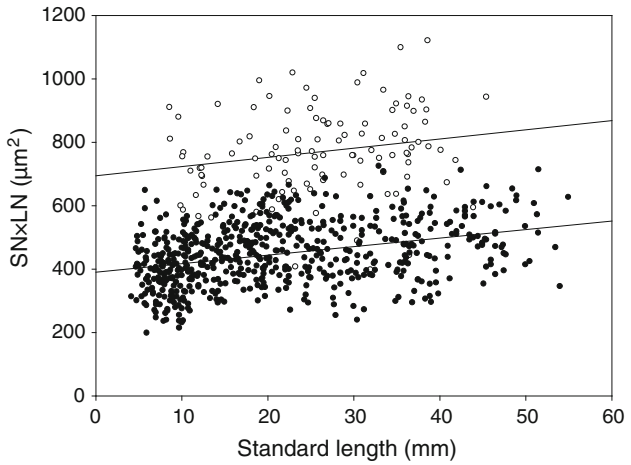

Fig. 3 Regressions of SN × LN on SL for genetically identified *B. saida* (*closed symbols* SN × LN = 2.684 SL + 390.145, $r^2 = 0.111$, $n = 602$, $p < 0.0001$) and *A. glacialis* (*open symbols*, SN × LN = 2.905 SL + 693.989, $r^2 = 0.040$, $n = 110$, $p = 0.0365$)

Discussion

Using easily identified adults, Madsen et al. (2009) found that most *B. saida* (96 %) are homozygote bearing alleles 130 bp at *Gmo8*, whereas most *A. glacialis* (90 %) are heterozygote at that locus. In their study, *Gmo8* misclassified none of the 97 *B. saida* as *A. glacialis*. But two of their 136 *A. glacialis* (1.5 %) bore the 130-bp allele homozygote signature typical of *B. saida* and therefore could be misidentified as *B. saida* using *Gmo8* as the sole identification criteria. In the present study, assuming that the 18 microsatellites analysis buttressed by *Cytb* determination provided the correct identification, *Gmo8* erroneously assigned 5 of 250 true *B. saida* (2 %) to *A. glacialis*, compared to 0 % in Madsen et al. (2009). By contrast, *Gmo8* misidentified as much as 38 % (17 out of 45) of *A. glacialis* as *B. saida*, compared to 1.5 % in Madsen et al. (2009). Given the relative scarcity of *A. glacialis* in our collections (15 %), this error rate implies that, in samples from the Beaufort Sea region, about 6 % of the fish (17/17 + 245) identified as *B. saida* based on *Gmo8* could be *A. glacialis*.

A first assumption in our approach to discriminate *B. saida* and *A. glacialis* based on otolith nucleus area is that the fish used in building the logistic regression were accurately identified to species by molecular analyses. The 18 microsatellites and *Gmo8* analyses diverged on the identification of 22 fish out of 295. *Cytb* was successfully amplified in 17 of these 22 ambiguous fish. In all of the 17 cases, *Cytb* identification confirmed the identification by the 18 microsatellite analysis. Hence, we are confident that out of the 523 fish sampled in 2004 and 2008 and used in building the logistic regression, 295 were correctly identified to species by the correspondence analysis of the 18 microsatellites. Assuming that the proportions of *B. saida* (84.7 %) and *A. glacialis* (15.3 %) were the same in the remaining 228 fish determined by *Gmo8* only, 4 (2 %) of the 193 *B. saida* were likely misidentified as *A. glacialis*

Table 2 Number of young gadids classified to each species by the logistic regression (numerator) and by molecular identification (denominator) by year, combination of years, and total of all years

Fish collected in 2004 and 2008 were pooled to build the logistic regression model. Fish collected in 2002 and 2003 were not used in building the model. Corresponding percentages are given in parentheses

Method	Years	Genetic species	Logistic regression (otolith nucleus area)	
			B. saida	*A. glacialis*
Gmo8	2004 + 2008	*B. saida*	191/191 (100 %)	0/191 (0 %)
		A. glacialis	4/37 (11 %)	33/37 (89 %)
18 microsatellites	2004 + 2008	*B. saida*	244/250 (98 %)	6/250 (2 %)
		A. glacialis	12/45 (27 %)	33/45 (73 %)
Gmo8	2002	*B. saida*	130/131 (99 %)	1/131 (1 %)
		A. glacialis	2/14 (14 %)	12/14 (86 %)
Gmo8	2003	*B. saida*	29/30 (97 %)	1/30 (3 %)
		A. glacialis	1/14 (7 %)	13/14 (93 %)
Total	All years	*B. saida*	594/602 (99 %)	8/602 (1 %)
		A. glacialis	19/110 (17 %)	91/110 (83 %)

and 13 (38 %) of the 35 *A. glacialis* as *B. saida*. Thus, we estimate at 3.3 % (17/523) the overall error in the molecular identification of the fish used in building the logistic regression. Curiously, while molecular misidentification should be more frequent in the fish analysed by *Gmo8* only, identification by the logistic regression agreed better with the *Gmo8* identification (100 % for *B. saida* and 89 % for *A. glacialis*) than with the 18 microsatellites analysis (98 % for *B. saida* and 73 % for *A. glacialis*) (Table 2).

A second assumption of our approach is that nucleus area is fixed at hatch and does not vary afterwards. In both species, a slow but statistically significant increase in nucleus area with increasing fish length seemed to invalidate this assumption. A first potential explanation for this increase is that the nucleus grows after hatch, an interpretation that runs contrary to the notion that the otolith grows by the successive addition of daily layers onto a fixed and static nucleus (e.g. Campana and Neilson 1985). A more plausible interpretation is that a large nucleus is associated with a large size at hatch, which is selected for during the early life as reported in several studies (e.g. Vigliola and Meekan 2002; Raventos and Macpherson 2005; D'Alessandro et al. 2013). We conclude that the apparent increase in lapillus nucleus with increasing length reflects the selective survival of the larger fish at hatch rather than the implausible growth of the nucleus.

A final assumption of our approach to discriminate *B. saida* and *A. glacialis* based on otolith nucleus area is that the same logistic regression can be used for other regions and years. In the Beaufort Sea, the logistic regression built with fish sampled in 2004 and 2008 reclassified the fish sampled in either 2002 or 2003 with the same or slightly higher success (Table 2). Factors such as temperature (e.g. Høie et al. 1999) and pCO_2 concentration (e.g. Maneja et al. 2013) may affect otolith size at hatching. Hence, the precise parameters of the logistic regression may change for other regions of the Arctic Ocean, in which case a reestimation of the parameters of the logistic regression may be advisable.

Typically, collections of larval and juvenile *B. saida* from arctic continental shelves comprise from 0 to 11 % of *A. glacialis* misidentified as *B. saida* (Sekerak 1982; Bouchard and Fortier 2011). In the present study, two simple conservative properties of the lapillus nucleus, the longest and the shortest diameters, allowed to correctly classify 99 % of *B. saida* and 83 % of *A. glacialis* ranging in development from hatching to the juvenile stage. Hence, the analysis of the otolith nucleus has the potential to reduce the error in the discrimination of the two species to negligible levels. For example, by correctly identifying 8 of the 10 *A. glacialis* in a sample of 100 arctic gadids assumed to be *B. saida*, our approach would bring the error from 10 % to about 2 %. In studies of the early growth and hatch date frequency distribution of *B. saida*, the otoliths of several hundred larvae and juveniles are typically analysed (Fortier et al. 2006; Bouchard and Fortier 2008; Bouchard and Fortier 2011), providing a large sample size to assess the frequency of occurrence of *A. glacialis* by adding two simple measurements of the nucleus. We conclude that our otolith-based method constitutes an inexpensive alternative to molecular analyses for discriminating *B. saida* and *A. glacialis*. Reliable identification should help elucidate the early life of *A. glacialis* and the interactions between the two species during planktonic life.

Acknowledgments We thank the numerous colleagues who contributed to field sampling, as well as the crew of the research icebreaker CCGS *Amundsen*. H. Cloutier, S. Puckett, and E. Rondeau provided technical assistance. S. L. Talbot at the Alaska Science Center, U.S. Geological Survey, Anchorage, Alaska, USA, designed the primers used in the amplification of *Cytb*. This study is a contribution to the programs of Québec-Océan at Université Laval, the Network of Centres of Excellence ArcticNet, and the Canada Research Chair on the response of marine arctic ecosystems to climate warming.

References

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier

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

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

Campana SE, Neilson JD (1985) Microstructure of fish otoliths. Can J Fish Aquat Sci 42:1014–1032

D'Alessandro E, Sponaugle S, Cowen R (2013) Selective mortality during the larval and juvenile stages of snappers (Lutjanidae) and great barracuda *Sphyraena barracuda*. Mar Ecol Prog Ser 474:227–242

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

Høie H, Folkvord A, Johannessen A (1999) Maternal, paternal and temperature effects on otolith size of young herring (*Clupea harengus* L.) larvae. J Exp Mar Biol Ecol 234:167–184

Madsen M, Fevolden S-E, Christiansen J (2009) A simple molecular approach to distinguish between two Arctic gadoid fishes *Arctogadus glacialis* (Peters, 1874) and *Boreogadus saida* (Lepechin, 1774). Polar Biol 32:937–939

Maneja R, Frommel A, Geffen A, Folkvord A, Piatkowski U, Chang M, Clemmesen C (2013) Effects of ocean acidification on the calcification of otoliths of larval Atlantic cod *Gadus morhua*. Mar Ecol Prog Ser 477:251–258

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

Nelson RJ, Bouchard C, Madsen M, Praebel K, Rondeau E, Schalburg K, Leong J, Jantzen S, Sandwith Z, Puckett S, Messmer A, Fevolden S-E, Koop B (2013) Microsatellite loci for genetic analysis of the arctic gadids *Boreogadus saida* and *Arctogadus glacialis*. Conserv Genet Resour 5:445–448

Raventos N, Macpherson E (2005) Effect of pelagic larval growth and size-at-hatching on post-settlement survivorship in two temperate labrid fish of the genus *Symphodus*. Mar Ecol Prog Ser 285:205–211

Scherrer B (2009) Biostatistique, 2e édn, vol 2. Gaëtan Morin, 592 pp

Sekerak AD (1982) Young-of-the-year cod (*Boreogadus*) in Lancaster Sound and western Baffin Bay. Arctic 35:75–97

Suzuki KW (2013) Identification guide to Arctic ichthyoplankton. http://www.arcticnet.ulaval.ca/pdf/identification_guide_arctic_ichthyoplankton.pdf

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

Thanassekos S, Robert D, Fortier L (2012) An individual based model of Arctic cod (*Boreogadus saida*) early life in Arctic polynyas: II. Length-dependent and growth-dependent mortality. J Mar Syst 93:39–46

Vigliola V, Meekan MG (2002) Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. Oecologia 131:89–93

Walkusz W, Paulic JE, Williams WJ, Kwasniewski S, Papst MH (2011) Distribution and diet of larval and juvenile Arctic cod (*Boreogadus saida*) in the shallow Canadian Beaufort Sea. J Mar Syst 84:78–84

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