

A Statistical Discrimination Method using Sagittal Otolith Dimensions between Sibling Species of Juvenile Cod *Gadus morhua* and *Gadus ogac* from the Northwest Atlantic

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Abstract

Greenland cod (*Gadus ogac*) and Atlantic cod (*Gadus morhua*) co-exist and are caught in the same fisheries in coastal waters off western Greenland and eastern Canada. Juveniles (<15–20 cm total length, TL) are similar in morphology and are not easily distinguished in fisheries research surveys or when examining stomach contents of common predators such as seals, small toothed whales, seabirds and gadoid fish. Often only the skeleton and otoliths remain in predator stomachs. Examination of variation in otolith length, width, height and weight in relation to body size and zoogeographic differences indicated otoliths of *G. ogac* are consistently larger and heavier than otoliths of *G. morhua* from Greenland and Canadian fish of both sexes and all sizes examined. Discriminant function analyses (DFA) was used to determine if otoliths removed from fish with known identity, can be individually discriminated based on otolith dimensions. Otoliths from the two species can be statistically discriminated based on otolith dimensions alone, primarily otolith length and height with relatively high certainty (71–80%). Discrimination certainty approaches 100% if body size is included. Simple additive statistical models with only a few descriptors and their discriminant functions with linear decision surfaces are provided and should simplify the identification of intact otoliths.

Keywords: Atlantic cod, cross-validation, DFA, discriminant function analysis, east Canada, *Gadus morhua*, *Gadus ogac*, Greenland cod, otolith identification, prey identification, species discrimination, stomach contents, West Greenland.

Introduction

Using otoliths for species discrimination and fish prey identification in stomach contents

Investigations of species interactions in marine ecosystems and food habitats of seabirds, marine mammals and marine fishes almost always require analyses of stomach content data. In many cases the size, biomass, and hence the importance of prey cannot be directly determined because prey are often partly digested. However, the size of individual fish prey and hence their importance in the diet can partly be estimated from the otoliths, three pairs of small carbonate stones,

or ‘ear bones’ located in the inner ear of teleost fishes (Degens *et al.*, 1969; Härkönen, 1986; Campana, 1999). The largest otoliths, the sagitta, often remain undigested in stomachs of marine birds and fishes after muscular tissue and bones have been digested and are valuable indicators of the diet of piscivorous marine predators if they can be identified (Jobling and Breiby, 1986; Elliot *et al.*, 1990). Fish otoliths contain only small percentages of water and organic matter (Templeman and Squires, 1956; Williams and Bedford, 1974; Degens *et al.*, 1969; Campana, 1999) and their dimensions are often correlated with fish length (Templeman and Squires, 1956; Mini, 1967; Gauldie and Nelson, 1990; Hunt, 1992; Lombarte and Leonart, 1993). In addition, size and shape, which vary among species,

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are reported to be species and sometimes stock specific (Boulva, 1972; Morrow, 1979; Frost, 1981; Härkönen, 1986; Hunt, 1992; Campana and Casselman, 1993; Lidster *et al.*, 1994; Begg and Brown, 2000; Stransky, 2005; Doering-Arjes *et al.*, 2008). Otoliths can be cleaned, dried, measured and weighed to great accuracy and are often collected in routine fisheries investigations for age determination as well as from predator stomachs (Williams and Bedford, 1974; Nielsen and Andersen, 2001). Hence, it is often possible to obtain very precise information on length, weight, age and quantity of individual fish prey if otolith size, and to some extent also, if fish size relationships have been determined. Morphological characteristics of otoliths have been documented for many marine fishes (Frost, 1981; Campana and Casselman, 1993; Begg *et al.*, 2000). Among these is Atlantic cod (*Gadus morhua*) (Fridriksson *et al.*, 1934; Hunt, 1992; Lidster *et al.*, 1994; Cardinale *et al.*, 2004; Berg *et al.*, 2005; Doering-Arjes *et al.*, 2008; Stransky *et al.*, 2008) an important and well-studied predator (Daan, 1973; Palsson, 1983; Kikkert, MS 1993) that feeds primarily on crustaceans and fish in addition to being cannibalistic in the northwest Atlantic waters (Hansen, 1949; Palsson, 1983; Köster and Schober, MS 1990; Lilly, 1994; Methven, 1999; Nielsen and Andersen, 2001).

Cod otolith characteristics

Otolith length from Atlantic cod was found to be slightly curvilinear and to increase with length of cod between 40–320 mm FL (fork length) (Hunt, 1992; Lidster *et al.*, 1994). In addition, characteristics of the sulcus, the longitudinal groove on the medial surface of the otolith, were found to be helpful for distinguishing otoliths of some species of very small gadids (Härkönen, 1986; Lidster *et al.*, 1994). Also, otolith zones related to *e.g.* maturity and spawning variability by fish age can be characteristic of cod populations (Rollefsen, 1933; Fridriksson *et al.*, 1934; Hüsey *et al.*, 2009). Otolith length generally increases with fish length (*loc. cit.*) but this relationship is apparently not always a simple straight-forward one for fish in general (Mosegaard *et al.*, 1988; Secor *et al.*, 1989; Lombarte and Leonard, 1993; Cardinale *et al.*, 2004; Doering-Arjes *et al.*, 2008; Checkley *et al.*, 2009). Otolith lengths decline from 5% of the total length of fast growing haddock (*Melanogrammus aeglefinus*) at 14–17 cm to almost 3% at 74–77 cm (Templeman and Squires, 1956). Furthermore, older haddock have heavier and longer otoliths than younger fish of the same length indicating otoliths that have been carefully examined may assist with distinguishing different populations of fish (Templeman and Squires, 1956; Begg and Brown, 2000; Begg *et al.*, 2000). Morphological characteristics of otoliths from Atlantic

cod stocks have only been described in a few studies (*e.g.*, Boulva, 1972; Hunt, 1992; Lidster *et al.*, 1994; Cardinale *et al.*, 2004; Berg *et al.*, 2005; Doering-Arjes *et al.*, 2008; Stransky *et al.*, 2008). Studies describing otoliths of its sibling, *Gadus ogac* Richardson 1836, Greenland or rock cod, appear to be limited to Boulva (1972) and Härkönen (1986). For adult fish around 40 cm TL, Boulva (1972) distinguished otoliths between *G. ogac* and *Arctogadus borisovi* and *A. glacialis*. However, thorough comparative descriptions of otolith characteristics of juvenile *G. morhua* and *G. ogac* are not available, and characteristics to individually discriminate them from otoliths of other co-existing gadoids are not well known (Härkönen, 1986; Lidster *et al.*, 1994; Campana, 2004). This is partly due to small juveniles of these sibling species not being previously distinguished (Methven and McGowan, 1998). These two species co-exist in coastal and bank areas off eastern Canada and western Greenland (Nielsen and Morin, 1993; Andersen *et al.*, 1994; Methven and McGowan, 1998; Laurel *et al.*, 2003) where distributions overlap and where they are exposed to the same predators including larger fish, seals, and small whales (*e.g.*, Hansen, 1949; Nielsen, MS 1992a,b; Beck *et al.*, 1993; Hammill *et al.*, 1997; Stenson *et al.*, 1997; Lawson *et al.*, 1998; Methven, 1999; Vikingsson and Kapel, 2000; Nielsen and Andersen, 2001).

Objective and research hypotheses

The overall objective of our study is to identify characters that allow otoliths from individual *G. morhua* and *G. ogac* to be distinguished from each other taking into account possible zoogeographic variation between fish in Canadian and Greenland waters. Additionally we quantify otolith length, width and height relationships with respect to fish length such that species identity can be determined from individual otoliths removed from a predator's stomach or from young fish surveys in Greenland and Canadian coastal waters (*e.g.*, Nielsen and Andersen, 2001; Methven *et al.*, 2003). We focus our efforts on juveniles less than 3 to 4 years of age with some as small as 40–50 mm, because this is the stage where otolith-fish relationships are poorly known and where otoliths are poorly described as well as where juveniles of the two species are not easily distinguished. The following three research hypotheses are tested in this study:

H_{01} : Otoliths from juvenile and medium-sized specimens of Atlantic cod (*G. morhua*) and Greenland cod (*G. ogac*) from both Greenland and Canada can be identified to species based on otolith dimensions alone (otolith length, width and height) using discriminant function analysis (DFA) and principal component analysis (PCA).

H_{02} : In order to distinguish otoliths from these two species using DFA and PCA, it is necessary to obtain additional information, *i.e.* fish length (total length or skeletal length) in addition to otolith dimensions mentioned above. Consequently, the relationships between otolith size and fish body size differ between *G. morhua* and *G. ogac*.

H_{03} : As otolith twisting seems to be a significant factor in otolith difference between the two species (S. A. Horsted, former Director of Greenland Fisheries Research Institute, Copenhagen, Denmark; pers. comm.) the total otolith height is a major influential factor in discrimination of otoliths between the two species. These relationships can together with otolith information (otolith dimensions, fish size/age, zoogeographic location) be used in discriminating specimens from the two species.

Materials and Methods

Sampling off West Greenland

Gadus morhua and *G. ogac* were collected off west Greenland during experimental gillnet surveys in coastal waters (<40 m depth) during July 1987–1989 and during deeper water (30–200 m) long line surveys in autumn from 1987–1989. Sampling details and localities are given in Table 1 and Fig. 1. Gillnets had ten equal sized panels with mesh sizes of 16.5, 18.5, 24, 28 and 34 mm bar length (two panels per mesh size). The longline fishery was conducted with lines having 400 hooks (Mustad 2330 BD, No. 6) that were mounted on 50 cm snoods at 2-m intervals and baited with 11–17 cm recently thawed capelin (*Mallotus villosus*). This sampling resulted in a broad size range of cod (typically <30–40 cm TL from gillnets and >30 cm TL from longlines) being collected for otolith analyses. Refer to Hovgård (1996) and Nielsen (MS 1992a,b) for further details of the gillnet survey and to Hovgård and Riget (1992) and Nielsen and Andersen (2001) for details of the longline survey. Otoliths from 148 *G. morhua* (14–66 cm TL) and 138 *G. ogac* (20–70 cm TL) were arbitrarily selected among a very high total number of otoliths sampled (Nielsen, MS 1992a,b). Fish were measured (TL nearest cm), weighed (± 5 g) and identified by the presence (*G. morhua*) or absence (*G. ogac*) of white pigment along the lateral line (Vladykov, 1945; Jensen, 1948). Sagitta otoliths were removed from randomly selected cod according to the area sampled, length group and sex. As a general rule, one otolith each from a female and a male from each 1 cm TL-class were selected within the length ranges sampled. Further details of otolith sampling and age determination are given by Nielsen (MS 1992a,b).

Sampling off eastern Canada

Three haul seines (Methven and Schneider, 1998) with 9 mm stretch mesh in the codend were used to catch age 0 and age 1 cod in coastal Newfoundland waters (Table 1 and Fig. 1). Most *G. morhua* and *G. ogac* were collected in water less than 10 m deep. Eighty-eight *G. morhua* (56–196 mm TL) and 92 *G. ogac* (64–258 mm TL) were collected. Fish were placed on ice shortly after being caught and were kept cold prior to having otoliths removed in the laboratory. All were identified to species using characters in Methven and McGowan (1998). We identified the smallest specimens of *Gadus* examined in this study (<75 mm) using a character not previously reported that was verified using starch gel electrophoresis. This character is based on the extent of pigmentation on finrays and membrane at the base of the posterior finrays on the first anal fin of *G. ogac* and *G. morhua*. The extent of pigmentation is greater in *G. ogac* than in *G. morhua* over the size range 40–75 mm. To test the validity of this character, we identified 100 *G. ogac* ($n = 48$) and *G. morhua* ($n = 52$) between 40–63 mm using starch gel electrophoresis that determined differences in the electrophoretic mobilities of creatine kinase and esterase proteins as previously demonstrated by Renaud *et al.* (1986). Each specimen was then re-identified using the extent of pigmentation on the first anal fin character in a blind test where the identification established by electrophoresis was not known to the person identifying the fish. Length and weight of individual fish were determined shortly after fish were identified and collected. Fish were measured for total length (mm) and blotted dry wet weight (0.1 g) prior to having otoliths removed. Each pair of sagittae was carefully cleaned using tap water and was stored dry in individually numbered envelopes.

Laboratory analyses

All otoliths from Greenland and Canada were carefully measured and aged in the laboratory in Denmark by the same person to ensure consistency of all length and weight measurements. Only completely intact sagittae were measured and used in this study. Otoliths were selected randomly from each envelope, there being no preference for otoliths from the right or left side of each fish. Cardinale *et al.* (2004) reported no significant differences between left and right side sagitta otoliths from *G. morhua*.

The maximum distances across each otolith with respect to otolith length (dimension 1), width (dimension 2), and height (dimension 3) were determined (Fig. 2) using a computerized Global Lab Image System (Data Translation, 1995). Otolith dimensions were

Table 1. Summary of *G. ogac* and *G. morhua* collections from different localities covering the two main areas of eastern Canada and western Greenland coastal waters where these two species co-exist.

Period	Area	Locality	Gear	Species	Fish TL range (mm)	No. of otoliths
Jul–99	Canada	Little Mosquito Cove 47°50'N 53°53'W	14 m seine	<i>G. ogac</i>	42 – 153	7
Apr–99	Canada	Bellevue 47°38'N 53°43'W	9 m seine	<i>G. ogac</i>	107 – 147	13
Apr–99	Canada	Bellevue 47°38'N 53°43'W	9 m seine	<i>G. morhua</i>	80 – 154	34
Oct–99	Canada	Little Mosquito Cove 47°50'N 53°53'W	25 m seine	<i>G. ogac</i>	86 – 180	49
Oct–99	Canada	Little Mosquito Cove 47°50'N 53°53'W	25 m seine	<i>G. morhua</i>	61 – 188	27
Oct–99	Canada	Baine Harbour 47°22'N 54°54'W	25 m seine	<i>G. ogac</i>	187 – 227	3
Oct–99	Canada	Little Mosquito Cove 47°50'N 53°53'W	25 m seine	<i>G. ogac</i>	120 – 180	3
Dec–99	Canada	Northeast Arm, Placentia 47°16'N 53°55'W	25 m seine	<i>G. ogac</i>	155 – 155	1
Dec–99	Canada	Little Mosquito Cove 47°50'N 53°53'W	25 m seine	<i>G. ogac</i>	174 – 183	2
Dec–99	Canada	Little Mosquito Cove 47°50'N 53°53'W	25 m seine	<i>G. morhua</i>	140 – 162	6
Dec–99	Canada	Sandy Harbour 47°40'N 54°20'W	25 m seine	<i>G. ogac</i>	187 – 245	5
Dec–99	Canada	Spanish Room 47°12'N 55°05'W	25 m seine	<i>G. ogac</i>	181 – 259	3
Dec–99	Canada	Baine Harbour 47°22'N 54°54'N	25 m seine	<i>G. ogac</i>	228 – 242	2
Dec–99	Canada	Little Bay, Marystown 47°09'N 55°06'W	25 m seine	<i>G. morhua</i>	136 – 197	20
Dec–99	Canada	Little Bay, Marystown 47°09'N 55°06'W	25 m seine	<i>G. ogac</i>	197 – 227	5
July 1987–89	Greenland	Quaqortoq, ~60°N (1F), Shore, W. Grl	Exp. Gillnet	<i>G. ogac</i>	270 – 300	15
July 1987–89	Greenland	Quaqortoq, ~60°N (1F), Shore, W. Grl	Exp. Gillnet	<i>G. morhua</i>	270 – 270	1
July 1987–89	Greenland	Quaqortoq, ~60°N (1F), Inshore, W. Grl	Exp. Gillnet	<i>G. ogac</i>	200 – 270	24
July 1987–89	Greenland	Nuuk, ~64°N (1D), Inshore, W. Grl.	Exp. Gillnet	<i>G. morhua</i>	140 – 170	13
July 1987–89	Greenland	Nuuk, ~64°N (1D), Shore, W. Grl.	Exp. Gillnet	<i>G. morhua</i>	200 – 200	3
July 1987–89	Greenland	Nuuk, ~64°N (1D), Shore, W. Grl.	Exp. Gillnet	<i>G. ogac</i>	200 – 240	4
Autumn 1987–89	Greenland	Fyllas Bank ~64°N (1D), Offshore, W. Grl.	Longline	<i>G. ogac</i>	200 – 700	87
Autumn 1987–89	Greenland	Fyllas Bank ~64°N (1D), Offshore, W. Grl.	Longline	<i>G. morhua</i>	340 – 660	61
July 1987–89	Greenland	Sisimiut, ~67°N (1B), Inshore, W. Grl.	Exp. Gillnet	<i>G. morhua</i>	160 – 270	32
July 1987–89	Greenland	Sisimiut, ~67°N (1B), Shore, W. Grl.	Exp. Gillnet	<i>G. morhua</i>	210 – 320	38
July 1987–89	Greenland	Sisimiut, ~67°N (1B), Shore, W. Grl.	Exp. Gillnet	<i>G. ogac</i>	230 – 270	8

computerized using a high resolution monochrome CCD video camera, mounted on a microscope and connected to a PC framegrabber card. Otoliths were placed with sulcus side up on a glass slide to measure dim1 and dim2 (length and width) and between two glass slides when estimating dim3 (height). The microscope magnification was adjusted to the size of the otoliths to obtain optimal high resolution. Measurements on images of small otoliths were made using a marker for each start and end point. Measurement units were calibrated with a calibration ruler (Graticule Ltd, Tonbridge, England). Larger otoliths were measured using an electronic digital sliding ruler (Mitutoyo 0–25 mm electronic ruler with precision 0.001 mm). Measurements of otolith length, width and height were obtained with a general precision of ± 0.1 mm. Otolith weight was measured in mg with a general precision of ± 0.2 mg.

Data and statistical analyses

Data were initially plotted to determine the extent of variation in body size-otolith (length, width, height, weight) relationships for *G. morhua* and *G. ogac* from off western Greenland and eastern Canada. Substantial overlap (discussed in Results) in otolith measurements between species and areas necessitated the use of statistical DFA based on a principal component analyses to correctly classify individual otoliths to species based on fish size, *i.e.*, for unambiguous species specific pattern recognition and discrimination according to fish size. The statistical method was initially tested for pattern recognition using the otolith dimensions alone, and later when using otolith dimensions in addition to fish size.

The PCA and DFA analyses compared principal

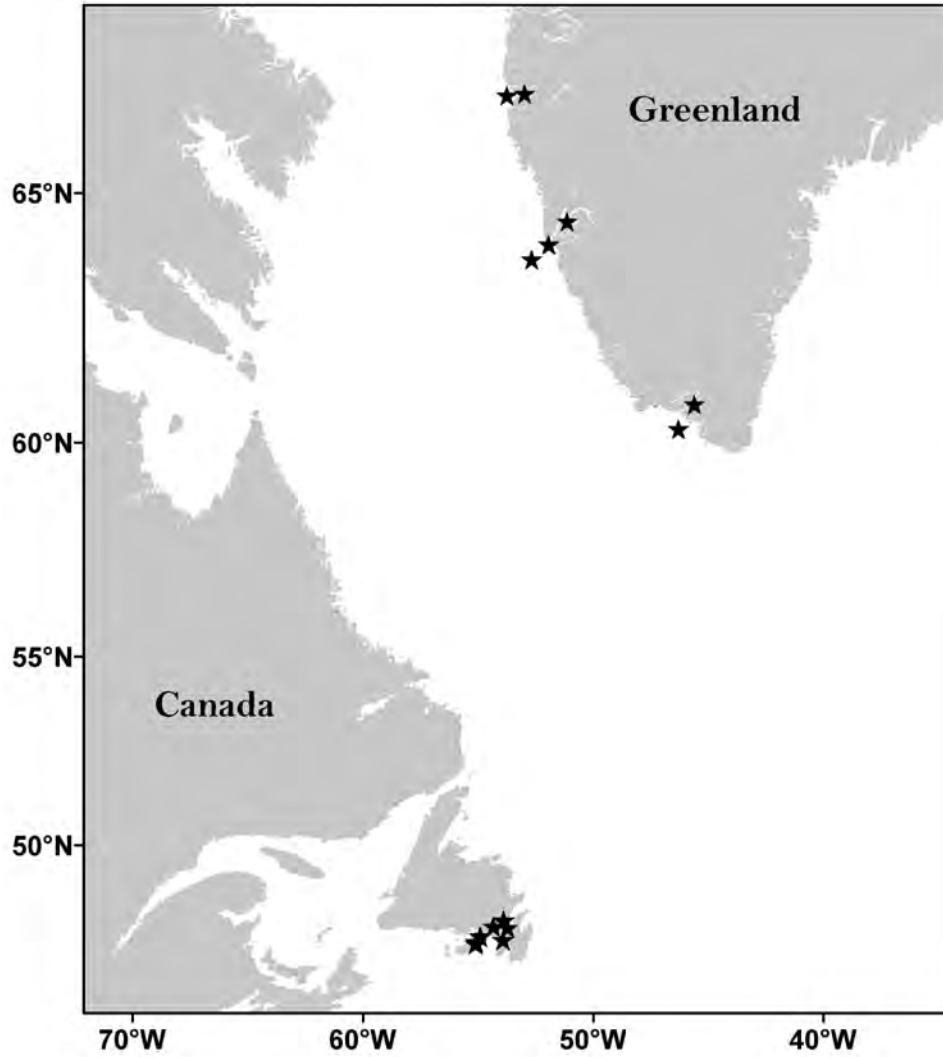


Fig. 1. Location of sampling sites where otoliths were collected from juvenile *G. ogac* and *G. morhua* in the coastal areas of western Greenland and eastern Canada.

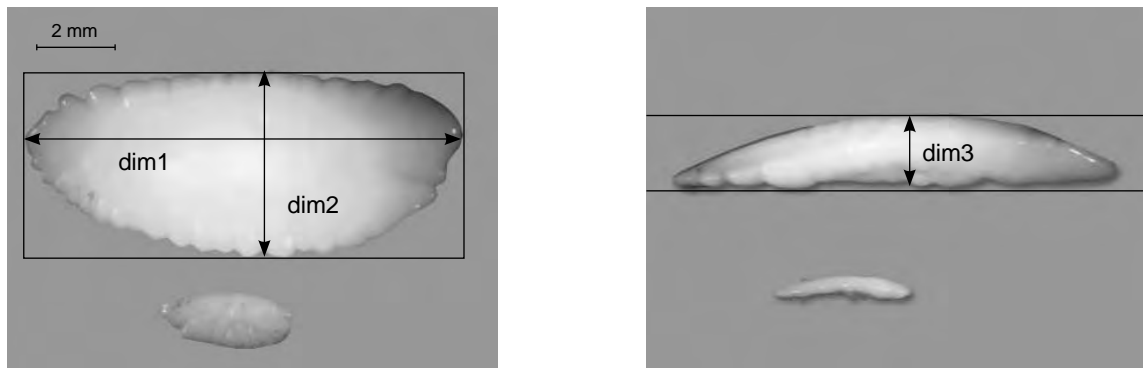


Fig. 2. Digital photograph of sagitta otoliths showing the measurements (dimensions) analysed in this study: dim1: otolith length, dim2: otolith width, and dim3: otolith height. The larger sagitta otolith is from a female *G. ogac* (28 cm TL) from Quaqqortoq, West Greenland. The smaller otolith is from a *G. morhua* (9 cm TL) from Bellevue, Canada. Magnification: 0.5 x 1.0 x 1.0. 1 mm corresponds to 62 pixels.

Table 2. Series of statistical discriminant function models (modified from Equation 1) used in this study. The models were tested with linear (lda) and quadratic (qda) DFA and cross-validation tests, respectively, in order to identify the best model to describe the variability in data and give the highest probability of classification and discrimination of otoliths from *G. ogac* and *G. morhua*. The otolith dimensions dim1, dim2, and dim3 refers to otolith length, width, and height, respectively, and length refers to fish length.

Model No.	Model	Only otolith dimensions	First order interactions
1	Species~Length×dim1×dim2×dim3	No	Yes, 3
2	Species~Length+dim1+dim2+dim3	No	No
3	Species~Length+dim2+dim3	No	No
4	Species~Length+dim1+dim2	No	No
5	Species~Length+dim1+dim3	No	No
6	Species~Length+dim1	No	No
7	Species~Length+dim2	No	No
8	Species~Length+dim3	No	No
9	Species~Length	No	No
10	Species~dim1×dim2×dim3	Yes	Yes, 2
11	Species~dim1×dim3	Yes	Yes, 1
12	Species~dim1+dim2+dim3	Yes	No
13	Species~dim1+dim2	Yes	No
14	Species~dim2+dim3	Yes	No
15	Species~dim1+dim3	Yes	No
16	Species~dim1	Yes	No
17	Species~dim2	Yes	No
18	Species~dim3	Yes	No

+ indicates additive models

× indicates models with first-order interactions.

components among individual fish. Analyses were carried out with the R-functions princomp (PCA), linear DFA discrimination (lda), and quadratic DFA discrimination (qda) using R-2.3.1 statistical software (Ithaka and Gentleman, 1996; R, 2007). Refer to Venables and Ripley (1996) for additional details. Variables included were fish species tested with respect to dependence of fish otolith dimensions and fish size according to country (area). This was done to determine the precise functional relationship between the otolith dimensions as well as for the relationships between fish-otolith-dimensions (length, height, width), otolith weight, and fish size. In principle, the quadratic DFA requires data to have an underlying normal distribution (Venables and Ripley, 1996). To obtain normality, all variables were log-transformed. The Shapiro-Wilks test (Sokal and Rolf, 1995) in the R-2.3.1 Statistical Package was used to test for normality.

A series of linear and quadratic DFAs were tested to

identify the best model to describe variability in the data. The linear DFA model used with different modifications was of the general form:

$$ldl = lda(\text{species} \sim \text{length} + \text{dim1} + \text{dim2} + \text{dim3}) \quad (\text{eq 1})$$

where *ldl* is the linear discrimination function, *lda* is linear discriminant analysis, length is total fish length (TL cm), and dim1 is otolith length (mm), dim2 is otolith width (mm), and dim3 is otolith height (mm).

Modifications of the model are summarized in Table 2. Some DFAs included fish length (models 1–9), others did not (models 10–18). All models were basically additive models, but several included first-order interactions shown in Table 2. All models were tested both by linear (lda) and quadratic (qda) analysis. The aim was to obtain the highest discrimination possible by using the simplest model with as few descriptors and interaction terms as

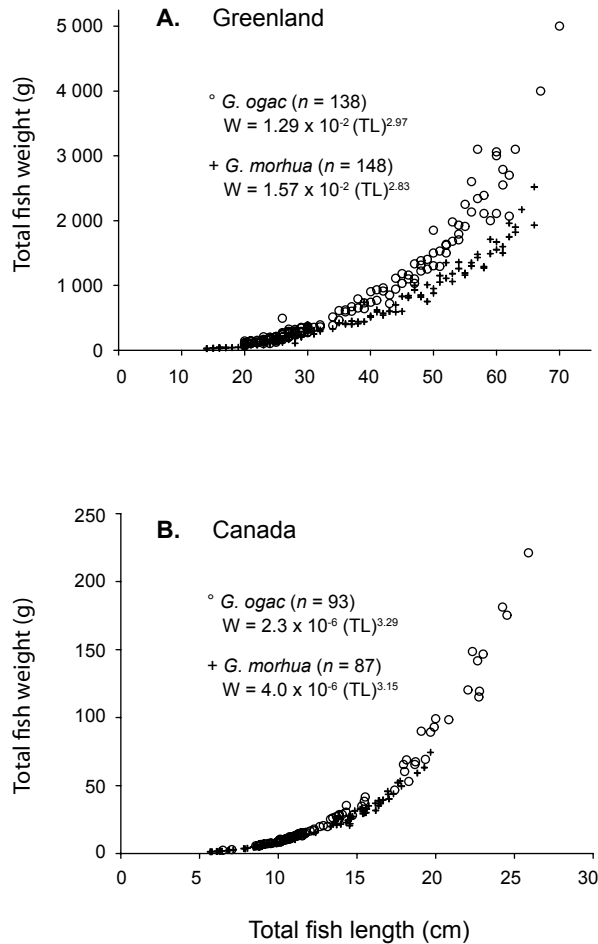


Fig. 3. Total fish length-weight relations for *G. ogac* and *G. morhua* from (A) Greenland and (B) Canada.

possible in order to avoid complex decision surfaces from the model (potentially highly influenced by noise in data) which are difficult to biologically explain and justify. The fewer descriptors, the higher robustness in relation to use and application of the model to new, future data.

The outcome of the cross-validation method is the probability (or proportion) of correct classification with 95% confidence limits as determined by the DFA model. Cross-validation was performed to validate the results obtained by the linear DFA models listed in Table 2. In this procedure, 50% of the observations were chosen at random to estimate a discrimination function and establish a prediction surface that was used to test how well one can predict the species when using the other half of the data. This was repeated 100 times to establish the probability of success (success frequency) of correct otolith classification. The final DFA models and resulting decision surfaces were selected based on their simplicity and ability to discriminate between species with the highest probability.

Results

Visual examination of data for species discrimination

Gadus ogac are heavier at a given length than *G. morhua* (Fig. 3). This is most apparent at sizes greater than 30 cm, but is also evident with somewhat more overlap at sizes less than 150 mm (Fig. 3). Both species have similar and nearly isometric somatic body growth (Fig. 3) indicating that either fish length or fish weight can be used in the discriminant function analyses described below. The larger and more robust appearance of *G. ogac* is also evident in its otoliths which are slightly longer and heavier than otoliths removed from same sized *G. morhua* (Figs. 4 and 5). Overlap is evident for fish from Greenland and Newfoundland and is most apparent for otoliths from Greenland fish less than 30 cm and for otoliths from Newfoundland fish less than *ca.* 150 mm (Figs. 4 and 5).

Overlap in otolith measurements, most apparent for the smallest and most difficult specimens to identify (Figs. 3 and 5) was not due to problems with misidentification. The smallest *G. ogac* and *G. morhua* examined in this study (40–75 mm) were distinguished from each other based on the extent of pigmentation at the base of the posterior most finrays and membrane of the first anal fin with pigmentation being more extensive in *G. ogac* than in similar size *G. morhua*. The error rate in a blind test using this character was 2% with 2 of 100 specimens being identified incorrectly when compared to identifications established using starch gel electrophoresis (Renaud *et al.*, 1986, Methven and McGowan, 1998). Both specimens that were misidentified were actually *G. morhua* when identifications were positively established using starch gel electrophoresis.

Statistical analysis of species discrimination

Fish otolith measurements (otolith length [dim1], otolith width [dim2], otolith height [dim3]) were tested for normality using the Shapiro-Wilk test. Otolith measurements from *G. ogac* were determined to be normally distributed (Greenland: $W = 0.9670$, $p = 0.0256$; Canada: $W = 0.9750$, $p = 0.0100$). Otolith measurements from *G. morhua* were only normally distributed for fish from Canada (Greenland: $W = 0.9883$, $p = 0.2983$; Canada: $W = 0.9723$, $p = 0.0490$). The linear DFA therefore seems to be the most fit method to use when evaluating results of the combined material (*G. morhua* and *G. ogac*) given that the quadratic DFA in principle assumes an underlying normality of the data.

Results of the statistical models tested (Table 2) in the cross-validation procedure indicate the quadratic discriminant functions perform slightly better than the linear discriminant functions when only the otolith

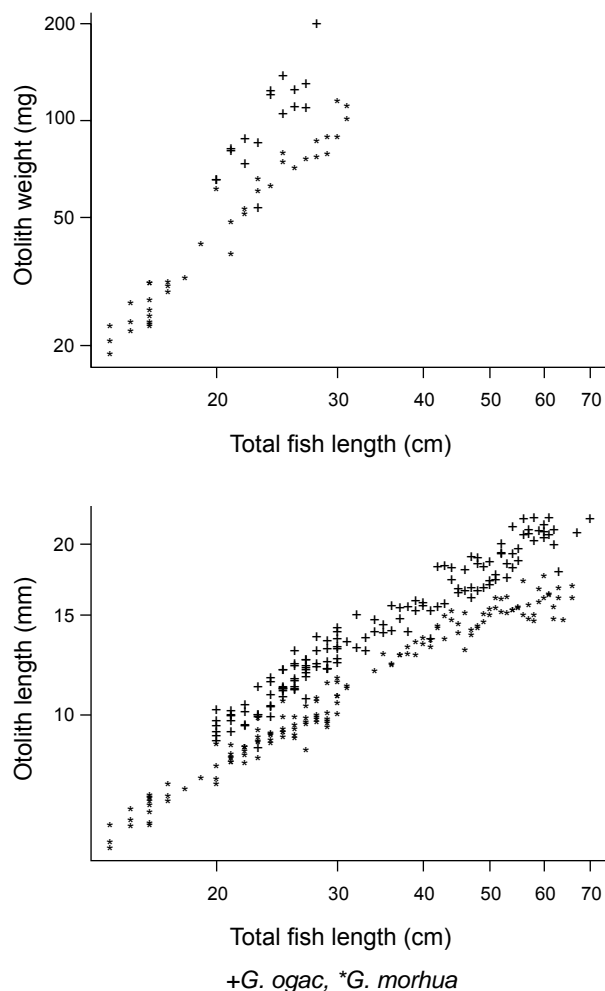


Fig. 4. Sagitta otolith length and weight plotted against total fish length (cm) on logarithmic transformed scale for *G. ogac* and *G. morhua* from coastal waters of west Greenland.

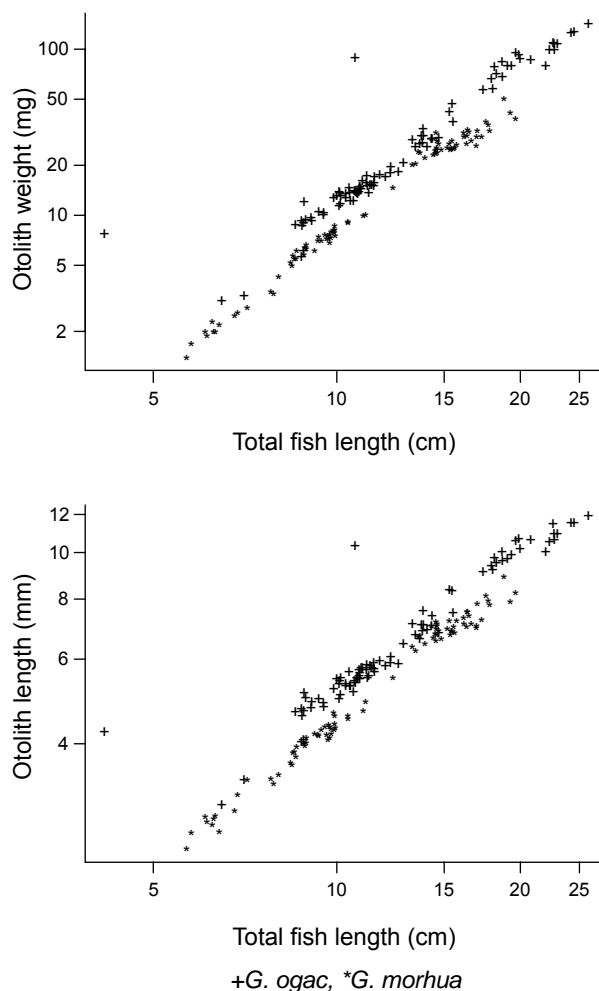


Fig. 5. Sagitta otolith length and weight plotted against total fish length (cm) on logarithmic transformed scale for *G. ogac* and *G. morhua* from coastal waters off Newfoundland, Canada.

dimensions are included for both the additive and multiplicative models (Figs. 6 and 7). Quadratic discrimination based on otolith length and otolith height in an additive model gives a better discrimination in the cross-validation with probabilities of 0.71 (Greenland) and 0.80 (Canada) than the model where all otolith dimensions (length, width, height) are included which is the case for both areas (countries). The probability of correct classification using linear discrimination for the same additive model (otolith length and otolith height), is approximately 0.76 for Canada and 0.60 for Greenland (Figs. 6 and 7). The probability does not increase when all otolith dimensions are included.

Furthermore, when fish length is included in the analyses, the linear discrimination is preferred for fish from both Greenland and Canada (Figs. 6 and 7). When fish length is combined with information on otolith dimensions, the probability of correct classification of otoliths to species increases significantly to 0.9–1.0 in the

cross-validation tests. Hence the DFAs can discriminate very well between species for both areas (countries) if fish length is known and included in the analyses (Figs. 6 and 7). For the most fit models the correlation increases only slightly from 0.92 in purely additive models to 0.93 in models including interaction terms for the Canadian fish. For the Greenland fish, the discrimination between species for the most fit models is 0.98–0.99 for additive models and models including interaction terms (Figs. 6 and 7). When looking at the overall material from both Greenland and Canada, the model giving best discrimination between species in the cross-validation is model 1 (Table 2) where otolith length, width and height in addition to fish length are included and where the first order interaction effects between all these variables are included. However, the resulting decision surface from this model is very complicated to explain biologically. Our aim has been to obtain a high discrimination of individual fish from both Greenland and Canada by using the simplest model with as few descriptors and interaction

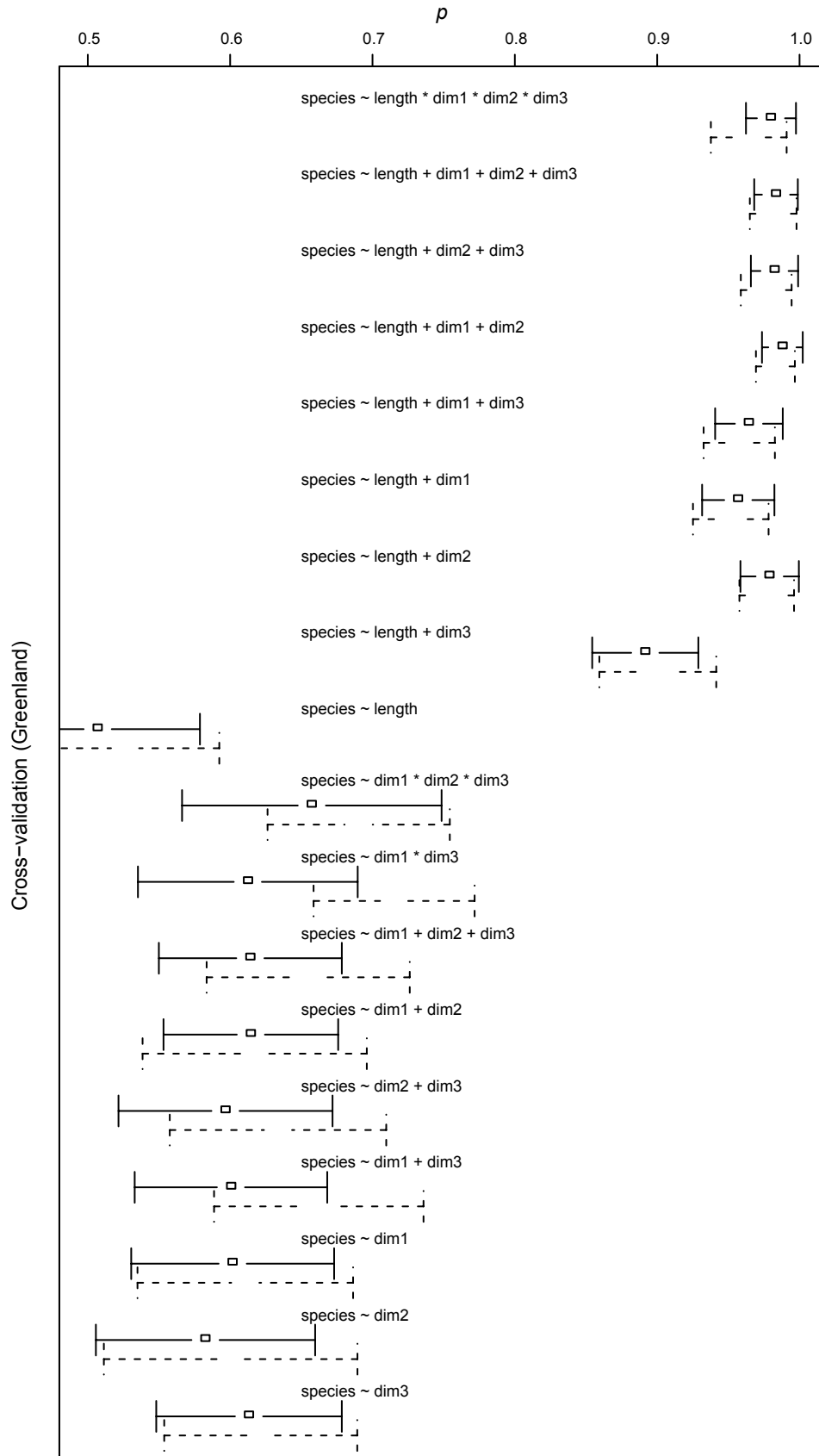


Fig. 6. DFA and cross-validation statistical test results for species discrimination in Greenland. 95%-confidence limits for the probability of correct classification for linear discrimination (lda, solid line) and quadratic discrimination (qda, dashed line). Results for 18 different statistical discriminant function models are shown. The otolith dimensions dim1, dim2, and dim3 refers to otolith length, width, and height, respectively, and length refers to fish length.

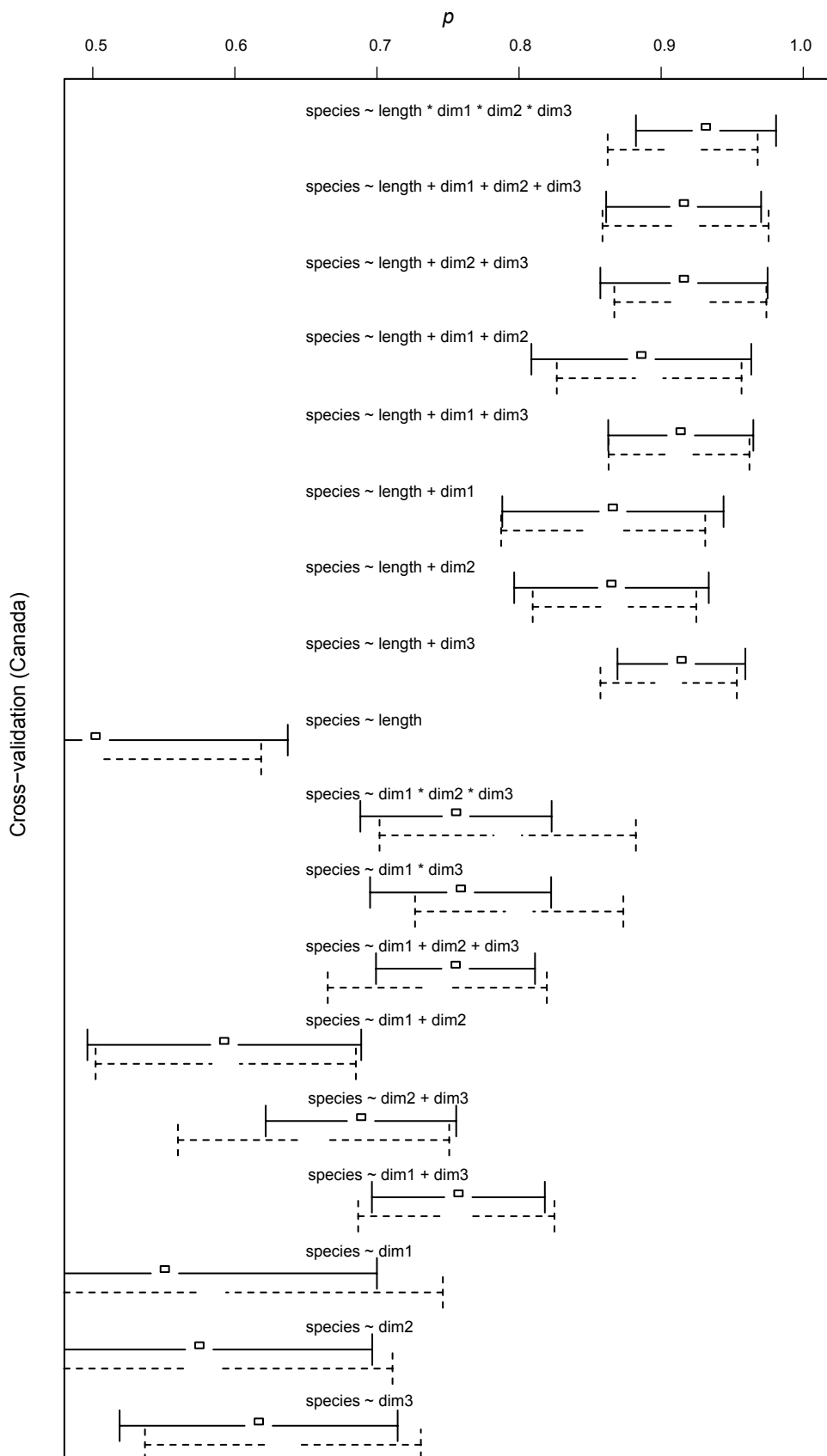


Fig. 7. DFA and cross-validation statistical test results for species discrimination in Canada. 95%-confidence limits for the probability of correct classification for linear discrimination (lda, solid line) and quadratic discrimination (qda, dashed line). Results for 18 different statistical discriminant function models are shown. The otolith dimensions dim1, dim2, and dim3 refers to otolith length, width, and height, respectively, and length refers to fish length.

terms as possible in order to avoid complex decision surfaces which are difficult to biologically explain and justify. In multiplicative models, the decision surfaces are often very heterogeneous and there is an increased risk that the surfaces are influenced heavily by variability (noise) in the otolith measurements in the dataset. The latter makes multiplicative models less robust to other data applications. Models with fewer descriptors and simple decision surfaces have a higher robustness when new data sets are examined. On this basis, the additive models are preferred. The discrimination of individual fish between species in the combined material is high in the purely additive model 5 that includes otolith length and otolith height as well as fish length with a probability of correct classification between species of 0.97 in Greenland and 0.92 in Canada.

The power of this classification is considered to be high as relatively few variables are used on numerous individual fish to provide consistent results for fish from both Greenland and Canada. The power of the DFA and the cross-validation for detecting group differences relies on the ratio of observations (n) to number of variables (p). Stability and power increase as the ratio $n:p$ increases (Cadrin, 2000). Furthermore, the power of the present classification is confirmed to be high by the significance of the cross-validation tests performed (see below) indicating robustness in the statistical results.

The linear decision surfaces and linear equations from which species identification can be determined are shown in Fig. 8. As there are differences in the results obtained between the Greenland and Canadian material, decision surfaces for both areas (countries) are given for several models. Based on the above principle of simplicity, Fig. 8 illustrates the results from the final selected additive DFA models. The probability of correct classification for the linear and quadratic discrimination, respectively, is given in Fig. 8 and is 0.59 (lda) and 0.68 (qda) for Greenland and 0.76 (lda) and 0.76 (qda) for Canada. When fish length and otolith width (model 7) are included for the Greenland material, the probability of a correct classification is increased to 0.98 (lda) and 0.98 (qda). When fish length and otolith height (model 8) are included for the Canadian material, the probability of correct classification is increased to 0.93 (lda) and 0.93 (qda). The statistics in relation to prior probabilities of groups as well as slope and intercept of the linear discriminant functions for these selected models are shown in Table 3. The linear functional relationships can be exemplified by model 15 for Canada as follows:

$$\text{If } Y > 0.805X - 1.343 \quad \text{for } G. \textit{ogac} \quad (\text{Eqn } 2)$$

$$\text{If } Y < 0.805X - 1.343 \quad \text{for } G. \textit{morhua} \quad (\text{Eqn } 3)$$

where X is the logarithm of dim1 and Y is the logarithm of

dim3 (See Table 3). Similarly, other intercepts and slopes of the other models from Table 3 can be put into the above functional relationship in order to discriminate individual fish to species for fish from Canada or Greenland.

These results lead to partial acceptance of hypothesis H_{01} because it is only partly possible to discriminate between species based on otolith measurements alone, while the probability of correct classification and discrimination is very high when fish length is included. This finding leads to acceptance of hypothesis H_{02} . When otolith height data are included, the probability of correct classification increases especially in the Canadian material where the smallest individuals of the two species were tested. This leads to acceptance of hypothesis H_{03} which appears to be especially valid for the smallest juvenile fish (<150 mm TL).

Discussion

Robustness and power of the results

Several different models were tested in the statistical DFA cross-validation procedures to determine otolith identification to species for individual fish. When only the otolith dimensions were included in the analyses, species recognition certainty was approximately 70% to 80% for the Greenland and Canadian fish, respectively. Only two of the three otolith dimensions (otolith length and height) were necessary to obtain the highest degree of species separation. For both areas (countries), the species discrimination in the cross-validation performs significantly better when information on otolith dimensions is combined with total fish length. In this case, the probability of correct classification of species increases to levels between 0.9–1.0 in the cross-validation tests.

The power of the overall species classification success from the present results is strong. First, there are relatively few variables used in the analyses. These were taken from numerous individual fish and provide consistent results between the two main distribution areas sampled. The additive linear models containing a few variables and interaction terms are the simplest to explain biologically and hence are to a lesser degree influenced by variability (possible noise) in the data, *i.e.*, are more biologically robust. Finally, the strong power of the present species recognition and classification is also evident from the statistical significance of the cross-validation tests performed on the full material indicating high robustness in the statistical results.

The ability to distinguish between otoliths of these two species is an important finding given that species identification has proven difficult where these species co-occur in fisheries surveys and in stomach samples from predators. In this context, possible zoogeographical

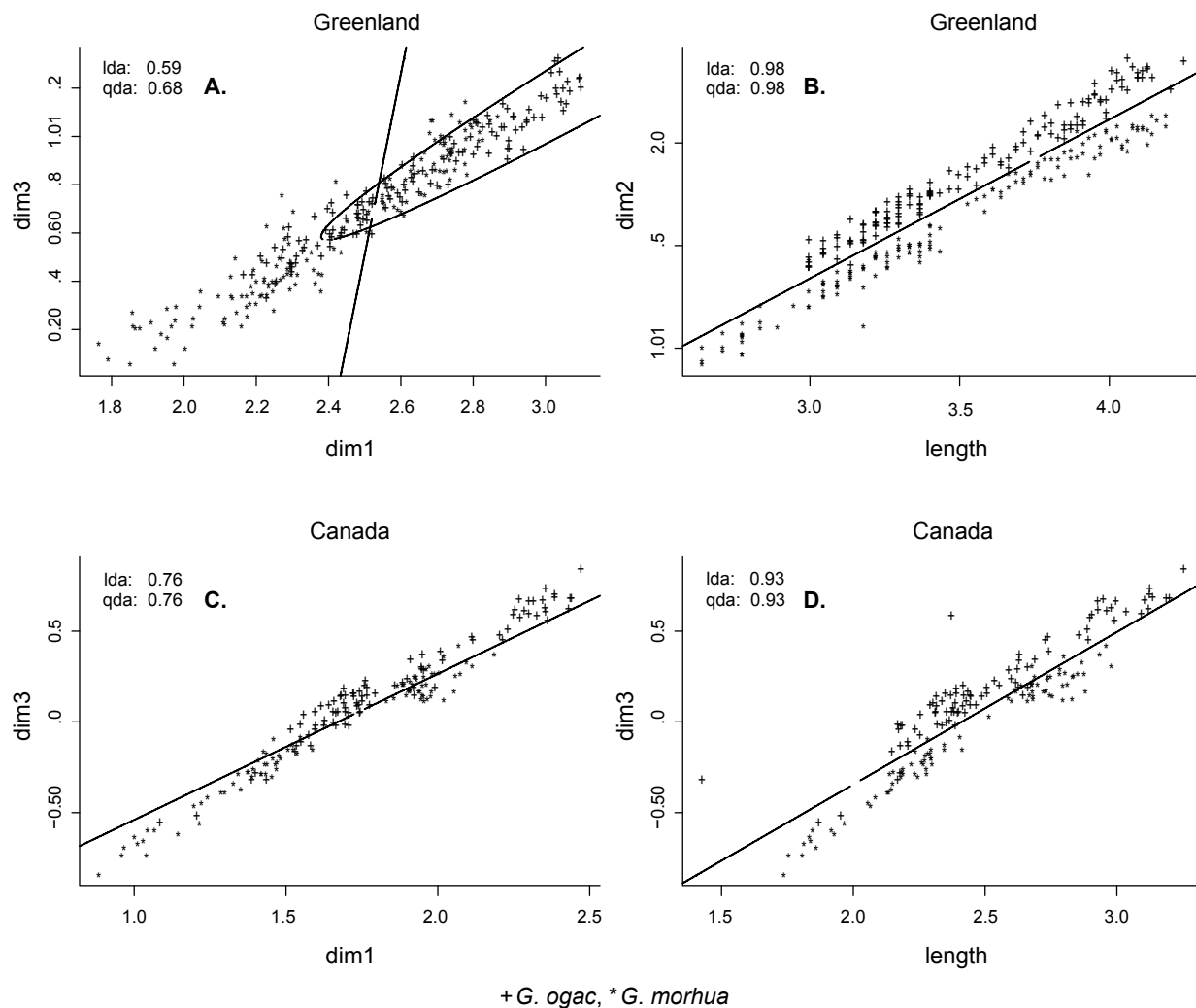


Fig. 8. Plots of decision surfaces for selected additive models. The probability of correct classification for linear (lda) and quadratic (qda) discrimination are given in the corner of each panel. As there are differences in the results obtained between the Greenland (A, B) and Canadian (C, D) material, decision surfaces from several models are shown. The left side of the figure (A, C) shows linear decision surfaces when only otolith length (dim1) and otolith height (dim3) are included in an additive model for the Greenland and Canadian material (model 15). The decision surfaces shown on the right side of the figure (B, D) are when fish length and one otolith dimension are included.

variation in otolith dimensions must be examined and potential impacts of different environmental and hydrographical conditions in different habitats need to be considered (e.g., between western Greenland / eastern Canada). This study solves this problem by providing a robust statistical quantitative method and simple model for individual fish otolith discrimination and species separation in different areas when only basic and easily obtainable otolith dimensions and fish length are available, for example in the stomach contents of predators. Prey skeletal remains are often present and available for a longer period after ingestion from which fish length and basic otolith measurements can be used to determine species and hence composition of prey in stomachs of predators. The results show that species and prey identity

of individual fish can be determined with certainties exceeding 90%, making the method efficient in relation to data from future stomach sampling programs and young fish surveys.

Context of other methods used for species separation of *G. ogac* and *G. morhua*

A methodology based on morphometrics for species separation has been reported for larger fish (Methven and McGowan, 1998). For specimens of intact fish taken in fisheries surveys (e.g., Hovgård and Riget, 1992; Methven *et al.*, 2003), two simple characters can be used to distinguish between *G. ogac* and *G. morhua*. Specimens larger than 100 to 140 mm can be distinguished by the

Table 3. Statistics for selected models in relation to prior probabilities of groups, intercept and slope of the linear discriminant functions providing decision surfaces for each selected model, as well as group means, and coefficients of linear discriminants (for log transformed variables – see also Fig. 8). The otolith dimensions dim1, dim2, and dim3 refers to otolith length, width, and height, respectively, and length refers to fish length.

Species	Variable	Model	Area	Prior prob. of groups	Intercept	Slope	Group means	Coef. linear discriminants
<i>G. morhua</i>	dim1	15	Greenland	0.5089	-18.1447	7.4906	2.3910	4.1388
	dim3						0.5867	-0.5525
<i>G. ogac</i>	dim1			0.4911			2.6411	
	dim3						0.8170	
<i>G. morhua</i>	dim1	15	Canada	0.4888	-1.3425	0.8048	1.6291	-11.4851
	dim3						-0.0816	14.2706
<i>G. ogac</i>	dim1			0.5112			1.8657	
	dim3						0.2091	
<i>G. morhua</i>	length	7	Greenland	0.5089	-0.9843	0.7746	3.4474	-14.2943
	dim2						1.5876	18.4550
<i>G. ogac</i>	length			0.4911			3.5435	
	dim2						1.8585	
<i>G. morhua</i>	length	8	Canada	0.4888	-2.0232	0.8399	2.4318	-9.6733
	dim3						-0.0816	11.5172
<i>G. ogac</i>	length			0.5112			2.5378	
	dim3						0.2091	

presence of white pigmentation along the lateral line of *G. morhua* and by its absence in *G. ogac* (Vladykov, 1945; Jensen, 1948; Methven and McGowan, 1998). The results in the present paper also indicate species-specific differences in fish condition for larger fish in both areas (countries). However, the latter can be influenced by environmental conditions (Dutil *et al.*, 1999). For *Gadus* specimens smaller than those examined by Methven and McGowan (1998), *i.e.*, less than 87 mm, we report that the extent of pigmentation at the base of the posterior most finrays on the first anal fin is greater in *G. ogac* than in *G. morhua* over the size range 40–75 mm. The error rate in a blind test using this character was only 2% when specimens were identified to species using starch gel electrophoresis (Renaud *et al.*, 1986; Methven and McGowan, 1998). Hence intact specimens of these two species can be distinguished at sizes as small as *ca.* 40 mm, approximately the size of settlement off eastern Canada. However, these species are more difficult to distinguish when determining their abundance and composition in predator stomachs.

Visual examination of body weight versus length showed distinct discrimination between individuals of the two species for the larger size groups investigated (>150 mm TL), but not for the smaller, juvenile specimens. Weight was also one character used to distinguish between *G. ogac* and *G. morhua* over the size range 87–135 mm when examined using discriminant function analyses (Methven and McGowan, 1998). Results presented in the present paper indicate both species are close to achieving isometric body growth (which will be somatic growth for the juveniles). Possible discrimination between larger specimens based on their body length-weight relationship might be influenced by sexual maturation and growth of the gonads. Furthermore, this method is not useful for individuals in predator stomach contents where the body weight estimate will be biased by digestion and the more rapid decomposition (degradation) of the soft body tissue (protein, lipid, muscle, and fat tissue, pigments, etc.) compared to the bone and cartilage tissue used in the length measurement of prey organisms. Also, otolith dimensions and weight at a given fish body length is most

different for the larger specimens, while these interspecific differences are not as obvious for the smaller individuals. Consequently, visual examination is not adequate to identify un-ambiguous distinctive patterns for species discrimination by size for the smallest fish.

Otolith characteristics and relevant factors introducing variability in otolith morphology

Twistedness of otoliths appears to be an important character to discriminate between otoliths of *G. morhua* and *G. ogac* (S. A. Horsted, Greenland Fisheries Research Institute, Copenhagen, pers. comm., and based on an initial blind test otolith separation trial). In general, otoliths are more twisted as otolith height increases. When otolith height (being a measure of the degree of otolith twistedness) is included in the model, the probability of correct classification increases (Figs. 6 and 7). This is especially so for the smallest specimens examined from Canada. Boulva (1972) reported that the otoliths of *G. ogac* are curved, and found that ratios of otolith width to otolith thickness indicated little resemblance between *Arctogadus* and *G. ogac*. He found that regression between otolith width and thickness showed statistical significant ($p < 0.001$) different slope and intercept between the species which was most profound for larger otoliths. Lidster *et al.* (1994) made comparative descriptions and identified by eye features and characteristics that might be helpful in distinguishing sagittal otoliths from juvenile *G. morhua* and *Boreagadus saida*, but no quantitative and statistical models were established to support this and reveal statistical significant differences between species. A main feature of *G. morhua* otoliths is the curvature along the longitudinal axis where the lateral surface was more concave and had more furrows in addition to containing crenulation around the entire periphery, compared to *B. saida* otoliths. Also, the *G. morhua* otolith had a more developed sulcus compared to *B. saida*. However, they did not describe how they avoided confusion between small *G. morhua* and *G. ogac* in their material from coastal waters in Newfoundland where these two species are widely and densely distributed and co-exist. The above characteristics will evidently not enable distinction between *G. morhua* and *G. ogac* otoliths as they both possess extensive curvature as described above, and *G. ogac* otoliths also have a well-developed sulcus. Also when Hunt (1992) analyzed Atlantic cod otoliths for fish down to 9 cm in length to be distinguished from species of haddock (*Melanogrammus aeglefinus*), pollock (*Pollachius virens*) and silver hake (*Merluccius bilinearis*) sampled from surveys and fishery in Canadian waters, he did not discuss potential confusion of small *G. morhua* and *G. ogac* in his material.

As the two species co-exist and reproduce in the same areas of both western Greenland and eastern Canada coastal waters (Nielsen and Andersen, 2001; Nielsen and Morin, 1993; Andersen *et al.*, 1994; Methven and McGowan, 1998), possible zoogeographical variation in otolith dimensions and potential impacts of different environmental and hydrographical conditions in different habitats needs to be considered in an otolith separation study. Environmental conditions and habitat variables such as temperature, carbon dioxide levels and feeding conditions, as well as demographics such as age, sex, stock, and year class specific effects, have been found to impact (allometric) growth in otolith shape being a possible confounding effect in otolith shape analyses in other studies on, for example, sea trout, haddock and Atlantic cod (*e.g.*, Campana and Casselman, 1993; Begg and Brown, 2000; Begg *et al.*, 2000; Simoneau *et al.*, 2000; Cadrin, 2000; Cardinale *et al.*, 2004; Doering-Arjes *et al.*, 2008; Checkley *et al.*, 2009). The obtained results of intra- and inter-specific differences are robust in this respect as the tested individuals were carefully selected and designed to have broad geographical coverage in the overlapping distribution areas of Greenland and Canada. Greenland and Canadian individuals were tested separately, but the tests showed consistent results across areas.

Several individuals from each fish length group of a very broad fish size spectrum of both sexes and species were sampled to take account of possible size-specific differences and regional effects including potential variability (noise) from intraspecific size variation. Also, to avoid confounding effects of fish length and age, a broad and continuous spectrum of both size and known age fish were included in the analyses. Several years and year-classes (covering the same year classes by species) were examined. The two species have been sampled at the same sites over the sampling period (multiple years) and during the same periods (seasons) covering both inshore and coastal waters by area. This increases the likelihood that the fish have been exposed to broad and similar environmental conditions in each area, as well as there is broad coverage of different types of habitats and environments in each area, to average out potential effects of such factors. Finally, right and left sagittal otoliths were sampled arbitrarily to avoid effects from this factor as well.

Context of methodologies for otolith analyses and discrimination

Several methods are used in otolith discrimination studies for different purposes. For discrimination of individual fish and for species recognition and classification, the multi-variate analysis of variance of

linear otolith descriptors in DFA and cross-validation tests have been used in the present study.

Comparison of univariate body dimensions has been shown to be ineffective to discriminate different groups (stocks) of the same species, because distributions usually overlap extensively (King, 1985; Cadrin, 2000). In stock separation, bivariate allometry modeling of relative growth of any two morphometric features has been widely used to separate populations within the same species, however, the assumption of homogeneous growth within a morphological character is a problem here (Cadrin, 2000). Multivariate allometry of otolith form, which is usually multi-dimensional and with geographic variation, has been used in simultaneous multivariate analysis of a representative array of characters to detect and identify shape differences among groups of fish (*e.g.*, Teissier, 1960; Cadrin, 2000). Here the PCA method is typically used on several log transformed dimensions. The first principal component (PC1) typically indexes a weighted combination of all variables interpreted as an index of general size variation because all dimensions increased during (isometric) growth and were positively correlated (Teissier, 1960; Cadrin, 2000). The second principal component (PC2) typically indexes the residual variance due to shape differences between groups arising from (allometric) developmental change in form. Group separation based on interpreting size and shape from PCA is reasonable if PC1 accounts for large successive portions of morphometric variance with stable eigenvectors, however, there is a risk for confounding the within-group and among-group variability in pooled-group PCA (Cadrin, 2000).

An alternative method for multi-group analysis is the DFA as used in the present study. DFA tests significance of group differences in variation and co-variation of several variables by deriving a weighted combination of the variables which maximizes differences among taxonomic groups (Bolles and Begg, 2000; Cadrin, 2000). Unlike PCA, which emulates a multivariate allometric equation, DFA is not as biologically interpretable (Cadrin, 2000). As in the present study, the DFA from specimens with known group or species membership (intrinsic data) can be used to classify specimens with unknown membership and allows for testing classification accuracy through cross-validation.

Development of computer-aided image analysis systems have facilitated progress and diversification of morphometric studies and geometric analysis approaches as well as the capability to increase the number of observations in relation to the number of variables

(MacLeod, 1990; Rohlf, 1990; Cadrin and Friedland, 1999). Geometric methods including shape analyses are categorized as either “outline methods” or “landmark methods” (Cadrin, 2000). The most common outline method involves fitting of Fourier series to the point coordinates along the perimeter of the morphometric feature, and the Fourier coefficients have commonly been used as multivariate observations for DFA covering outer otolith and scale shape for discriminating fish stocks (Friedland, MS 1996; Cadrin, 2000; Stransky *et al.*, 2008). Also, geometric landmark coordinate methods have been used to quantify deformations of morphometric points in coordinate space (*i.e.*, to quantify change in shape from a reference and an “average” shape) to study ontogenetic changes and geographic variation in fish otoliths. Several authors have investigated shape of otoliths to classify groups and populations of fish to stocks and species (*e.g.*, L’Abée-Lund, 1988; Campana and Casselman, 1993; Begg and Brown, 2000; Stransky, 2005; Stransky and MacLellan, 2005; Stransky *et al.*, 2008). This method was also used to make the fish ageing processes more objective and accurate and calibrate population age structure by including information on otolith morphometrics and otolith shape (Doering-Arjes *et al.*, 2008). Even though Fourier coefficients in outline methods and landmark methods can be used to discriminate stocks they are often criticized for not enabling clear biological interpretation of group differences. This is because Fourier coefficients in outline methods and the warps from a reference shape in landmark methods can only be considered as “biologically arbitrary variables” (Bookstein *et al.*, 1982; Lohmann and Schweitzer, 1990; Rohlf and Marcus, 1993; Rohlf, 1998; Cadrin, 2000). Critics point out that Fourier coefficients are not homologous among specimens, that landmarks must be fixed in coordinate space, and that landmark methods can only define morphological location and orientation of principal deformations to guide the choice of variables for more traditional analyses of linear distances such as DFA (Bookstein *et al.*, 1982; Rohlf and Marcus, 1993; Cadrin, 2000). External structures like shape of the otolith reflect phenotype expressions that are mainly influenced by species, sex, age, year class, stock and environment (Begg and Brown, 2000; Doering-Arjes *et al.*, 2008). Accordingly, Fourier transcription methods are most efficient when discriminating between groups or stocks of fish (*e.g.*, Stransky *et al.*, 2008), but are not so well suited and easy interpretable when the aim is to identify and classify individual fish to a certain species as in the present study.

In conclusion, form projection and shape analysis methods cannot be biologically interpreted as easily as the few multivariate variables with linear distances used

in the DFA and cross-validation tests in the present study. This is because the typically complex and large number of Fourier-transformed descriptors may result in over-parameterization of models in shape analysis of average population distinctions. Another reason for not using form projection analysis with image recognition methods here is the lower efficiency of this method when a main discrimination character is twistedness of the otoliths where otolith height has significant influence. The *G. ogac* otoliths appear to be more twisted than otoliths from *G. morhua*. Form projection of otolith morphometrics, where normalized and size corrected elliptical Fourier descriptors of otolith outline shape is typically used resulting in digital silhouette images and multivariate shape analysis (Stransky and MacLellan, 2005), is not optimal for determining twistedness and height of the otoliths. Accordingly, this method is not optimal for species discrimination in present context.

Implementation of the method and future studies

The present method and results are easy to implement in fish stock monitoring (*e.g.*, in fisheries research surveys and in commercial fishery on board and/or market sampling) as well as in stock assessment and management of cod resources in the investigated areas. Our method is very efficient for application. The highest level of identification is achieved when both body size and otolith dimensions are analysed together in the same model. With respect to identification of stomach contents, determining the fish length will sometimes not be as reliable as in relation to other monitoring. The results indicate a simple and efficient method for species discrimination by use of a few, easily identifiable, and obtainable otolith and fish body characters, and it provides a simple to use and robust functional relationship and statistical discrimination procedure. Furthermore, the method is relatively simple to explain biologically, and the cross validation feature increases the power and robustness of the classification method.

However, future studies should be performed to investigate a range of other possible morphological relationships, various vectors across the surface of the otolith, and shape analysis features that would produce discrimination models that would not be over specified, which is the risk of Fourier shape analyses and related methods. As twistedness may be an important character in species difference there might very well be potential in identifying other morphological relationships and shape analysis features. In the present study, we have focused on finding a few very simple, robust, easily identifiable and easy to explain biologically characters that are not easy to mis-identify and mis-interpret in the estimation process.

Such easy to identify and easy to measure characteristics are cost-effective both with respect to resource and time demands for processing, and they increase the likelihood of implementation of the method.

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The authors of this paper have equal status.

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