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Separation of Norwegian coastal cod and Northeast Arctic cod by otolith morphometry

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Abstract

For stock assessment purposes, Atlantic cod (*Gadus morhua*) from the coastal and offshore regions off northern Norway is usually allocated to Norwegian coastal cod (NCC) and Northeast Arctic cod (NEAC) by internal morphological features of their otoliths. As this classification is subject to individual interpretation, this study investigated an alternative objective approach for the separation of the two cod groups, using otolith shape analysis. Otolith samples from coastal areas along northern Norway and from the Barents Sea were analysed by univariate shape descriptors and Elliptical Fourier Analysis (EFA). When combining those methods, the classification score was 89% for NCC and 90% for NEAC. When genetic typing data (Pan I marker) were used as reference, the classification scores were reduced to 83% for NCC and 76% for NEAC. These results imply that differences in internal otolith morphology are translated to a large extent into the outer shape, but that those cannot directly be linked to genetic structure. Environmental conditions, however, seem to have a considerable influence on how otolith growth increments and consequently otolith shapes are formed. As the various fjord systems in Norway provide local habitats and as differences within the NCC with regard to genetic structure and life-history parameters had been found in earlier studies, variation of NCC otolith shapes between three coastal regions was also examined. The region classification scores for reader-typed NCC varied between 60% and 81%. Apart from the outer shape analyses, experimental work on the detection of internal (annuli) shapes was carried out and will be presented as work in progress.

Keywords: Stock identification; Otolith shape analysis; Northeast Atlantic; Norway; Atlantic cod; *Gadus morhua*

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Introduction

There are several stocks of Atlantic cod (*Gadus morhua* L.) in the Northeast Atlantic that are managed as separate units. The largest stock at present is the Northeast Arctic cod (NEAC) which has its nursery and feeding area in the Barents Sea. The Norwegian Coastal Cod (NCC) is found in fjords and along the coast of Norway. The NCC has been drastically reduced in recent years, and there is great concern that overfishing is taking place (ICES, 2006). In 2006, the NCC stock was also introduced on the national red list of threatened species as a 'near threatened' stock (Kålås et al., 2006). Catch regulations for the NCC have unfortunately had limited effect on preventing unsustainable fishing pressure on the stock, since a mixed fishery with the NEAC, especially during the spawning season, is taking place. Current regulations have also left considerable cod quota for the coastal vessels to be taken at the end of the year, thus resulting in nearly pure NCC catches since few NEAC are close to the coast and available for these fishers at this time of the year. The International Council for the Exploration of the Sea (ICES) has recommended no catch of NCC since 2004 (ICES, 2006), but for social-economic reasons, limited quotas are still given.

The NEAC stock undertakes long migrations, from the central part of the Barents Sea to the northern part of the Norwegian coast for feeding, to the Lofoten area and as far south as the coast of Møre to spawn, after having reached sexual maturity at an age of approximately seven years (Bergstad et al., 1987). During these migrations to and along the coast, the NEAC spatially overlap with the NCC stock. The NCC, which is more typically found within fjords, displays less migratory behaviour (Berg and Pedersen, 2001). The two stocks do not mingle randomly at the spawning grounds. Separate groups from the different stocks may stay simultaneously at local spawning grounds within small areas, but NEAC are more abundant in deeper waters (Nordeide, 1998).

The two stocks have different life history characters. Faster growth of NCC compared to NEAC has been observed for larval, juvenile and adult fish (van der Meeren et al., 1994; Svåsand et al., 1996; Otterlei et al., 1999). The NCC mature at ages of 5-6 years at lengths of 40-50 cm (Berg and Pedersen, 2001), while NEAC generally mature at 6-8 years of age at 75-90 cm length (Bergstad et al., 1987). Several genetic studies have revealed significant differentiation between NEAC and NCC, indicating that they are genetically distinct populations (e.g. Sarvas and Fevolden, 2005 and references therein). Natural markers and morphological features have also been used to investigate stock-specific differences of Norwegian cod. Body shape differences exist and are well known to fishermen, where NEAC are generally longer and thinner than NCC (Svåsand et al., 1996). Meristic differences between the stocks have also been identified, such as the NCC having lower vertebrae numbers than NEAC (Løken and Pedersen, 1996). Otterlei et al. (2002) found that the otoliths (ear stones) of juvenile NCC are larger than those of NEAC at a given fish length.

In the fisheries assessment, fish from the two stocks are routinely distinguished through otolith morphology (Mjanger et al., 2000). The stock separation criteria developed by Rollefsen (1933 and 1934) are still used and are based on differences in shape and relative size of the two innermost translucent zones. The NCC has an even and oval shaped first translucent zone, often looking like "a glowing halo", and a large second translucent zone. The NEAC has an elongated first winter zone with a small bulge on one side, and a smaller second translucent zone (Figure 1). Fish otoliths that do not show the typical morphology for NEAC and NCC are classified either as uncertain

NCC or uncertain NEAC (Jakobsen, 1987). A fifth otolith type has been characterized as "the Svalbard type", which refers to NEAC often found in the Svalbard area with clear translucent bands (Mjanger et al., 2000). Acknowledging that the typing of otoliths is subject to reader-specific experience and inferred variation of stock allocation for the fisheries assessment, Berg et al. (2005) applied digital image analysis to quantify the stock differences in internal otolith morphology. So far, however, it is unclear if the reported differences in the shape of the innermost growth increments are translated to the otolith outline shape of older fish.

Otolith morphometric analyses have previously been used for taxonomic studies and species identification (e.g. Campana, 2004; Stransky and MacLellan, 2005; Schulz-Mirbach et al. 2006). As modern image analysis techniques allow efficient 2D outline analyses of several hundred samples, otolith shapes have been extensively used for stock discrimination (e.g. Cadrin and Friedland, 2005; Stransky, 2005; Turan, 2006). Campana and Casselman (1993) were the first to apply Fourier analysis (see Lestrel, 1997, for a review) of the otolith outlines to investigate intraspecific variation of Atlantic cod. Further studies on cod around the Faroe Islands (Cardinale et al., 2004), Iceland (Jónsdóttir et al., 2006; Petursdottir et al., 2006) and the northern North Sea and west of Scotland (Galley et al., 2006) also reported small-scale differences between cod populations using otolith shape analysis.

By analysing otoliths of Norwegian cod from a selection of distribution areas across all life stages, this study was aimed at investigating if there are differences in the outer shape of NCC and NEAC otoliths, allocated by otolith reader types and genetic typing. In the case of consistent differences between stocks, this method would provide an alternative quantitative technique to allocate fish to NCC or NEAC, without the necessity to section the otoliths. As there are indications that NCC may comprise several more or less discrete stocks (Fevolden and Pogson, 1997; Pogson and Fevolden, 2003; Skarstein et al., in press), and as growth and maturity at age was reported to differ between fjords in northern Norway (Berg and Albert, 2003), variation of NCC otolith shapes between fjord areas was also examined.

Material and Methods

Sampling and selection of material

Cod otoliths from fish randomly sampled in the Barents Sea and the Vestfjorden area (Figure 2) were selected from the routine survey conducted by the Institute of Marine Research (IMR) in Bergen, Norway, during the winter cruise 2001, and from commercial fishing vessels where IMR collected fish samples during 2000 and 2001 (Table 1). The fish were caught by bottom trawl, net, long line or Danish seine. Fish classified as NCC (type 1) and NEAC (type 5) by the basis of inner otolith morphology by the age readers (see Table 1 for all types) were selectively picked. The NEAC were selected from stations in the Barents Sea where an overweight of type 5 otoliths was apparent. The NCC otoliths were picked from stations close to the coast east and west of the Vestfjorden area where this phenotype dominated. Genetically typed fish were sampled in trawls on cruises with research vessels from the University of Tromsø, Norway, from 1999 to 2001. The samples were caught in five areas close to the coast or within fjords (Balsfjorden, Porsanger, Nordkapp, Varanger),

plus one sample from Svalbard (Figure 2), and were a sub-sample of the material used by Sarvas and Fevolden (2005). Stock allocation of these samples, based on inner otolith morphology interpreted by the age readers, showed there was an overweight of the NCC type at Varanger, Balsfjorden and Porsanger with 87% classified as either certain or uncertain NCC. For fish caught off Nordkapp, 80% of the otoliths was classified as certain or uncertain NEAC type. The Svalbard samples were all allocated to the Svalbard type. The allocation of otolith types was carried out by two experienced age readers at IMR.

Fish length varied between areas (Table 1). In the Barents Sea, which contained only NEAC, fish ranged in size from 10 to 90 cm. The other areas displayed smaller size ranges, so only otoliths from fish lengths between 30 and 70 cm were used in the further analyses in order to minimise morphometric variation. The age of these fish varied between 2 and 13 years. In the analyses of univariate fish and otolith measurements, all fish were included.

Image and shape analysis

Otolith outlines were digitised using an image analysis system consisting of a high resolution monochrome CCD video camera, mounted on an Olympus® microscope and connected to a PC framegrabber card. The microscope magnification was adjusted to the size of the otoliths to ensure as high resolution as possible, varying between 30x and 50x. The image analysis system was calibrated in horizontal and vertical direction separately to avoid possible distortion effects of the lens system. The otoliths were positioned onto a piece of plastiline in a consistent manner, with the sulcus (convex) side up and the rostrum to the left in horizontal line. High-contrast video images were produced using transmitted light, delivering dark two-dimensional objects with bright background. The video signal was captured in the Ulead VideoStudio® (version 8.0) software, and the images analysed in ImageJ (version 1.37). The otolith outline was detected by intensity thresholding (dark otolith against a bright background) and a four-connection principle (horizontal and vertical line segments, only). From the detected points, 1000 equidistant points were interpolated.

A set of univariate descriptors was calculated based on the digitised x-y coordinates. In addition to otolith length (OL, major axis length), otolith width (OWI, minor axis length), the perimeter (OP) and area (OA) were recorded. All otoliths were weighed with a precision of 0.1 mg. All univariate descriptors appeared to be reasonably normally distributed (Kolmogorov-Smirnov test of normality; $p > 0.05$) with equal group variances (homogeneity of variance, Levene's test; $p > 0.05$), while the populations differed in their mean (fixed-effect model). Age-, length- and weight-specific differences in univariate descriptors between NCC and NEAC groups were tested by analysis of variance (ANOVA).

The digitised outline coordinates were forwarded to Elliptical Fourier Analysis (EFA; Kuhl and Giardina, 1982), using C++ modules based on the algorithms of Ferson et al. (1985). The principle methodology of Fourier analysis has been described elsewhere (e.g., Lestrel, 1997) and is therefore not presented here. The EFA represents a fitting of harmonic functions to the original otolith outlines with an ellipse as the first approximation step. The different outlines are standardized with regard to orientation, size and starting point (Kuhl and Giardina, 1982), so that three of the four Elliptical Fourier Descriptors (EFDs) describing the first harmonic ellipse are constant for all outlines. This reduces the total number of EFDs to be used in the analysis by three.

Only the first 10 harmonics (37 EFDs) were included in the statistical analysis, as these were responsible for over 99% of the shape variation (Lestrel, 1997). Before analysing the EFDs for differences between NCC and NEAC, the distribution of these data was tested. As all EFD amplitudes appeared to be reasonably normally distributed (Kolmogorov-Smirnov test of normality; $p > 0.05$), no transformation of the EFD data was carried out.

Fish size correction and multivariate analyses of Fourier descriptors

All otolith morphometrics and Fourier descriptors were significantly ($p < 0.05$) related to fish size. To ensure unbiased comparisons between groups, the size-effect was statistically removed by using residuals of linear variate *vs.* fish size (total length) regressions with pooled among-group slopes across all sampled specimens. Power-functions were used to remove the size-effect in fish (FW) and otolith weight (OW), providing residuals to be used in the analyses. A log-log scale was used to ensure a residual variance independent of fish size. Size-corrected variates were compared between groups (species or regions) using linear discriminant function analysis in SYSTAT 9.0 (SPSS Inc., 1999). The rate of the classification experiments was calculated using jackknifed cross-validation (SPSS Inc., 1999). To visualise the shape differences between the groups, average otolith shapes were plotted for each group by means of the reproduced outlines of the averaged normalised EFDs within a group.

Genetic typing

For genetic classification of the samples, previously analysed frequencies of alleles and genotypes at the scnDNA pantophysin gene (*Pan* I) were used (Sarvas and Fevolden, 2005). Total DNA was extracted from gill arches, which were preserved in 96 % ethanol, and variation at the locus was scored using the PCR based assay as described in details by Fevolden and Pogson (1997). The *Pan* I locus possesses two allelic classes and three genotypes are scored in the RFLP (restriction fragment length polymorphism) analyses using the restriction enzyme *Dra* I. They are the two homozygotes *Pan* I^{AA} and *Pan* I^{BB}, and the heterozygote *Pan* I^{AB}. In the Barents Sea, inhabited by NEAC, the *Pan* I^{BB} genotype is totally predominating whereas in Norwegian fjords, with mostly coastal cod, the *Pan* I^{AA} genotype predominates (Fevolden and Pogson, 1997; Sarvas and Fevolden, 2005). The frequency difference of the two homozygous genotypes between coastal and offshore areas persists over years and can be seen in different age groups of cod (Sarvas and Fevolden, 2005). Heterozygotes, *Pan* I^{AB}, occur both among NCC and NEAC, normally in higher frequencies among the former.

Results

Univariate measurements

Individuals classified by otolith readers as either NCC or NEAC showed consistent differences both in size- and age-specific somatic and otolith characteristics. Only weight-at-length was not significantly ($p > 0.05$) different between NCC and NEAC for fish < 65 cm total length (TL; Fig. 3a). In contrast, NCC had significantly higher mean OW between 30-70 cm TL ($p < 0.05$, Fig. 3c) and consistently higher but less pronounced length-specific mean OP, OA and OL/OWI (Fig. 3e) than NEAC. When analysed by age, NCC between 1-4 years had significantly higher mean FW ($p < 0.05$, Fig. 3b), OW (Fig. 3d), OA, and OP. Interestingly, the pattern reversed consistently in fish older than 5 years with higher mean values for NEAC than NCC. The OL/OWI ratio, however, was always higher in NCC than NEAC over the entire investigated age range.

Classification by otolith reader types

All discriminant analyses of the EFDs were highly significant ($p < 0.001$). The classification score of the discriminant analysis using the reader types as reference ranged from 73% to 90% (Table 2). The lowest score was obtained for reader type 1 when the alternative was reader type 5 and only the univariate descriptors were applied. The highest score was obtained for reader type 1 when the alternative was reader type 5 and only EFDs were used, and for reader type 5 when both univariate descriptors and EFDs were used.

The discrimination between all four otolith reader types on the basis of EFDs was much poorer, ranging from 22% score for reader type 4 to 75% for reader type 5 (Table 3). Misclassification was highest between otolith types 1, 2 and 4. The corresponding canonical scores plot (Figure 4) also shows considerable overlap between these groups and higher affinity of the type 2 and 4 groups to the type 1 otoliths than to the type 5 otoliths. On the first discriminant axis, types 1 and 5 are moderately well separated.

The sampling regions of NCC, as defined by otolith reader type 1, have classification scores ranging from 60% (Finnmark) to 81% (Balsfjorden; Table 4). The lowest misclassification rates were observed between Balsfjorden and Vestfjorden, and these regions were also furthest apart on the canonical scores plot (Figure 5). The samples from Finnmark cluster between Balsfjorden and Vestfjorden.

The average shapes of type 1 and type 5 samples (Figure 6, upper panel) visualise the differences in the outlines of NCC and NEAC otoliths, especially between rostrum and antirostrum and along the postrostrum tip. Shape variation between NCC otoliths from different fjord regions (Figure 6, lower panel) is highest on the dorsal outline between the antirostrum and the postrostrum, where an intermediate position of the Finnmark otolith outlines between the Balsfjorden and Vestfjorden otolith shapes is apparent. The Balsfjorden otoliths separate from otoliths of the other two regions along the dorsal and ventral sides closer to the postrostrum.

Classification by genetic types

When grouping the samples by *Pan* I types, the discriminant analysis based on the univariate variables and the EFDs combined resulted in poorer classification scores than based on the reader types. Excluding the heterozygotes *Pan* I^{AB}, the NCC score was 83% and the NEAC score was 76%. When the heterozygotes were included and only EFDs were applied, the classification score ranged from 51% for *Pan* I^{AB} to 62% for *Pan* I^{BB} (Table 5). Misclassification rates were highest (around 29%) between the types *Pan* I^{AA} and *Pan* I^{AB}. Consequently, the individual samples exhibited a large overlap (Fig. 7), especially between the *Pan* I^{AA} and *Pan* I^{AB} types and less pronounced between the *Pan* I^{BB} and the two other types.

Comparison of otolith-based stock classification with *Pan* I genotypes

The distribution of *Pan* I genotypes within each reader-defined group (i.e., 1, 2, 4, 5) varied considerably (Fig. 8a). Homozygote *Pan* I^{AA} (40%) and heterozygote *Pan* I^{AB} (45%) were the most frequent genotypes in type 1 otoliths, while *Pan* I^{BB} homozygotes were much rarer (14%). Conversely, type 5 otoliths were predominantly comprised by *Pan* I^{BB} homozygotes (63%) but very few *Pan* I^{AA} homozygotes. Intermediate otolith groups 2 and 4 (less certain NCC or NEAC characteristics) contained the highest percentage of heterozygote genotypes. The distribution of otolith groups within genotypes (Fig. 8b) suggested that *Pan* I^{AA} homozygotes consist almost entirely of fish with the NCC otolith type (NCC, type 1 + 2 = 85%), whereas *Pan* I^{BB} homozygotes appeared to fall into cod specimens classified either as type 5 (46%) or type 1 (33%).

Discussion

There is growing evidence that local cod populations exist and may be discerned based on morphological otolith characteristics (Cardinale et al., 2004; Jonsdottir et al., 2006). In the case of NCC and NEAC, experienced readers rely on annual growth structures in cross-sections of the otoliths, but the present study suggests that basic univariate measurements on whole otoliths may already yield a very high classification success of the two stocks. For each fish length class, NCC-typed otoliths were both significantly wider and longer than NEAC-types, had a bigger area, perimeter and a higher otolith weight, that yielded a maximum classification rate of 81%. This is consistent with a number of other studies on gadoids reporting a high utility of basic otolith morphology for stock/population separation (Begg and Brown, 2000; Campana and Casselman, 1993; Jonsdottir et al., 2006; Petursdottir et al., 2006). Compared to NEAC-type fish, age-specific measurements in NCC-types showed consistently higher fish weight and length, and also a higher otolith weight, length, and width until 4 years of age, while for fish older than 5 years the pattern was reversed. This indicates consistent differences in the life history between both stocks, possibly elicited by an earlier maturation of NCC than NEAC (Berg and Pedersen, 2001). The separation between NCC and NEAC increased markedly when not only the univariate measurements and ratios were applied, but also the multivariate shape descriptors, i.e. EFDs. An overall correct classification of the otolith reader types 1 and 5 of more than 90% means that the two Norwegian cod stocks can be distinguished by very cost-effective and objective image analysis of the whole otoliths.

When the uncertain otolith types (2 and 4) were included in the discriminant analyses, the classification rate between NCC and NEAC was 3-6% lower than using the certain types 1 and 5 only. This decreased classification rate indicates that the uncertain types are rather determined by unclear internal growth increment patterns than being reflected in the outer otolith shapes. The average shapes of NCC and NEAC otoliths show only slight but consistent differences on several sectors on the outlines, leading to the high classification rates observed. It should be noted, however, that the variation between individual otoliths within NCC and NEAC was considerably high, and that the differences in average shapes of NCC otoliths between fjord regions was similar to the NCC-NEAC differences.

Strikingly, consistent patterns of discrimination were found for both methods of grouping the individual samples, i.e. the otolith reader typing and the genetic types. The differentiation of the NEAC from the NCC and "intermediate"-type cod (otolith reader types 2 and 4, genetic type Pan I^{AB}) resulted in the highest observed correct classification rates within each analysis. This is in concordance with Berg et al. (2005) and Wennevik (2006) who also found a high correspondence between the genetic typing and otolith classification by the age readers. However, a substantial number of the Pan I^{BB} genotypes was classified as NCC by the age readers, which gives a conflicting stock allocation. A possible explanation is that all the genotyped fish were caught close to the coast. The cod genotyped as Pan I^{BB} in these areas experience environmental conditions that differ from those of the more typical NEAC found in the central Barents Sea, and their otolith zonation patterns are likely to resemble the NCC type.

The observed differences in outer otolith morphology between NEAC and NCC represent an unknown combination of genetic and environmental differences (Swain et al., 2005). Although differences in inner otolith morphology between the two stocks are well documented (Berg et al., 2005; and references therein), little is known about the basis for the different otolith shapes. There is a genetic component of otolith growth (Söllner et al., 2003), but for stocks within a species with low genetic heterogeneity like NCC and NEAC, there is likely to be an environmental and physiological basis for the differences in otolith morphology. Co-rearing of fish from the two stocks at identical environmental conditions has shown that differences in otolith size at given fish length exist between the two stocks at the larvae and early juvenile stage (Otterlei et al., 2002). This is a strong indication of genetic influence on otolith characteristics, which also has been reported for other cod stocks (Cardinale et al., 2004). Campana and Casselman (1993) found that growth rate was contributing to differences in cod otolith shapes to a large extent. In this study, the growth trajectories differed between the stocks, with NCC growing faster until age 5 and slower beyond this age, compared to NEAC.

Environmental influences on the observed differences between the two stocks are also likely to play a major role, as the semi-enclosed fjords compared to the open water masses further north in the Barents Sea imply different environmental conditions. Water temperature is a key factor influencing most physiological properties of poikilotherm animals like fish. The Barents Sea consists of a mixture of Arctic water from the north, inflowing Atlantic water and the coastal current from the south (Loeng, 1991). The NEAC in the Barents Sea experience mean annual temperatures of 4-5°C, with seasonal amplitudes of 2-2.5°C (Ottersen et al., 1998; Stiansen et al., 2005). Along the coast where the NCC is found, the mean annual temperatures are generally 1-2°C higher, and the seasonal amplitudes are also larger (Sætre et al., 2003). However, the temperature

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can vary substantially within fjord areas. Deeper in the fjords, the water can be much colder during wintertime than in the outer regions (Hegseth et al., 1995). Since NEAC stay in deeper waters than NCC (Nordeide, 1998), they will not experience the warmer temperatures at shallower waters in the summer which the NCC does. The observed larger otolith weight at fish length of NCC is probably a response of the higher temperatures they experienced (Mosegaard et al., 1988; Otterlei et al., 2002).

Migration has an energy cost, which also leads to increased active metabolism. Otolith growth is closely coupled to fish physiology, and altered metabolism can thus infer morphological differences in the otoliths (Wright, 1991; Bang and Grønkjær, 2005). NCC and NEAC exhibit seasonal differences in migratory behaviour. During summer, NEAC feed close to the ice border in the north where temperature is low, but during winter, they migrate further south to warmer water masses, first the mature fish to spawn and later a lot of immature fish to feed on the nearshore spawning capelin (Bergstad et al., 1987; Ottersen et al., 1998). As a consequence, most of the NEAC experience the lowest seasonal temperature during summer and autumn, and the highest temperature during winter (Godø and Michalsen, 2000). This is in contrast to the NCC which experience the highest temperatures during summer and lowest during winter. How this is translated into seasonal and overall growth differences between the stocks is unknown, but it can potentially contribute to stock specific otolith morphologies. Nevertheless, recent work shows that NCC and NEAC exhibit the same seasonality in the hyaline and opaque otolith growth, showing that ontological mechanisms (e.g., nearly similar spawning and feeding season) may dominate over different migration behaviour and ambient temperature regimes (Millner et al., unpublished data).

Our analyses also proved that differences in otolith morphology exist between NCC from different areas. Again, this can be caused by genetic and environmental factors, and the interaction between these effects. There are generally large temperature differences between fjords, but also within fjord systems (Hegseth et al., 1995). Water temperature in general decreases further north. Within a fjord, the temperature is colder in winter and warmer in summer in the inner part of fjords compared to outer and more exposed fjord areas. Thus, physiological differences driven by ambient temperature differences between the fjord areas can play a major role. Significant differences in growth and sexual maturity of cod within fjord systems have also been observed, suggesting that they could be considered as different stocks (Berg and Pedersen, 2001; Berg and Albert, 2003). Co-rearing of offspring of cod caught at different locations along the Norwegian coast showed large differences in growth potential, suggesting existence of genetically distinct sub-populations along the coast (Salvanes et al. 2004). Laboratory experiments have also demonstrated interaction between the genotype and environmental conditions on growth of southern NCC (Imsland et al., 2005). Genetic analyses showed that both on a larger (Skarstein et al., in press) and narrower (Pogson and Fevolden 2003) geographic scale, fjord samples may be different from one another.

In conclusion, this study showed that NCC and NEAC can be distinguished by outer otolith morphology with high certainty, providing an alternative method of stock allocation without the necessity to section or break the otoliths. The decreased classification rates when using genetic typing data indicate that differences in otolith morphology cannot directly be linked to
genetic structure. Differences in environmental conditions seem to have a considerable influence on how otolith growth increments and consequently otolith shapes are formed.

The moderate separation of NCC by fjord regions also suggests a considerable heterogeneity among the cod inhabiting the coastal areas in northern Norway.

NCC and NEAC are often occurring together close to the coast, and this is a challenge for fisheries management since stock size of NCC is critically low. Effective stock separation tools are therefore needed. Our results show that NCC can be effectively separated from NEAC based on outer otolith morphology. The traditional way of separating NCC from NEAC by reading otoliths is both time consuming and expensive. However, for the purpose of stock identification, fish age is not always necessary. Automated systems for image capturing and analyses of whole otoliths can be very effective if large numbers of otoliths can be processed in a short time, e.g. by using moving transport bands (Harbitz, 2007). Otolith data from fish where other separation tools also are available (like genetics) can be used as a powerful template for further analyses. If inclusion of NCC is suspected to occur in NEAC targeted fishery, otolith samples could be ordered from the fishing fleet and analysed immediately. Collecting the otoliths is a very simple task that unlike genetic samples does not require much training or equipment. Otoliths from individual fish can also be pooled in larger quantities for an effective sampling procedure.

If a cost-effective automated image capturing and analysing technique is established, it will also open for the possibility of analysing very large numbers of otoliths. Fine-scale spatial resolution of otolith morphology variation can thus be revealed. This can add to a better and more detailed knowledge of fish population structure and help in identifying local fish populations that require management focus to avoid depletion. Analyses of large quantities of otoliths enables also long time series of otolith morphology to be established, providing valuable information such as temporal stability of otolith outer shape.

During the project on outer otolith shape analyses, thin-sections of the analysed otoliths were photographed, and several image analysis procedures were explored to facilitate automated inner ring (annuli) detection, in order to analyse the annuli shapes for comparisons between NCC and NEAC. The first objective was to automatically detect the otolith core (nucleus), see Annex 1. In a further step, a routine was developed to enable the detection of the two innermost annuli to separate between NCC and NEAC (see Annex 2). This work is currently in progress in order to apply the developed routines to a large set of available images.

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Tables

Table 1. Norwegian cod otoliths by sampling area, otolith reader types and genetic types. For data analysis, only otoliths from fish with total lengths of 30-70 cm were included. The sampling years, mean lengths and ages (± 1 standard deviation), as well as the age range for these samples are given. The samples were allocated to otolith types (1 = certain Norwegian coastal cod [NCC], 2 = uncertain NCC, 3 = Svalbard type, 4 = uncertain Northeast Arctic cod [NEAC], 5 = certain NEAC) and genetic types (*Pan* I^{AA} allele, typical for NCC; *Pan* I^{AB} heterozygote allele; *Pan* I^{BB} allele, typical for NEAC).

Area	Code	Number of fish (total)	Selected number of fish	Year(s)	Mean lengths	Mean age	Age range	Otolith reader types					Genetic types		
								1	2	3	4	5	Pan I^{AA}	*Pan* I^{AB}	*Pan* I^{BB}
Svalbard	SVA	32	7	2000	33.7 (± 3.9)	3.1 (± 0.3)	3-4			7					7
Barents Sea	BAR	408	273	2001	52.6 (± 11.6)	4.9 (± 1.1)	3-7			2	1	270			
Varanger	VAR	108	76	2001	51.0 (± 11.2)	5.4 (± 2.1)	2-11	52	15		7	2	52	14	9
Nordkapp	NOK	152	150	2001	46.6 (± 7.1)	4.1 (± 0.9)	3-7	18	11	1	26	94	15	58	73
Porsanger	POR	49	41	1999	58.0 (± 5.1)	6.8 (± 2.2)	4-13	25	10		5	1	13	22	3
Balsfjorden	BAL	309	230	2000-2001	45.5 (± 7.7)	5.3 (± 1.5)	2-10	158	41		25	6	73	97	35
Vestfjorden, West	VEW	48	40	2001	60.7 (± 7.1)	5.0 (± 0.9)	3-7	38			1	1			
Vestfjorden, East	VEE	71	70	2000	57.8 (± 3.8)	4.3 (± 0.9)	3-7	67		1		2			
Total		1177	887		50.5 (± 10.3)	4.9 (± 1.5)									

Table 2. Correct jackknifed classification success of the linear function discriminant analysis between stocks according to the otolith reader types (see Table 1 for codes), based on univariate otolith measurements and indices and the Elliptical Fourier Descriptors (EFDs) of the first 10 harmonics.

Stock (otolith types)	n	Univariate only	EFDs only	Univariate + EFDs
NCC (1+2)	435	74%	87%	88%
NEAC (4+5)	441	78%	80%	84%
NCC (1)	358	73%	90%	89%
NEAC (5)	376	80%	86%	90%

Table 3. Jackknifed classification matrix of the linear function discriminant analysis between otolith reader types (see Table 1 for codes), based on the Fourier descriptors of the first 10 harmonics. The percentages in rows represent the classification into the otolith reader types given in columns, sample sizes are given in parentheses. Overall classification success is 57.0%, Wilks' $\lambda = 0.4092$.

Otolith reader types	Classification by Fourier descriptors									
	1		2		4		5		Total	
1	**50.8**	(182)	26.3	(94)	17.3	(62)	5.6	(20)	100.0	(358)
2	36.4	(28)	**31.2**	(24)	24.7	(19)	7.8	(6)	100.0	(77)
4	26.2	(17)	32.3	(21)	**21.5**	(14)	20.0	(13)	100.0	(65)
5	5.6	(21)	5.1	(19)	14.9	(56)	**74.5**	(280)	100.0	(376)

Table 4. Jackknifed classification matrix of the linear function discriminant analysis between sampling regions (see Figure 1), based on the Fourier descriptors of the first 10 harmonics. The percentages in rows represent the classification into the sampling regions of Norwegian coastal cod (otolith reader type 1) given in columns, sample sizes are given in parentheses. Overall classification success is 74.0%, Wilks' $\lambda = 0.2764$.

Sampling regions	Classification by Fourier descriptors							
	Finnmark		Balsfjorden		Vestfjorden		Total	
Finnmark	**60.0**	(57)	16.8	(16)	23.2	(22)	100.0	(95)
Balsfjorden	15.8	(25)	**81.0**	(128)	3.2	(5)	100.0	(158)
Vestfjorden	20.0	(21)	4.8	(5)	**75.2**	(79)	100.0	(105)

Table 5. Jackknifed classification matrix of the linear function discriminant analysis between genetic types (see Table 1 for codes), based on the Fourier descriptors of the first 10 harmonics. The percentages in rows represent the classification into the types given in columns, sample sizes are given in parentheses. Overall classification success is 56.0%, Wilks' $\lambda = 0.5171$.

Genetic types	Classification by Fourier descriptors							
	$Pan\ I^{AA}$		$Pan\ I^{AB}$		$Pan\ I^{BB}$		Total	
$Pan\ I^{AA}$	**58.2**	(89)	28.8	(44)	13.1	(20)	100.0	(153)
$Pan\ I^{AB}$	29.5	(56)	**51.1**	(97)	19.5	(37)	100.0	(190)
$Pan\ I^{BB}$	11.8	(14)	26.1	(31)	**62.2**	(74)	100.0	(119)

Figure legends

Fig. 1. Otolith thin-section pictures of typical Norwegian coastal cod (upper picture) and Northeast Arctic cod (lower picture). The length of the white bars represents 1 mm of the original otolith size.

Fig. 2. Map showing the sampling stations for Norwegian coastal cod and Northeast Arctic cod.

Fig. 3. Length- and age-specific means (error bars represent ±1 standard deviation) of fish weight (a, b), otolith weight (c, d), and the otolith length/width ratio (e, f) of Norwegian coastal cod (NCC, grey circles) and Northeast Arctic Cod (NEAC, black triangles). Asterisks denote significant differences between means ($p < 0.05$, one-way ANOVA).

Fig. 4. Linear discriminant analysis scores for the classification of Norwegian cod by otolith reader types (see Table 1 for codes), based on Fourier descriptors of their otolith shapes. The first discriminant axis explains 92.6%, the second axis 5.2% of the variation.

Fig. 5. Linear discriminant analysis scores for the classification of Norwegian coastal cod by sampling regions (FIN = Finnmark, including Varanger, Nordkapp and Porsanger; BAL = Balsfjorden; VES = Vestfjorden, West and East), based on Fourier descriptors of their otolith shapes. The first discriminant axis explains 79.7%, the second axis 20.3% of the variation.

Fig. 6. Upper panel: Average shapes of Norwegian coastal cod (otolith type 1) and Northeast Arctic cod (otolith type 5) by otolith reader types. Lower panel: Average shapes of Norwegian coastal cod by sampling regions.

Fig. 7. Linear discriminant analysis scores for the classification of Norwegian cod by genetic types, based on Fourier descriptors of their otolith shapes. The first discriminant axis explains 79.2%, the second axis 20.8% of the variation.

Fig. 8. (a) Relative distribution of the three Pan *I* genetic types within groups of Norwegian coastal cod (NCC) and Northeast Arctic cod (NEAC), as typed by otolith readers (see Table 1 for codes). (b) Relative distribution of these four otolith types within each of the three Pan *I* genetic types, i.e., Pan I^{AA} homozygotes, Pan I^{BB} homozygotes, and Pan I^{AB} heterozygotes. Numbers of individuals are given above each group of bars.
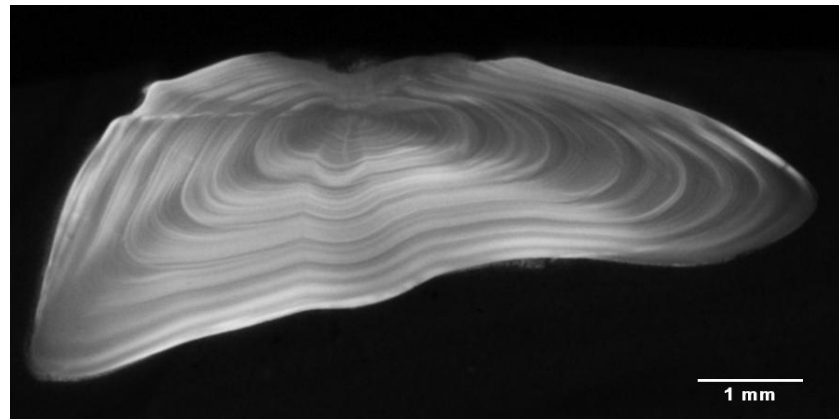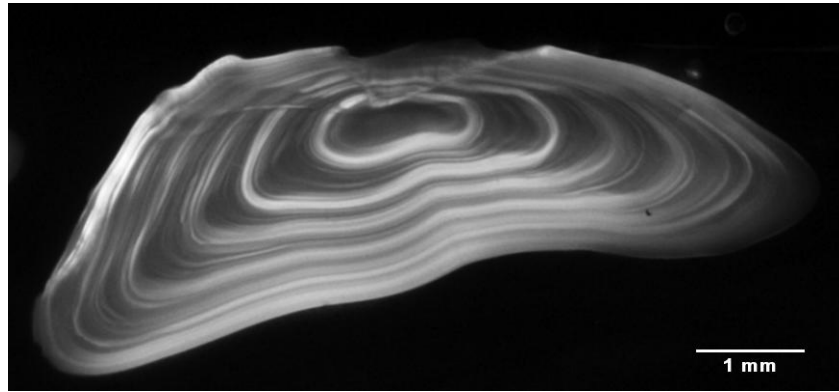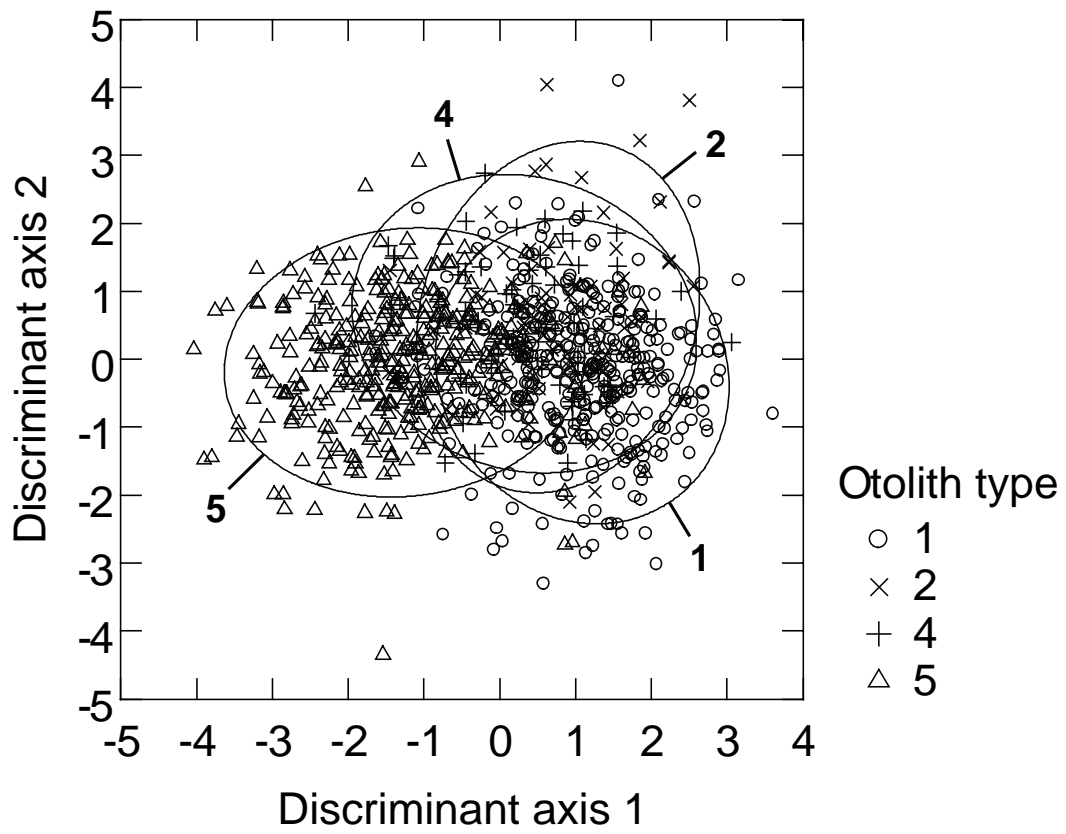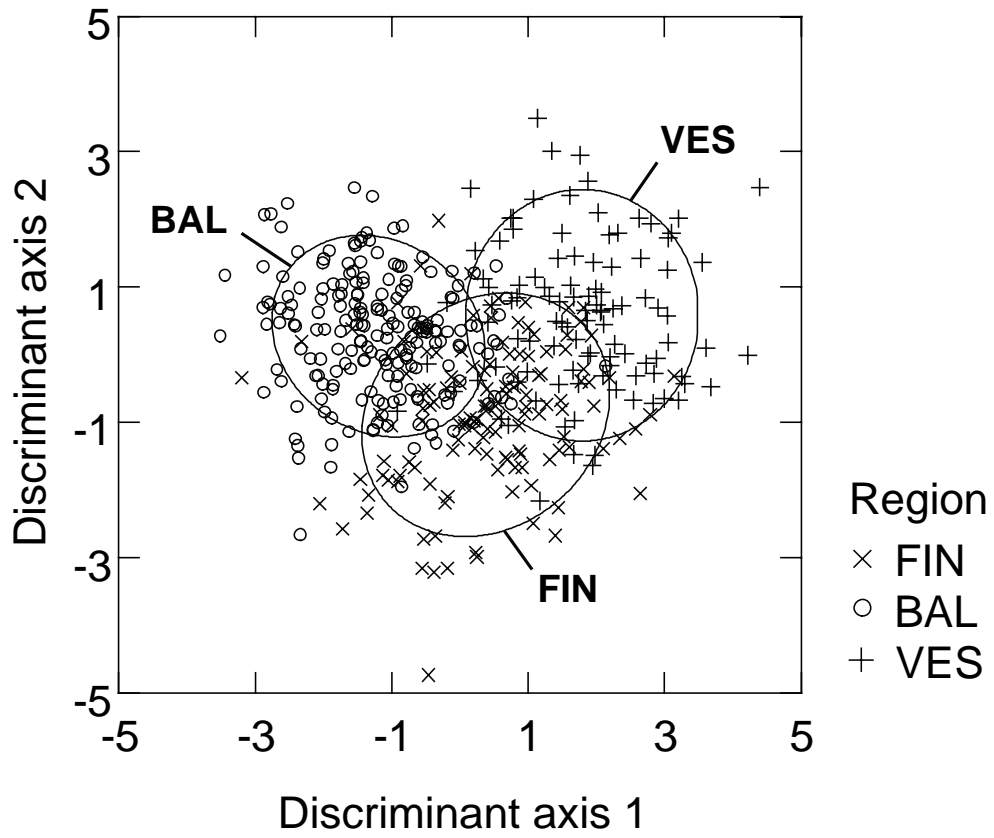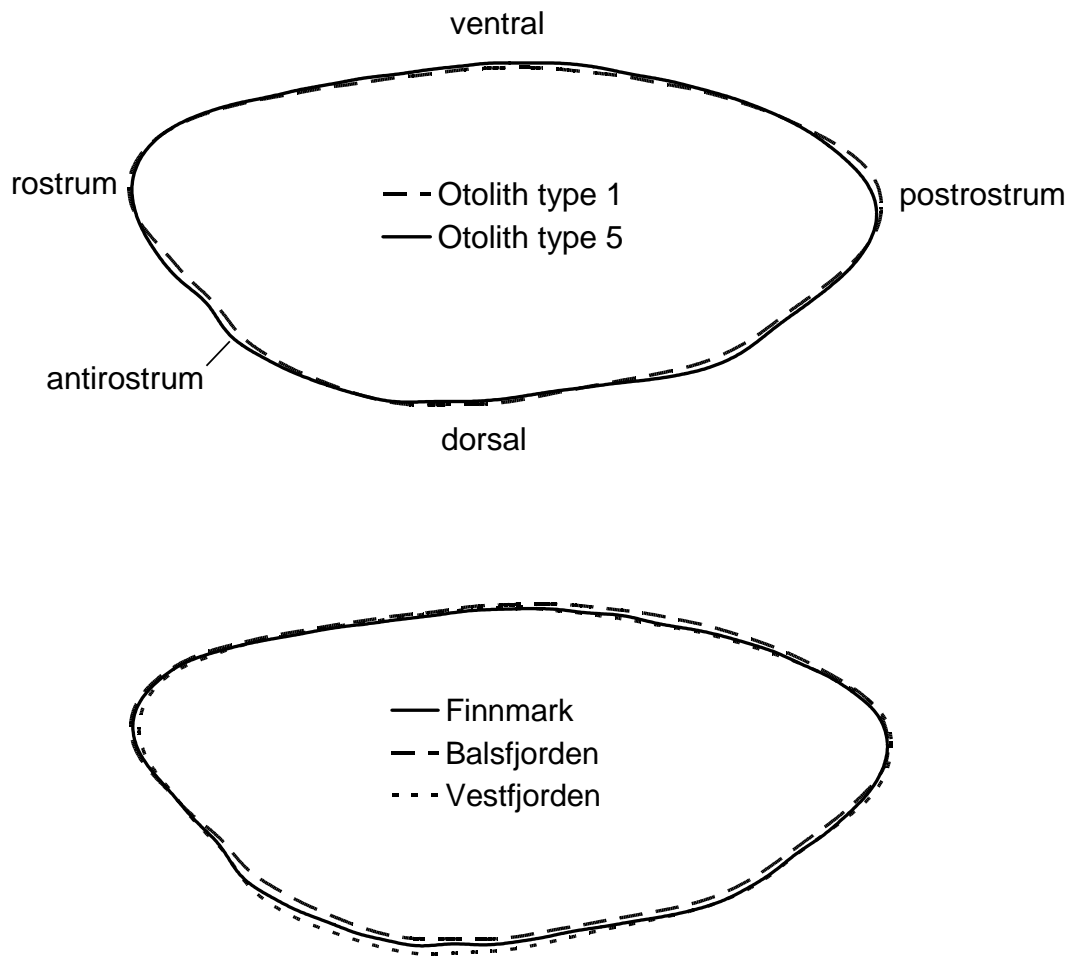
Figure 1.
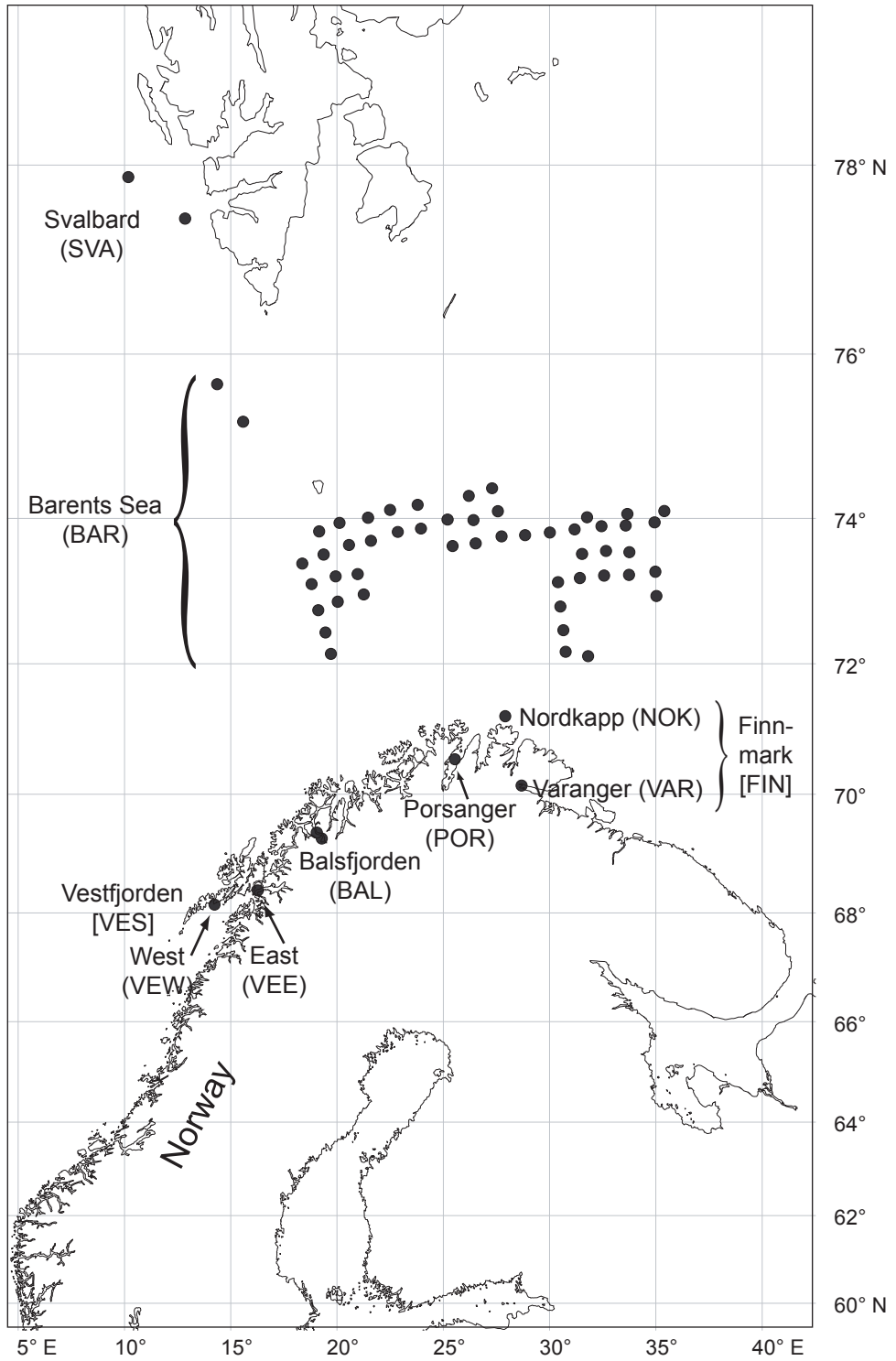
Figure 2.
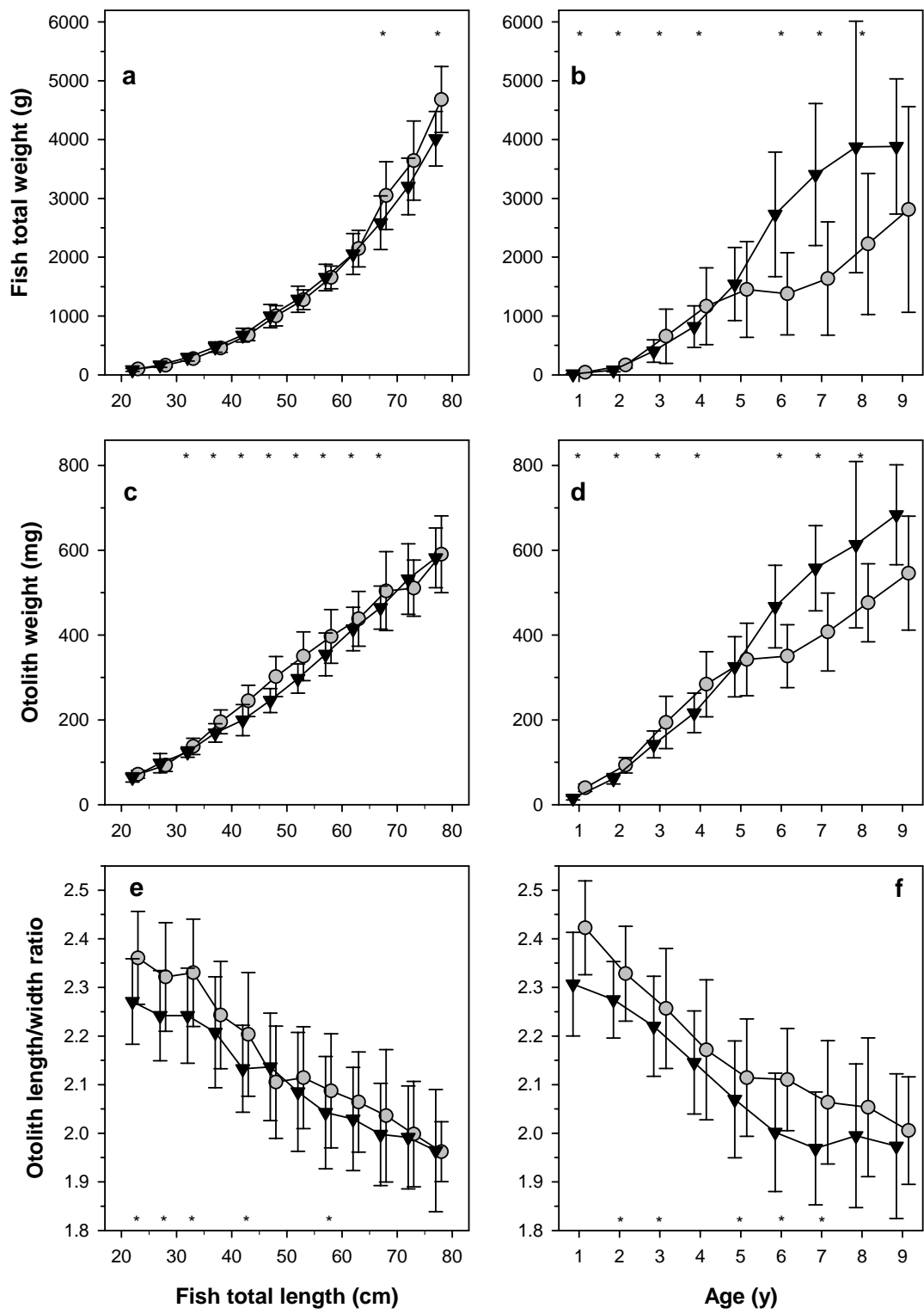
Figure 3.

Figure 4.

Figure 5.

ventral

rostrum

- - Otolith type 1
—— Otolith type 5

postrostrum

antirostrum

dorsal

—— Finnmark
- - Balsfjorden
- - - Vestfjorden

Figure 6.
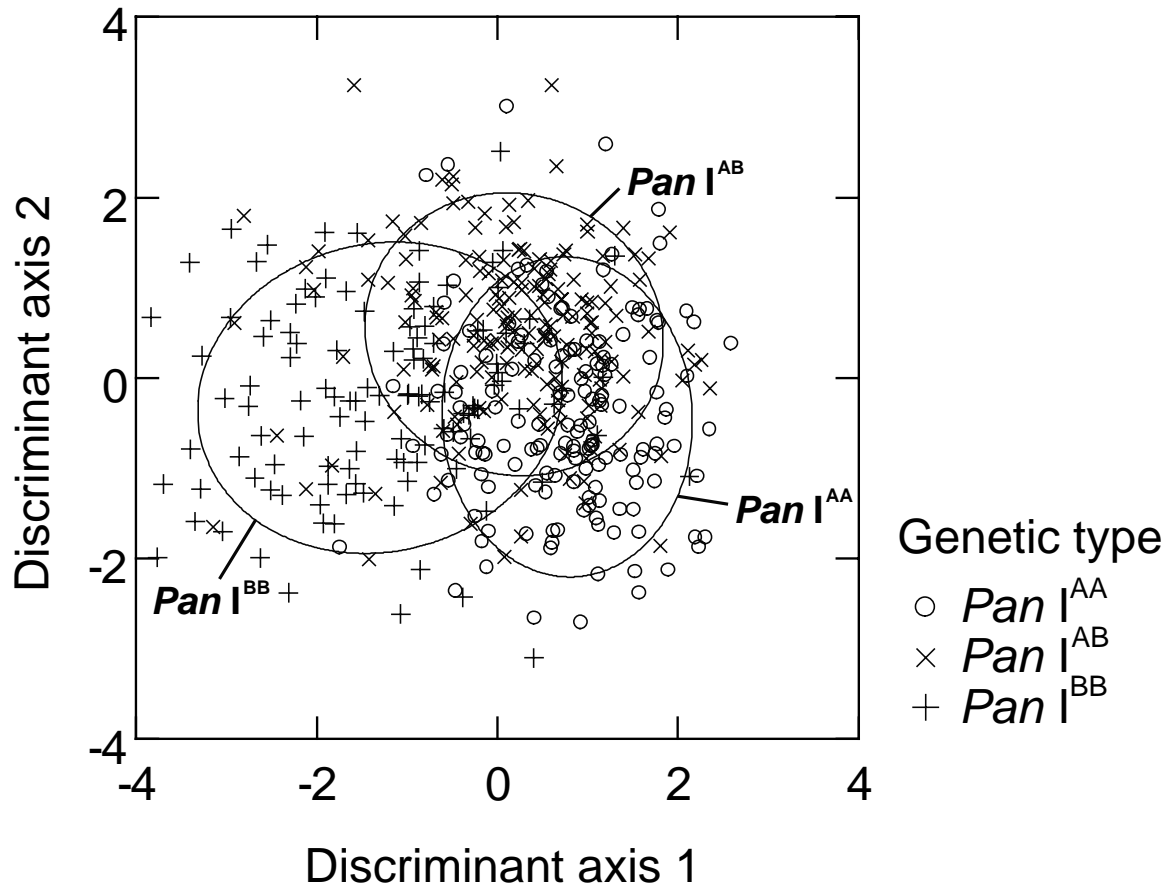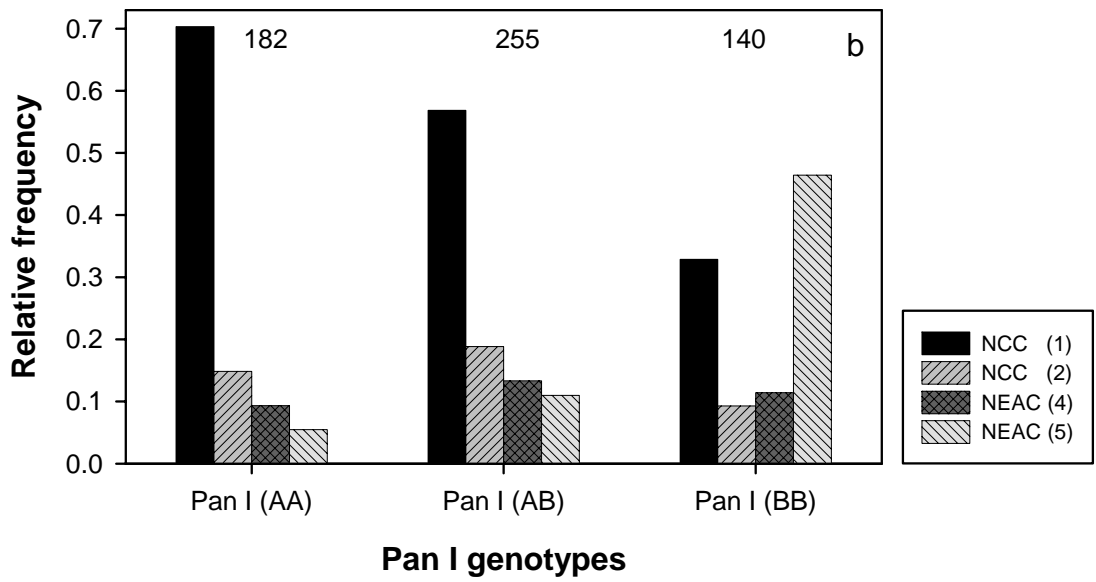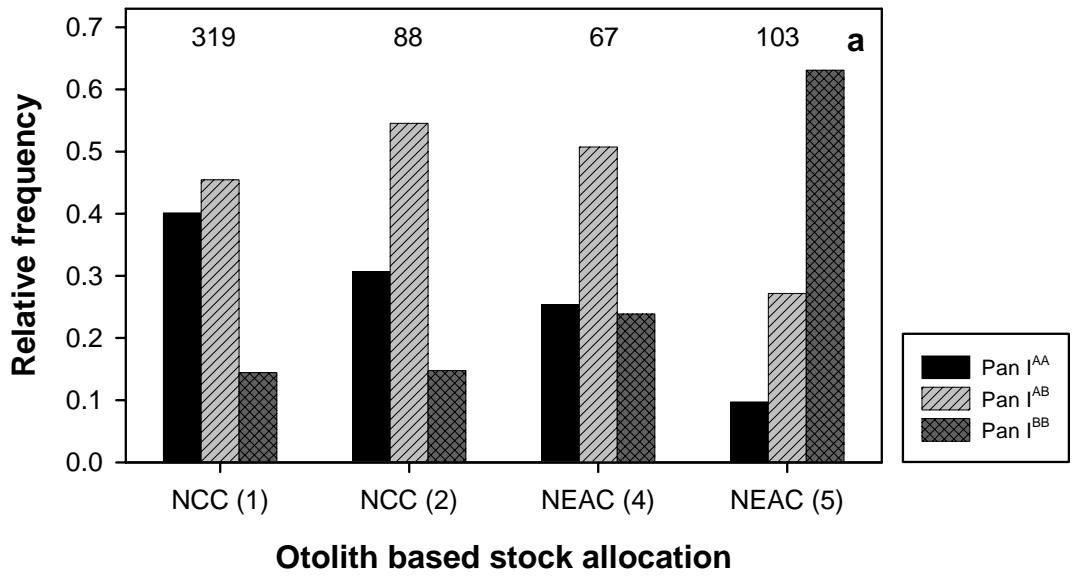
Figure 7.

Figure 8.

Annex 1:

**Automatic cod otolith core prediction [<u>work in progress</u>]**

The following procedure is an ad hoc approach where the predicted otolith core (nucleus) appears to lie within the first year zone (annulus) in a varied test sample of 312 otoliths.

1. Find the two most apart contour points P_1 and P_2 to define horizontal axis, $P_1 = (x_1,0)$ and $P_2 = (x_2,0)$.
2. Find area centroid (x_c,y_c) of domain delimited by contour.
3. Find the contour point (x_3,y_3) furthest apart from x-axis on the vertical line through the centroid.
4. Calculated distance $\Delta y = |\,y_c - y_3\,|$.
5. Predict core $C = (C_{px},C_{py})$ with $C_{px} = x_c$ and $C_{py} = y_c \pm 5\Delta y/12$, where the sign is chosen so that C is further apart from the x-axis than (x_c,y_c).

Automatic prediction of otolith core, (C_{px}, C_{py})
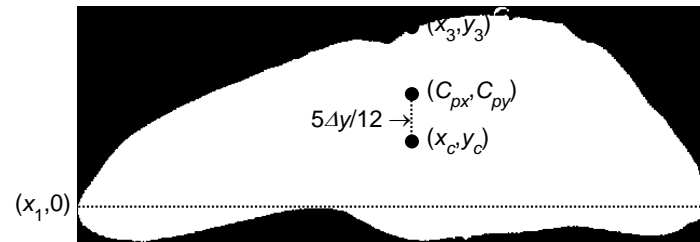
1) x axis defined by most apart contour points

$(x_1,0)$ $(x_2,0)$
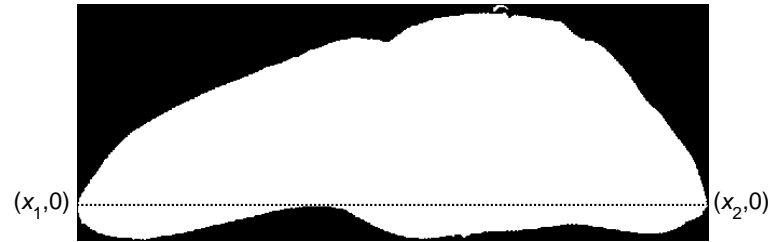
2) Area centroid (x_c, y_c)

$(x_1,0)$ • (x_c, y_c)
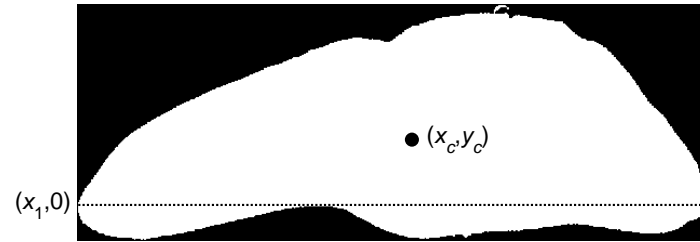
3) Contour point (x_3, y_3) and distance Δy

$\Delta y \rightarrow$
(x_3, y_3)
• (x_c, y_c)
$(x_1,0)$ $(x_2,0)$

4) Automatically predicted core (C_{px}, C_{py})

(x_3, y_3)
• (C_{px}, C_{py})
$5\Delta y/12 \rightarrow$ • (x_c, y_c)
$(x_1,0)$
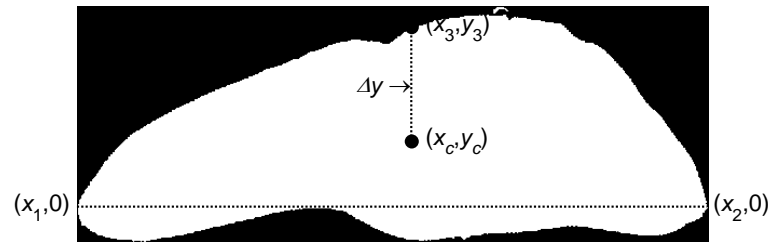
5) Automatically predicted core (C_{px}, C_{py})
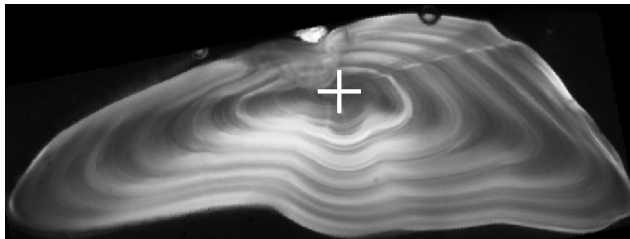
Automatic prediction of core
Ad hoc procedure
Appears to predict within 1st annual zone
test sample: 312 otoliths

Annex 2:

Automatic detection of the two innermost growth zones (annuli) in cod otolith thin-sections [<u>work in progress</u>]

After photographing the cod otolith thin-sections with transmitted light (Figure A2.1) and reflected light (Figure A2.2), the otolith core (nucleus) position is estimated (Figure A2.3) using the routine described in Annex 1. A polar plot (Figure A2.4) is constructed using the estimated otolith core and the boundary of the reflected light image. Each angle slice (a vertical line in the polar coordinate image) is analyzed, and an estimate of the derivative function of each angle slice is calculated. Using the derivative function, the peaks (year zones) are identified. The peaks are then clustered using several criteria, and the two innermost rings are identified (Figure A2.5).

Using the scaling parameter criterion suggested in Berg et al. (2005), the estimated scaling parameter is 1.65. In Berg et al. (2005), a threshold at 1.75 was established, where the otoliths with a scaling parameter larger than 1.75 were classified as NCC. Since the estimated scaling parameter is 1.65 for the otolith example in this Annex, it is classified as NEAC.
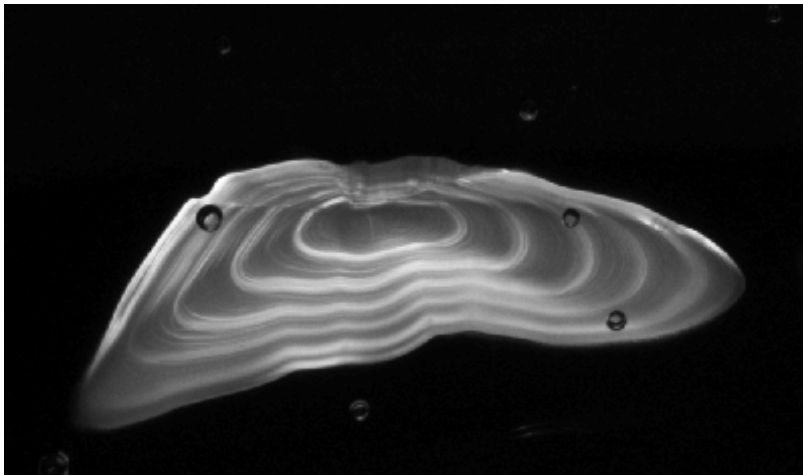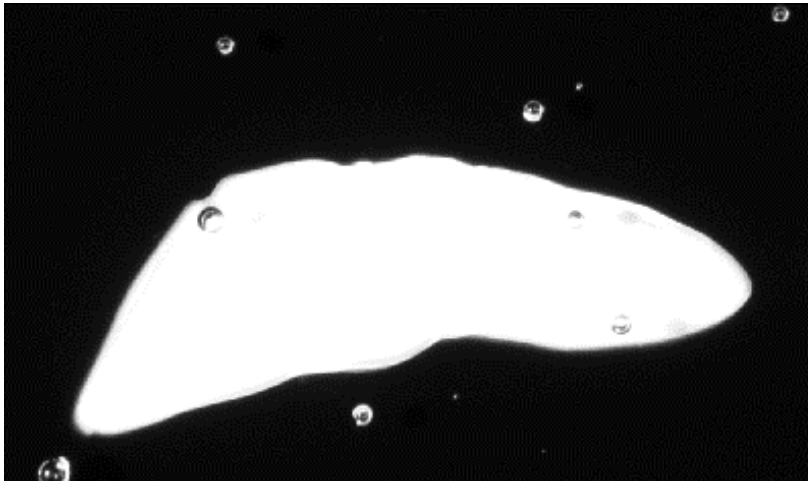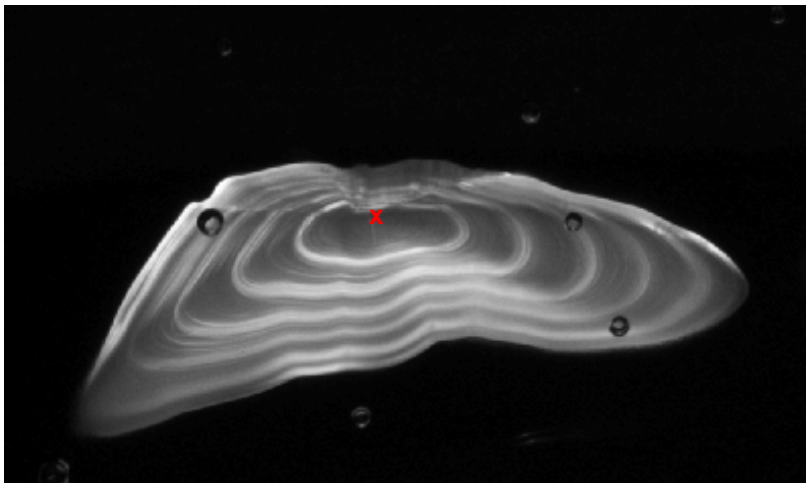

Figure A2.1: Cod otolith thin-section photographed using transmitted light.

Figure A2.2: Cod otolith thin-section photographed using reflected light. This image is used to extract the boundary of the otolith.

Figure A2.3: Cod otolith thin-section. The red cross denotes the detected otolith core using the automatic routine described in Annex 1.
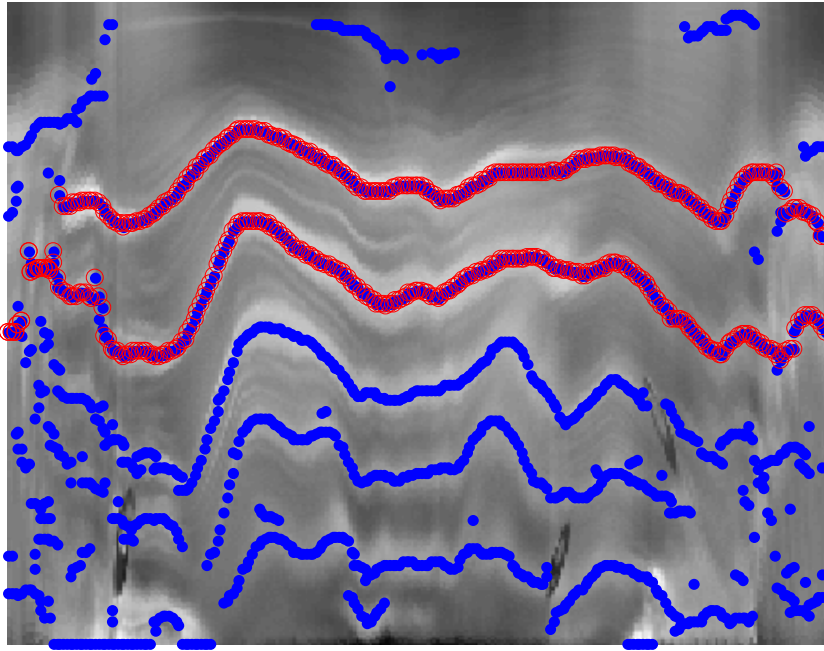
Figure A2.4: The otolith image in polar coordinates. The vertical axis is the distance from the otolith core, and the horizontal axis is the angle. The blue dots denote detected peaks in the image, and the red circles denote the detected two innermost rings.
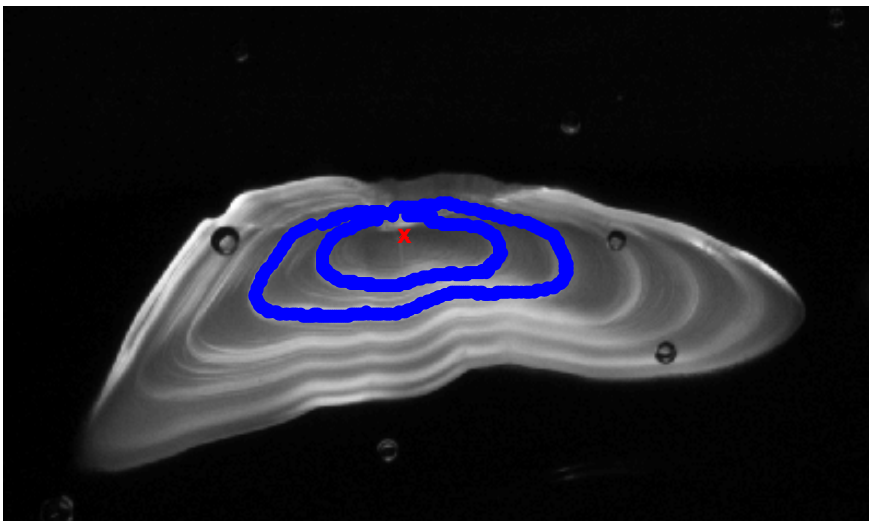

Figure A2.5: The detected two innermost rings (blue lines) and the otolith core (red cross).