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Using Otolith Morphometrics to Separate Small Walleye Pollock *Theragra chalcogramma* from Arctic Cod *Boreogadus saida* in Mixed Samples

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Abstract: Species identification errors occasionally occur when collecting biological information and ageing structures. Therefore, it is useful to have the ability to distinguish between species using only otoliths. Otolith morphometrics (area, perimeter, length, width, and number of scallops) and fish fork length were used to distinguish between walleye pollock *Theragra chalcogramma* and Arctic cod *Boreogadus saida* measuring between 8 and 20 cm caught in the eastern Bering Sea. Discriminant analysis of otolith morphometrics and fork length correctly classified verified walleye pollock and Arctic cod with 99% accuracy. The number of scallops and otolith area were the most effective individual otolith characteristics for distinguishing between species. Otolith shape analysis using Fourier methods with fork length was also attempted and was found to be less effective (94% accuracy) than otolith morphometrics and fork length combined. The discriminant functions were applied to an unverified production-aged juvenile walleye pollock sample from the eastern Bering Sea. Based on length-at-age of some specimens, it was thought that this sample might be contaminated with Arctic cod. Twelve of the 154 (8%) production-aged specimens were classified as Arctic cod by the best of these discriminant functions; however, none of the aged specimens displayed strong Arctic cod characteristics.

INTRODUCTION

Walleye pollock *Theragra chalcogramma* is an abundant species in the Bering Sea, which supports one of the largest single species commercial fisheries in the world. Stock assessment for this species relies upon age information generated from visual inspection of otoliths. Production-aged specimens collected in 1999 gave an older age-at-length than expected in walleye pollock measuring between 8 and 20 cm. Fourteen specimens with fork lengths between 10 and 15 cm that are typically assigned an age of 1 year appeared to have 2 or 3 growth marks, suggesting an age of 2 or 3 years. Possible causes for this growth pattern include 1) at-sea species misidentification, 2) unusual environmental conditions resulting in non-annular growth checks, 3) unusual environmental conditions that slowed fish growth, or 4) late-spawned walleye pollock with less than expected first-year growth. This research explores the possibility that a slower-growing gadid was misidentified as walleye pollock, which could result in an older age-at-length when counting annular rings. Although only a small percentage of the ageing specimens displayed an unusual growth pattern, species identification could be a widespread problem that would affect stock assessment of walleye pollock.

The species most similar in appearance to juvenile walleye pollock is Arctic cod *Boreogadus saida*. Arctic cod are slower growing and have a range that overlaps with walleye pollock. Walleye pollock are distributed from the north Bering Sea to central California (Hart, 1973), and Arctic cod are circumpolar in distribution and typically range into the Polar Sea south to the Pribilof Islands and northern Bristol Bay (Lowry and Frost 1981). The range of the 2 species overlaps in the eastern Bering Sea from the southern extent of the Arctic cod range north to Saint Lawrence Island. Walleye pollock less than 20 cm are typically 2 years or younger, whereas Arctic cod at 20 cm could be as old as 5 years (Lowry and Frost, 1981).

Using verified samples of walleye pollock and Arctic cod, we attempted to build a discriminant function for distinguishing these two similar species from their otoliths when the whole fish is not available for identification.

The possibility that walleye pollock were being misidentified as other gadid species was also investigated. Examinations of juvenile Pacific cod *Gadus*

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macrocephalus and saffron cod *Eleginus gracilis* indicated that otolith morphological differences described in Frost (1981) sufficiently distinguished these species for specimens less than 20 cm in length.

METHODS

Our verified collection is comprised of samples collected where the 2 species both occur, with the exception of 39 walleye pollock taken in southeast Bristol Bay. Specimens were collected using bottom trawl gear from 1998 to 2000 in the Eastern Bering Sea as part of the Alaska Fisheries Science Center's (AFSC) annual groundfish surveys (Figure 1). Otoliths (sagittae) from 264 walleye pollock and 199 Arctic cod were removed. Verified walleye pollock specimens ranged from 6.6 to 19.9 cm fork length and verified Arctic cod ranged from 9.4 to 19.8 cm fork length (Table 1). Whole fish were collected and frozen in the field, then thawed in the laboratory where field identification was confirmed using pyloric caeca counts, fork length was recorded to the nearest millimeter, and otoliths were removed.

A discriminant function estimated in this study was used to separate the 1999 production-aged walleye pollock specimens, possibly contaminated with Arctic cod. These unverified aged samples relied on the at-sea identification by AFSC survey scientists. Data collected with the unverified sample included fish length, weight, otolith characteristics, and haul data. Fork length of unverified specimens was measured to the nearest centimeter.

Otolith scallops were visually counted on the distal (or convex) side using a binocular microscope and reflected light. A scallop was defined as a groove on the otolith perimeter that extends up the distal side

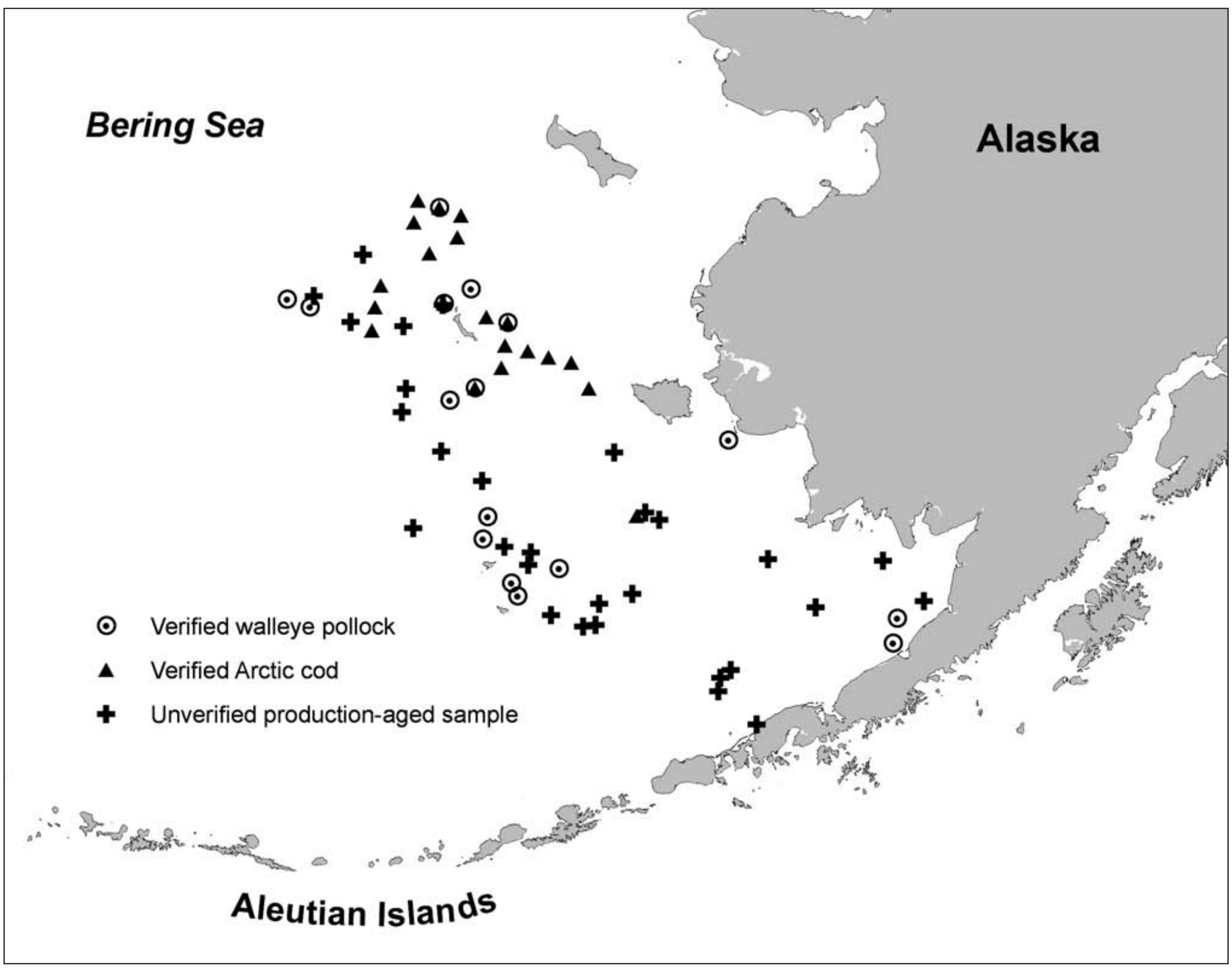

Figure 1. Collection sites for verified walleye pollock and Arctic cod (1998 and 2000) and unverified production-aged sample (1999).

Table 1. Length distributions of verified walleye pollock, verified Arctic cod, and unverified production-aged sample.

Collection	N	Mean length (cm)	Length range (cm)
Verified Arctic cod	199	13.7	9.4–19.8
Verified walleye pollock	264	12.9	6.6–19.9
Unverified production-aged sample	153	13.5	8.0–20.0

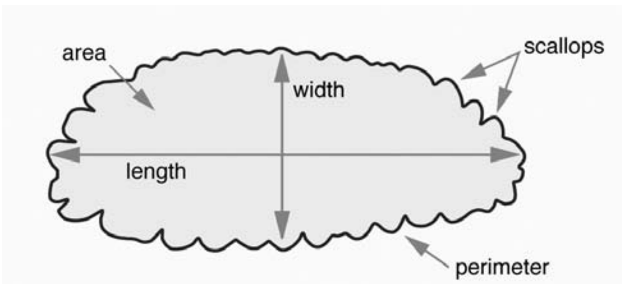

Figure 2. Otolith morphometrics viewed on a sagittal otolith.

(Figures 2 and 3). When both otoliths were available, scallop counts were averaged between the right and left otoliths.

Otolith area, perimeter, length, and width were measured using a digital camera on a dissecting microscope at 6X magnification under reflected light. Optimas 6.5[1] software was used to capture the image and to make and record otolith measurements and Fourier coefficients. Unless it was broken or missing, the left otolith was used for otolith measurements and Fourier analysis. Fourier coefficients describe the shape of an object as well as its topography by using radial measurements that can be statistically compared. In this study, 64 Fourier coefficients were recorded for each specimen.

[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Otolith shape morphometrics (area, perimeter, length, width, and number of scallops), Fourier shape analysis, and fork length were analyzed with a predictive discriminant function. Several discriminant functions were created using different combinations of variables to maximize classification accuracy.

Shape analyses using otolith morphometrics (area, perimeter, length, width, and number of scallops) were quantified and Fourier analysis was performed to determine if species-specific indicators could be used to identify similar fish species (Figure 2). Similar work was previously performed by Campana and Casselman (1993) to distinguish stocks of Atlantic cod *Gadus morhua* and by Rybock et al. (1974) to separate juvenile steelhead trout from rainbow trout.

Classification of species was accomplished using linear discriminant analysis with SYSTAT 10.0 software. This analysis estimates coefficients that allow computation of canonical scores which maximize separation between 2 or more groups and minimizes within group variability. The estimated canonical scores can then be used to classify subsequent samples. When there are 2 groups, a midline is calculated by averaging the group mean canonical scores and adjusted with a constant to place it at zero. A positive canonical score would classify that individual as species A, and a negative score would indicate species B. Jack-knifed classification was used to cross-validate prediction accuracy (SYSTAT 2000). Discriminant analysis assumes that a verified sample with the species correctly identified is available and that the distribution of the data is normal (SYSTAT 2000).

The effectiveness of the discriminant function was measured by the classification accuracy of the discriminant analysis. Using the jack-knife, the classification accuracy is the percentage of samples that are grouped back into their respective source groups by the canonical scores of the discriminant analysis. Log transformation of otolith area and perimeter was

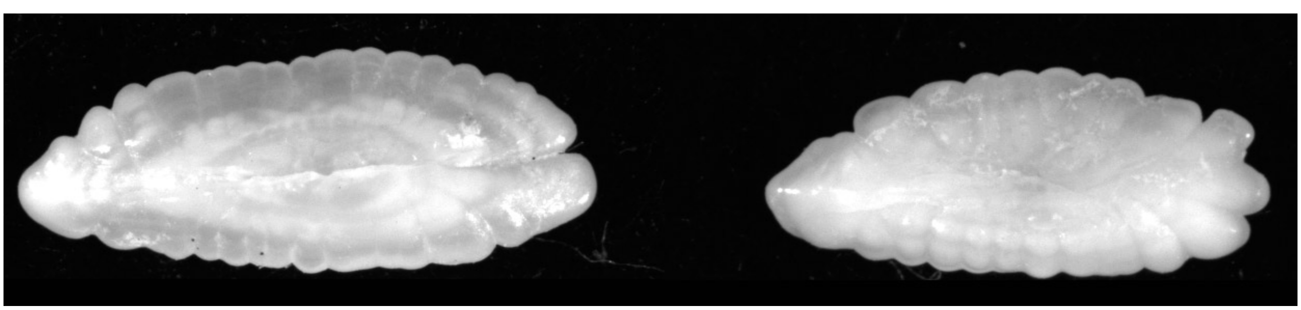

Figure 3. A walleye pollock (left) and Arctic cod (right) otolith at 6.3X magnification. Fork length and otolith length is 136 mm and 6.5 mm for the walleye pollock and 134 mm and 5.7 mm for the Arctic cod.

attempted, but did not improve classification accuracy. Once the equation was estimated, the production-aged sample was applied to the function to classify each specimen as walleye pollock or Arctic cod.

The variables analyzed in the discriminant function include fork length, otolith morphometrics (area, perimeter, length, width, and number of scallops), and Fourier coefficients. Otolith morphometrics were ranked by the "F-to-remove" generated from the discriminant analysis. The production-aged sample was classified by multiplying the fork length and otolith characteristics by the corresponding best-fit discriminant coefficients.

RESULTS

Six combinations of otolith morphometrics and fork length (Models 1-6) were analyzed on verified samples with the discriminant function. All 6 models were accurate to 94% or greater (Table 2), but the highest classification accuracy (99%) was with otolith area, number of scallops, and fork length (Model 1) (Figure 4). Individual otolith characteristics (length, width, area, perimeter, and scallop count) provided notable separation between species (Figure 5). Classification accuracy diminished slightly when otolith length, width, and perimeter were added to the discriminant function (Model 2). Scallop count was the most effective otolith characteristic for distinguishing the two species based on "F-to-remove" (Table 3). Walleye pollock were found to have a greater number of scallops than Arctic cod for a given fork length. Fork length was also an important variable, although 95% classification accuracy was achievable without fork length in the discriminant analysis by using scallops, area, length, and width (Model 5).

Table 2. Discriminant function equations for otolith morphometrics and classification accuracy. The canonical score (CS) was determined by summing the variables times the canonical coefficient (CC_n): $CS = FL \times CC_{FL} + Scallop \times CC_{Scallop} + Area \times CC_{Area} + Perimeter * CC_{Perimeter} + Major \times CC_{Major} + Minor * CC_{Minor} + Constant$.

	Canonical coefficients							
Model	Fork length (FL)	Scallop	Area	Perimeter	Length	Width	Constant	Accuracy
1	0.114	–0.175	–0.562				–4.526	99%
2	0.114	–0.171	–0.690	–0.226	0.201	3.691	–7.554	98%
3	0.110	–0.183			–2.027	0.479	0.507	96%
4	0.040	–0.285					1.524	95%
5		0.305	0.705		–1.313	–5.764	6.711	95%
6	0.157				–3.392		–0.751	94%

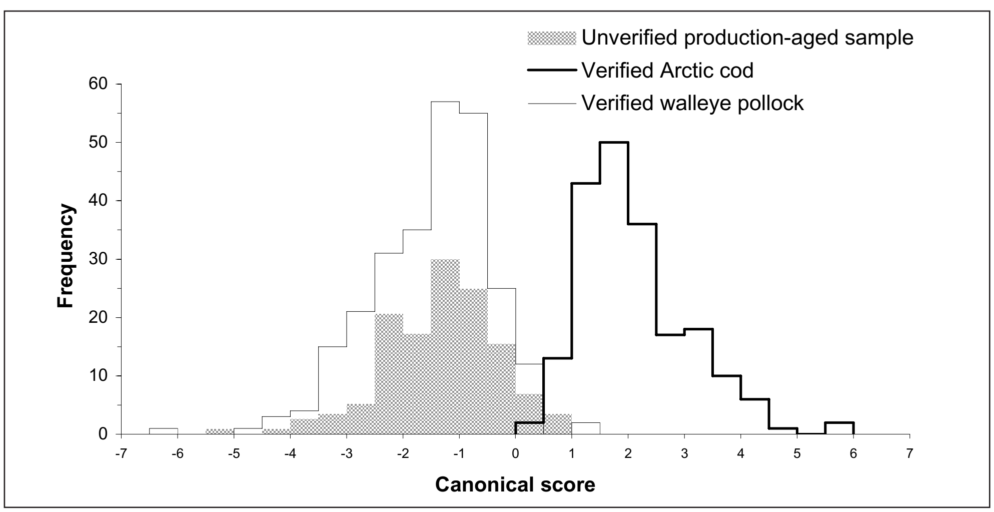

Figure 4. Canonical scores of verified walleye pollock, verified Arctic cod, and unverified production-aged samples. Variables using Model 1 include fork length, otolith area, and scallop count.

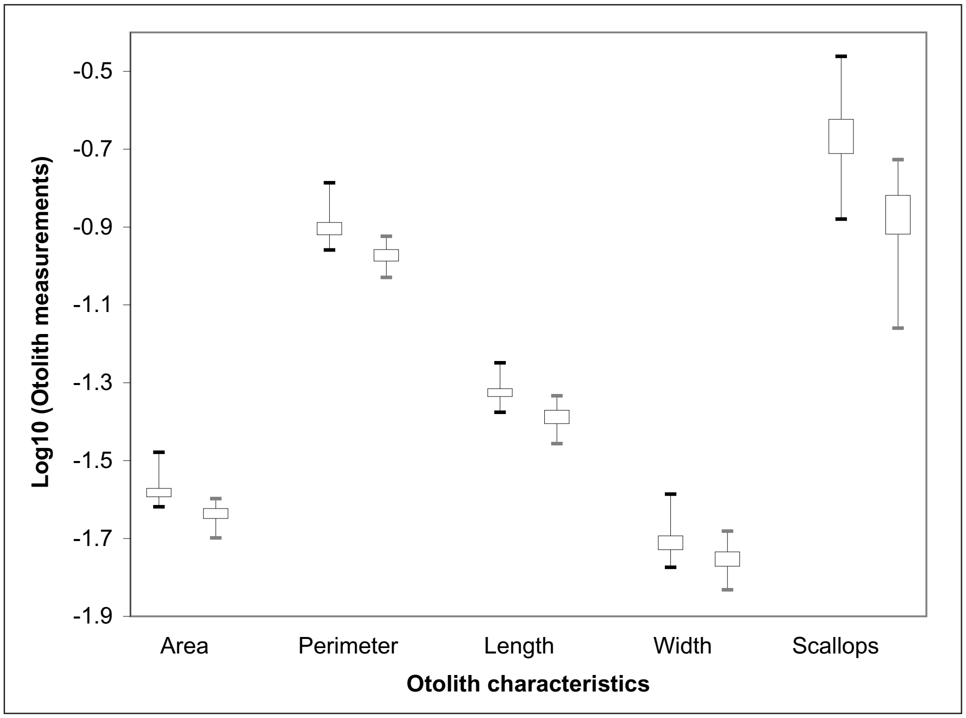

Figure 5. Otolith morphometrics (mean, minimum, and maximum) for verified walleye pollock (black) and Arctic cod (gray) corrected for fork length and log-transformed. The transformation used on a morphometrics measurement X was $Y = \log_{10}(X/FL)$ except for area: $Y = [\log_{10}(X/FL^2)]/2$. The $\log_{10}$ of the area was divided by 2 to fit all the otolith characteristics on one box plot graph.

Table 3. Individual ranking of otolith morphometrics from discriminant analysis. The following morphometrics were analyzed with fork length for comparison.

Variable	Rank	F-To-Remove	Tolerance	Accuracy
Scallops	1	1035	0.446	95%
Area	2	882	0.050	96%
Length	3	848	0.047	94%
Perimeter	4	841	0.060	96%
Width	5	291	0.086	82%

Linear discrimination with Fourier shape analysis accurately distinguished walleye pollock and Arctic cod 72% of the time. Accuracy improved to 94% when Fourier methods were coupled with fork length.

The most accurate linear discriminant function developed from verified samples (Model 1) was applied to 154 unverified production-aged walleye pollock specimens. The function classified 142 specimens (92%) as walleye pollock, and none of the 12 specimens classified as Arctic cod demonstrated strong Arctic cod characteristics (Figure 4). The canonical scores of the production-aged sample displayed a normal distribution similar to the distribution of verified walleye pollock.

DISCUSSION

Verified walleye pollock and Arctic cod were separated with very high accuracy using otolith morphometrics. The highest accuracy was achieved when otolith area, scallop, and fork length were included in the discriminant function (Model 1), but significant separation is possible with linearly measured and visibly countable characteristics. Decisions about species identification can be made using fork length, scallop count, and otolith length and width (Models 3 or 4), which can be quantified without an image analysis system. Separation can still be achieved for situations where fork length is not available by using otolith scallops, area, length, and width measurements (Model 5) in the discriminant function.

It was hoped that 64 Fourier shape descriptors would provide enough precision to recognize the difference in scalloping; however, Fourier analysis with fork length was less accurate (94%) than otolith dimensions and fork length. The otolith outlines of both species are similar, which may explain why discrete otolith measurements were a more efficient distinguisher than the Fourier analysis. Doubling the number of Fourier descriptors may improve edge precision so that scallop variability is better defined.

Other otolith morphometrics are useful for visual identification of the species but are not pronounced in juvenile specimens and therefore were not included in our analysis. For example, walleye pollock otoliths are slightly curved and have a pronounced caudal groove, whereas Arctic cod otoliths are flat and lack a caudal groove (Frost 1981; W. Walker, National Marine Fisheries Service, Seattle, personal communication). The quantitative method of species distinction in this study may be helpful in other applications of fish identification where key otolith characteristics cannot be identified by visual inspection, such as very small or degraded otoliths.

The canonical scores of the production-aged sample suggest it is unlikely that Bering Sea walleye pollock are misidentified as Arctic cod on a widespread basis (Figure 4). Of 154 specimens less than 20 cm fork length, 142 of 154 (92%) were classified as walleye pollock, and samples classified as Arctic cod were close to the canonical score midline and could be a result of normal variability.

Of the 154 specimens in the production-aged sample, 14 walleye pollock specimens were excluded from the Bering Sea walleye pollock ageing database because of observed irregular growth patterns. The discriminant function classified 13 of these 14 specimens as walleye pollock. The one specimen classified as an Arctic cod displayed moderate Arctic cod characteristics (CS 0.99). This specimen was collected in Bristol Bay, which is outside the typical distribution of Arctic cod, and did not exhibit clear scallop patterns.

From these results, we concluded that the initial identification of 1999 production-aged sample was correct and that unusual growth patterns observed in the aged sample were not due to species misidentification. It is possible that a small percentage of the ageing sample was contaminated with Arctic cod; however, it is unlikely to affect an age-based stock assessment.

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