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Exploring the use of otolith shape analysis to identify the stock spatial structure of dusky rockfish (*Sebastes variabilis*)

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Abstract

Dusky rockfish (*Sebastes variabilis*) is a commercially valuable groundfish species in Alaska waters, with its highest abundance and fishery catch occurring in the Gulf of Alaska (GOA), and lesser abundance and catch occurring throughout the Aleutian Islands and southeastern Bering Sea. Despite its commercial importance, information regarding stock structure of dusky rockfish has been data-limited. In this study, otolith shape analysis was used to evaluate the stock structure of dusky rockfish across five geographical subareas exhibiting ecological differences in the GOA and Bering Sea and Aleutian Islands (BSAI), where dusky rockfish are managed as two separate stocks. A combination of size and shape indices, wavelet and elliptic Fourier descriptors, were examined from left and right-side otoliths collected from these regions (n = 522). Individual variation existed across subareas. Wavelet and elliptic Fourier descriptors indicated that mean otolith shapes were partitioned between the two management regions but also showed a high degree of overlap among subareas. Classification accuracies of otoliths to their subarea of origin through linear discriminant analysis (LDA) were variable (6.3% to 73.5% and 15.4% to 65.8% correctly classified for the elliptic Fourier and wavelet analyses, respectively). The highest classification rates were found between the western GOA and eastern Aleutian Islands, contributing to the observed differences between management regions and providing some support for current management paradigms. Dusky rockfish exhibited low to moderate overall classification rates (43.9% to 52.2%), suggesting minimal stock structure within Alaska waters. This study highlights the utility of otolith shape analysis as a stock discrimination tool, and results will help refine further investigations and support fishery management in Alaska.

Keywords: Dusky rockfish, *Sebastes*, otolith shape analysis, population structure, stock assessment

1. Introduction

Knowledge of stock structure is critical to understanding population biology and dynamics, and is necessary for effective sustainable fisheries management. According to Hilborn and Walters (1992), stocks are defined as homogenous populations of fish, with individuals of these populations having similar life history characteristics. However, while the appropriate definition of a stock has remained a challenge to management (Cadrin et al., 2014), its concept remains fundamental to stock assessment and fisheries management. Implementing appropriate stock structure and spatial extent within assessments and fisheries management can, at least in principle, sustain productive fisheries, whereas ignoring or misspecifying stock structure can have potentially deleterious effects, including overfishing or failure to detect declines in a latent population (see Cadrin, 2020 for a review of case studies and best practices). Identifying the appropriate stock structure draws from a suite of complementary, interdisciplinary techniques that cover multiple aspects of the life history characteristics of a fish species, which includes addressing both genetic and phenotypic variation (Begg et al., 1999).

Otolith shape analysis has been used globally to discriminate stocks or identify stock structure for a variety of marine fish to inform management (Campana and Casselman, 1993), including mulloway (*Argyrosomus japonicas*; Ferguson et al., 2011); anglerfish (*Lophius piscatorius*; Cañas et al., 2012); European anchovy, *Engraulis encrasicolus*; Bacha et al., 2014); Patagonian toothfish (*Dissostichus eleginoides*; Lee et al., 2018); blue jack mackerel, (*Trachurus picturatus*; Moreira et al., 2019); and European hake (*Merluccius merluccius*; Moralis-Nin et al., 2022). For rockfishes (*Sebastes* spp.), otolith shape and morphometric analysis has been conducted for commercially important species across their range. Otolith shape analysis involves a quantitative geometric description using methods such as elliptic Fourier analysis of two-dimensional otolith shapes (Lestrel, 1997), thus capturing biological information that can be compared between populations within or between species. Use of basic external indices that describe the otolith size or shape have been used in combination with these more complex analyses to identify stock structure of commercially important species (Ferguson et al., 2011; Mapp et al., 2017; Mahê et al., 2019; Moreira et al., 2019). In the northwest and northeast Pacific Ocean, these studies have demonstrated the importance and utility of using otolith shape among rockfishes to distinguish between species (Zhuang et al., 2015; Park et al., 2023); to identify patterns of otolith shape from sympatric species to correlate morpho-types with ecological traits (Tuset et al., 2015); and to indicate differences between potential nearshore and offshore stocks (Vaux et al., 2019).

Dusky rockfish (*Sebastes variabilis*) is a commercially valuable rockfish found along and in outer continental shelf waters of Alaska (Williams et al., 2022). The highest abundances occur in the Gulf of Alaska (GOA), with the largest biomass estimates reported in the western GOA (von Szalay and Raring, 2018; Fig. 1). In the GOA, where dusky rockfish is part of a targeted rockfish (*Sebastes* spp.) trawl fishery and assessed through statistical catch-at-age modeling, fishery catches have remained below acceptable biological catches (ABCs) and overfishing levels (OFLs;

Williams et al., 2022). Dusky rockfish abundance is considerably lower in the Bering Sea and Aleutian Islands management region (BSAI), where it is assessed as part of a non-target, and comparatively data-poor multispecies rockfish complex using index-based methods (Sullivan et al., 2022). Dusky rockfish primarily occur in the Aleutian Islands within the BSAI management region and are rarely observed in the eastern Bering Sea (Hoff, 2016; Markowitz et al., 2022; Fig. 1). The biology of dusky rockfish is data-limited, although recent work showed that size structure and growth between sexes exhibited homogeneity across the Aleutian Islands (TenBrink et al., 2023). In the GOA, life history traits of dusky rockfish have been more broadly studied, but data gaps persist, including information on the spatial and temporal extent of these traits (Malecha et al., 2007; Williams et al., 2022).

Within the BSAI management region, dusky rockfish catch is the largest of any species within its multispecies rockfish complex (361 metric tons; approximately 60% of complex in 2021; Sullivan et al., 2022), even exceeding catches of shortspine thornyhead (*Sebastolobus alascanus*), which comprises approximately 95% of the stock complex's total estimated biomass. Dusky rockfish are primarily caught in the Atka mackerel (*Pleurogrammus monopterygius*) bottom trawl fishery. In recent years, high exploitation rates (catch/biomass) in the eastern Aleutian Islands (Sullivan et al., 2022; Fig. 1) have prompted concerns about localized depletion (Hanselman et al., 2007) and highlighted data gaps on dusky rockfish stock structure in Alaska waters (Lunsford et al., 2011). In addition, within the federal management range of dusky rockfish in Alaska, there are distinct ecological boundaries that exist. In the BSAI region, the Aleutian Islands is divided by eastern, central, and western ecoregions within this marine ecosystem (Ortiz and

Zador, 2021). The western GOA is a large coastal ocean system dominated by the Alaska Coastal Current, while the eastern GOA has a narrow continental shelf influenced by the northward-flowing Alaska Current (Stabeno et al., 2004). An ecological boundary has been found near 148°W in the GOA (Coffin and Mueter, 2016; Fig. 1). We therefore undertook an otolith shape analysis study to identify dusky rockfish stock structure throughout its range in two bordering management regions. The objectives of our study were to 1) use otolith shape analysis to determine if there are differences in otolith shape between management regions using two descriptor techniques, and 2) to test for differences in otolith shape among subareas of each management region that exhibit ecological and oceanographic differences.

2. Material and methods

2.1 *Study area and sampling*

A total of 522 paired sagittal otoliths from dusky rockfish specimens were collected from both fisheries-dependent and fisheries-independent sampling platforms with bottom trawl gear during 2019-2022 (Table 1). The fork length and weight of each dusky rockfish specimen was measured to the nearest centimeter and gram, respectively. The sex of each fish was determined by gonadal examination. Left and right otoliths were collected and stored in a 50% glycerol thymol solution prior to processing. Otoliths were collected across the GOA and BSAI management regions (Fig. 1). Spatial reconstruction of the study area was created through the R package “sf” (Pebesma, 2018; Pebesma and Bivand, 2023) and “ggplot2” (Wickham, 2016). Subareas within each region follow the numerical statistical areas associated with the GOA and BSAI fishery management plans (North Pacific Fishery Management Council; NPFMC, 2020a;

NPFMC, 2020b). From bottom trawl research surveys conducted by the National Marine Fisheries Service's Alaska Fisheries Science Center in the summer, the BSAI sampling occurred on the continental shelf and upper continental slope to a depth of 500 m from Attu Island in the west to Unimak Island in the east (Fig. 1; von Szalay and Raring, 2020). From the BSAI management region, otoliths were collected across the Aleutian Islands. The survey samples in multiple regions that exhibit distinct oceanographic and biological characteristics. The three Aleutian Islands ecoregions also encompass primary management or statistical subareas (Fig. 1). We define subareas for this study as western Aleutian Islands (WAI; 543), central Aleutian Islands (CAI; 542), and eastern Aleutian Islands (EAI; 541) that follow the aforementioned management areas. The GOA bottom trawl survey covered the continental shelf and upper continental slope to 700 m from the Islands of the Four Mountains to the west and east to Dixon Entrance (Fig. 1; von Szalay and Raring, 2018). Among the two GOA ecological divisions, there are five management statistical areas. In this study, we define our subareas in the GOA as western GOA (WGOA; 610, 620, 630) and eastern GOA (EGOA; 640 and 650). Fish were also sampled by fishery observers during Atka mackerel and rockfish bottom trawl fisheries in the GOA and BSAI throughout the calendar year.

2.2 *Otolith image acquisition and processing*

Undamaged otoliths were blotted dry and placed on a black surface with the sulcus facing downward and the rostrum (anterior) end pointing to the left. Under reflected light, a calibrated high-resolution image of the proximal face of either the left or right sagittal otolith from either sex was obtained with a digital microscope camera (Leica DMC4500) mounted on a

Leica stereo microscope MZ9.5. During this process a fixed, single magnification of 6.3× was used (10x eyepieces; 0.63x zoom; 1.0x main objective). Before shape analysis, each otolith image was edited to show the maximum amount of contrast between the otolith and background. Adobe Photoshop Elements version 18.0 was used to contrast the white otoliths with the black background, if necessary. Subsequent measurements were based on these captured images. Images of left and right-side otoliths were analyzed separately in this study. Few samples of right-side otoliths were collected from subarea WAI; therefore, right-side otoliths from the WAI were not included in subsequent analysis.

2.3 *Otolith shape analysis*

Otolith shape analysis was performed using the “shapeR” package (Libungan and Palsson, 2015) in R version 4.2.2 (R Core Team, 2022). In “shapeR”, the original jpeg-formatted images of each otolith were transformed into gray-scale and the outlines were detected using a threshold pixel value of 0.2. Each digitized image was visually evaluated to ensure that each outline accurately captured the edge of the otolith. If the digitized outline did not closely match the otolith outline (e.g., due to high pixel noise), the original image was manually edited and digitization was repeated. Contour smoothing was also performed (Libungan and Palsson, 2015).

Two types of otolith shape descriptors were used from the otolith outlines: wavelet and elliptic Fourier (Libungan and Palsson, 2015). Both descriptors were chosen due to their reported differences in performance when describing stock structure of fish species (e.g., Neves et al., 2023). Reconstruction of the otolith shape using wavelet and elliptic Fourier descriptors were generated and standardized with fish fork length to minimize ontogenetic effects (Libungan and Palsson, 2015). The level of wavelet and number of Fourier harmonics needed for a 98.5% accuracy of the otolith outline reconstruction was 5 and 12, respectively. In order to further minimize ontogenetic effects, samples were truncated to 38-50 cm fork length. This size range encompassed adult, mature fish captured from similar depth profiles. Wavelet and elliptic Fourier descriptors produced 64 and 45 standardized coefficients, respectively. Coefficients that had a significant interaction with fork length ($P < 0.05$) were excluded from analysis. The remaining standardized coefficients were used to compare otolith shape among subareas using canonical analysis of principal coordinates (CAP).

Four primary indices related to the size of the otolith were used (area, perimeter, otolith length, and otolith width; Fig. 2). Otolith weight was added as an index to account for differences in otolith characteristics such as thickness. From the primary size indices, six shape indices were calculated to determine if otolith shape varied among subareas within the GOA and BSAI management regions (Table 2). Data were standardized using fork length for each specimen as size effects can bias stock structure (Smith, 1992) using the common within-group slope (Lombarte and Lleonart, 1993),

$$M_S = M_O \left(\frac{\bar{x}}{x}\right)^b$$

where,

M_S = standardized (size-adjusted) measurement.

M_0 = original parameter (size or shape index).

$\underline{x}$= average size parameter (fork length) for all datasets.

x= size parameter (fork length) of each fish species.

b = slope of the regression between log M_0 and log x.

The standardized size and shape indices were evaluated for normality and homogeneity of variance. The data deviated from a normal distribution (Shapiro-Wilks test of normality, P < 0.025) and equality of variances across areas (Levene's test, P < 0.001); therefore, non-parametric tests were used for comparison analysis. The calculated shape indices were evaluated for collinearity using Pearson correlation coefficients, with a minimum value of ± 0.70 exhibiting significance between indices (Dormann et al., 2013). Roundness, ellipticity, and aspect ratio were all highly positively correlated (≥ 0.85), and form factor was positively correlated with circularity (≥ 0.95); therefore, only circularity, rectangularity, and roundness were retained for analysis.

2.5 Statistical analysis

A combination of univariate and multivariate analyses was used to describe otolith shape in this study. Non-parametric Kruskal-Wallis tests were used to test otolith shape between subareas for each size and shape index ("rstatix" R package; Kassambara, 2023). The eta-squared (η^2), based on the Kruskal-Wallis H-statistic, was used as a measure of the otolith shape effect size. Interpreting η^2 effect values followed Cohen (1988): 0.01 – 0.06 (small effect), 0.06 – 0.14 (moderate effect), and ≥ 0.14 (large effect). Welch t-tests were applied to each size and shape index to compare their overall means between the GOA and BSAI management regions. For multivariate analyses, the otolith shape variation was visualized and compared with a CAP on the standardized wavelet and elliptic Fourier coefficients using the “vegan” R package (Oksanen et al., 2013). ANOVA-like permutation tests of the standardized coefficients were used to examine the differences in otolith shapes from each subarea based on 1,000 permutations. To determine whether otoliths collected in different subareas could be distinguished based on their shapes (Klecka, 1980), a linear discriminant analysis (LDA) was applied to the standardized wavelet and elliptic Fourier coefficients (Libungan and Palsson, 2015). LDA is a supervised dimensionality reduction and data classification procedure and was conducted using the *lda* function within the “MASS” R package (Venables and Ripley, 2002) and PAST statistics software (ver. 3.19; Hammer et al., 2001). Predictive models were examined for accuracy using jackknifed cross-validation (‘leave-one-out’), which calculates an unbiased estimation of classification success. LDA was performed on different models that compared the performance of the standardized wavelet and elliptic Fourier coefficients and shape indices. A one-way permutational multivariate analysis of variance (PERMANOVA) was used to statistically test differences among subareas. PERMANOVA dissimilarity matrices were based on Euclidean distance and Type III (partial) sum of squares, and calculated using 9,999 random permutations (Anderson, 2005).

Results

3.1 *Otolith morphometric analysis*

The mean values for the standardized size and shape indices varied across subareas for both left-side and right-side otoliths (Fig. 3). With the exception of circularity, all size and shape indices were different among some subareas ($P < 0.05$; Kruskal-Wallis; Fig. 3). For the right-side, otolith area had the largest shape effect (Table 3), with those from the EGOA being the largest. Mean otolith length and mean otolith weight also exhibited moderate to large effects among right-side otoliths (Fig. 3). For left-side otoliths, roundness exhibited the largest effect, with those from subareas of the Aleutian Islands having the highest mean values, and those otoliths from the EGOA and WGOA having the lowest (Fig. 3). Mean otolith length, otolith width, and roundness from left-side otoliths, and mean otolith length and roundness from right-side otoliths were significantly different between the BSAI and GOA management regions (Welch t-tests; $P < 0.05$).

3.2 *Otolith shape analysis*

Outline reconstruction of the mean shape of otoliths using both standardized wavelet and Fourier coefficients were similar for each otolith side. For the left-side otolith, two main sections were identified where divergences occurred among subareas (Fig. 4A; Supplementary Fig. S1). These divergences were observed along the otolith rostrum and posterior side of the otolith. Among these sections, the mean otolith shape had the strongest variation at an angle approximately between 0°-45° and from this 300°-360° angle range. The mean otolith shapes of right-side otoliths showed divergences along the rostrum near 180° and the posterior ventral edge between -300°-360° (Fig. 4B; Supplementary Fig. S1). ANOVA-like permutations tests showed significant differences in the mean otolith shape of dusky rockfish between subareas from wavelet and elliptic Fourier coefficients among left and right-side otoliths ($P < 0.001$; Table 4).

CAP plots showed heterogeneity among the subareas with some distinction among management regions (Fig. 5). For left-side otoliths, the first two axes explained > 85% of the variation using wavelet and elliptic Fourier coefficients (Fig. 5). There was a high degree of overlap among otolith shapes, with variability in individual otoliths across subareas. A general ordination pattern was evident along the first canonical axis, showing two different cluster groups (WAI, CAI, and EAI; and WGOA and EGOA). Right-side otoliths exhibited a similar ordination pattern along the first canonical axis (Fig. 5).

Classification from jackknifed LDA showed an overall success of 45.6% and 52.2% for Fourier descriptors for the left-side and right-side otoliths, respectively (Fig. 6A, B). Wavelet descriptors exhibited similar results for each otolith side, but the success rate was slightly lower (Supplementary Fig. S2). Use of only shape indices performed poorly, with left and right-side otoliths exhibiting classification success of 34.5% and 24.8%, respectively (Supplementary Fig. S3). Both shape descriptors showed the highest classification rates in the WGOA and the EAI. Classification success was generally poor across the periphery of the study range in the WAI and EGOA for left-side otoliths (Fig. 6A), and correctly classified only 30.8% in the CAI for right-side otoliths (Fig. 6B). PERMANOVA pairwise tests revealed statistically significant differences between all subareas for right-side otoliths, with the exception of otoliths collected in CAI and EAI using Fourier descriptors (Table 5). There were also no differences observed for EGOA and WGOA using wavelet descriptors. PERMANOVA tests for left-side otoliths exhibited a general pattern of significant differences between otolith shape between management regions.

Discussion

What was previously known about dusky rockfish stock structure was based on an evaluation of known biological information by Lunsford et al. (2011), which included compiling available data from survey and fishery sources on life history, habitat, oceanography, distribution and population trends from the GOA. This prior study determined that dusky rockfish exhibited minimal stock structure. However, major deficiencies in the aforementioned data were noted, including any temporal or spatial study that determined if the dusky rockfish population was a single stock. The most recent stock assessment of dusky rockfish in the GOA continues to support a minimum stock structure hypothesis based on this previous information, but further research was deemed necessary to help evaluate this (Williams et al., 2022). For dusky rockfish in the BSAI management region, there has been a complete absence of information to determine stock structure (Sullivan et al., 2022); however, conservation concerns related to relatively high incidental catches of dusky rockfish in the Atka mackerel fishery in the eastern Aleutian Islands have prompted a need for further research into dusky rockfish stock structure in Alaska waters.

This was the first study that investigated otolith morphometry and shape analysis as discrimination tools to characterize stock structure for dusky rockfish across its range in Alaska. Our results indicated that otoliths from individual dusky rockfish were highly variable but differences were found in univariate measurements and mean otolith shape between management regions and, in some instances, among subareas. CAP results indicated a high degree of overlap among otolith shapes among subareas; however, a general ordination pattern was evident along the first canonical axis, with subareas clustered by management region (Fig. 5). These patterns were consistent between right and left-side otoliths and between the elliptic Fourier and wavelet shape descriptors (Fig. 5). The LDA of mean otolith shape indicated some separation between subareas. The highest classification rates for both elliptic Fourier and wavelet shape descriptors were observed in the WGOA and the EAI, whereas classification success was generally poor across the periphery of the study range in the WAI and EGOA (Fig. 6). Based on otolith shape analysis, our results appear to support the current fishery management paradigm of separate dusky rockfish stocks in the GOA and BSAI with overall low to moderate stock structure throughout Alaska waters.

Otolith shape analysis involving contour reconstruction provides a more complex knowledge on otolith shape variability (Tuset et al., 2021), but the use of size or shape indices in this study was not necessarily limited. Of the two shape descriptors, elliptic Fourier achieved slightly better results than wavelet. Differences between the two descriptors have been noted (e.g., Graps, 1995; Libungan and Palsson, 2015), and both were useful in discriminating among subareas. There have been few studies that compared both routinely-used descriptors (e.g., Neves et al., 2023), but our results agree with Sadighzadeh et al. (2012), who suggested that the elliptic Fourier descriptor is more efficient in describing variation within species. In the future, additional tools and methods to assess classification performance using Fourier analysis, such as machine-learning techniques (Smolinski et al., 2020) and combining geographical areas (Stransky, 2005), could improve accuracy.

The stock structure of dusky rockfish indicated by otolith shape analysis appears to be similar to that derived from other sets of life history traits (i.e, growth). Campana and Casselman (1993) concluded that otolith shape was strongly related to fish growth rate, and, consequently, that otolith shape might not differentiate well among stocks with similar growth rates. Growth of dusky rockfish in Alaska has not been studied extensively. Spatial variation has been reported, as Malecha et al. (2007) found significant differences in growth between areas in the GOA, but issues with sample sizes were noted from the eastern GOA. In the current stock assessment of dusky rockfish in the GOA, growth within the age-structured model is combined for both sexes and area (Williams et al., 2022). In the Aleutian Islands, TenBrink et al. (2023) found no differences in growth among samples collected across the eastern, central, and western subareas. Sexual dimorphism among otolith shape should be investigated further in dusky rockfish; however, as Vaux et al. (2019) found secondary sexual differences in otolith shape in deacon rockfish (*Sebastes diaconus*). A robust study on dusky rockfish growth rates and subsequent examination of sexual differences among otolith shape across its longitudinal range might help further detect spatial similarities or differences.

Differences in otolith shape has been linked to genetic (Vignon and Morat, 2010) and environmental effects, such as temperature (Lombart and Lleonart, 1993) and diet (Mille et al., 2016), which would affect the growth phase across sizes of fish. In our study, sampled across a very large area, regional environmental differences exist. For example, Samalga Pass in the Aleutian Islands, directly east of the Islands of the Four Mountains near the management border of subareas EAI and WGOA (Fig. 1), is a well-documented oceanographic barrier that separates the warmer, fresher, nitrate-poor water in the GOA from the colder, saltier, and nitrate-rich water of the Aleutian Islands (Ladd et al., 2005; Zimmerman and Prescott, 2021). Hunt and Stabeno (2005) described a strong discontinuity in the marine ecosystem east and west of Samalga Pass, including differences in the species composition of zooplankton, cold-water corals, groundfish, seabirds, and marine mammals. The oceanographic barrier at Samalga Pass may explain the higher classification rates in the WGOA and EAI. Several other large passes throughout the Aleutian Islands (Zimmerman and Prescott, 2021) may further isolate dusky rockfish within localized areas (e.g., subareas CAI and WAI), and a finer-scale spatial analysis of the data presented in this study could be used to analyze these patterns. In the GOA, spatiotemporal variation in the timing and magnitude of chlorophyll-a concentrations related to sea surface temperature, freshwater discharge, and other oceanographic variables (Waite and Mueter, 2013) may result in differential prey availability for dusky rockfish among subareas in that management region. A delineation near 148°W in the GOA separating eastern and western areas is created by two distinct downwelling regions (Coffin and Mueter, 2016). Behrenfeld and Falkowski (1997) found that carbon (^{14}C) productivity also shows regional boundaries between the two areas. Further research and increased sample sizes would be needed to test the hypothesis that otolith shape could be an indicator allowing the characterization of populations of dusky rockfish between areas with different ecological conditions.

All rockfish stocks in the U.S. Exclusive Economic Zone off Alaska, including Pacific ocean perch (*S. alutus*), northern (*S. polyspinis*), rougheye (*S. aleutianus*), blackspotted (*S. melanostictus*), and shortraker rockfish (*S. borealis*), are managed as separate stocks within single and multi-species assessments in the GOA and BSAI. However, data to support this separation are limited, and otolith shape analysis offers a promising, low cost method of stock delineation that could support management of many of these rockfish stocks. For example, high exploitation rates of blackspotted and rougheye rockfish in the western Aleutian Islands have prompted questions about stock structure and spatial management in that region (Spencer et al., 2010; Spencer et al., 2022). While preliminary results from a low coverage whole genome sequencing analysis suggest a lack of population genetic structure for blackspotted and rougheye rockfish in the Aleutian Islands, persistent low abundance coupled with increasing exploitation rates warrant further examination (Larson et al., 2021). Application of otolith shape analysis could provide additional information on demographic connectivity for this stock, which may have more relevance to fisheries management than genetic structure (Waples et al., 2008).

Defining stock structure is a critical piece in management decision making. The lack of defining spatial structure in stock assessment models may lead to misperceptions of stock status (Cadrin, 2020), eventually leading to erroneous management reference points that have failed to capture the spatial component when evaluating stocks in model development. Given that some degree of partitioning was observed in our multivariate analysis, this otolith shape analysis provides some support for existing fisheries management of dusky rockfish in Alaska, which separates catch recommendations between the GOA and BSAI (Sullivan et al., 2022; Williams et al., 2022). The subareas with the highest catches and biomass in each management region (subarea 541 in the EAI and subareas 630, 620, and 610 in the WGOA) have a low to moderate level of population connectivity, with a relatively high number of samples being classified in the other subarea if not correctly classified. If dusky rockfish in these subareas are connected, either through larval dispersal or adult migration, this could imply reduced management concern for subareas like EAI with high exploitation rates. However, given the mixed results of this study and the need to disentangle suspected sources of variation such as sex, sampling year, and associated spatial ecological differences, further analysis of dusky rockfish demographic rates, including movement patterns, habitat utilization (e.g., Conrath et al., 2019), and size and age structure, is warranted. Additional procedures, such as genomics or genetics (Vignon and Morat, 2010; Rodgveller et al., 2017; Vaux et al., 2019), body morphology (Düranni et al., 2022), and otolith elemental chemistry (Ferguson et al., 2011) would provide a more thorough understanding of dusky rockfish stock structure.

Credit author contribution statement

T. T. TenBrink – Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing, Visualization, Project Administration; **J. Y. Sullivan** – Methodology, Investigation, Writing, Visualization; **C. M. Gburski** – Data curation, Methodology, Visualization, Writing.

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Table 1. Otolith collections of dusky rockfish (*Sebastes variabilis*) by management region, subarea, sample sizes (*n*) of otolith side, and years of sampling used in this study.

Region	Subarea	Statistical Area	*n*		Year(s)
			Right	Left	
BSAI	Eastern Aleutian Islands	541	51	64	2019, 2020, 2021
	Central Aleutian Islands	542	26	41	2019, 2020, 2021
	Western Aleutian Islands	543		42	2019, 2020, 2021, 2022
GOA	Western Gulf of Alaska	610, 620,630	112	117	2019, 2020, 2021
	Eastern Gulf of Alaska	640, 650	37	32	2019, 2021
Total			226	296	

Table 2. Shape indices calculated for dusky rockfish (*Sebastes variabilis*) otoliths. F_L= Feret length; F_W = Feret width; O_A = otolith area; O_{AC} = otolith convex hull area; O_P = otolith perimeter.

Shape index	Formula
Aspect ratio	$F_L \ / \ F_W$
Circularity	$O_P^2 \ / \ O_A$
Ellipticity	$(F_L - F_W)/(F_L + F_W)$
Form factor	$4\pi O_A/O_P^2$
Rectangularity	$O_A/(F_L \times F_W)$
Roundness	$(4O_A)/(\pi F_L^2)$

Table 3. Summary table for non-parametric Kruskal–Wallis tests of the null hypothesis that each size or shape index from each otolith side is identical between five subareas of the Bering Sea and Aleutian Islands and Gulf of Alaska. The Kruskal-Wallis test statistic (*H*) approximates a Chi-square distribution. Eta squared (η^2) effect values followed Cohen (1988): 0.01 – 0.06 (small effect), 0.06 – 0.14 (moderate effect) and > 0.14 (large effect). Note: List includes indices that exhibited either a large or a moderate effect.

Variable	Otolith side	H (X^2)	Eta Squared (η^2)
Area	Right	40.4	0.168
Otolith length	Right	32.6	0.133
Otolith weight	Right	34.0	0.139
Area	Left	31.5	0.094
Otolith length	Left	31.6	0.094
Otolith width	Left	25.7	0.075
Roundness	Left	54.1	0.172
Otolith weight	Left	29.6	0.088

Table 4. ANOVA-like permutation tests from standardized wavelet and elliptic Fourier coefficients. df = degrees of freedom; SS = sum of squares; *F* = *F* value.

Otolith	Descriptor		df	SS	*F*	*P*-value
Left-side	Fourier	Model	4	0.09	4.04	0.001
		Residual	291	1.56		
	Wavelet	Model	4	12.53	5.11	0.001
		Residual	291	178.19		
Right-side	Fourier	Model	3	0.05	3.01	0.001
		Residual	222	1.30		
	Wavelet	Model	3	6.03	2.55	0.001
		Residual	222	175.97		

Table 5. Results of PERMANOVA testing for differences in otolith shape between subareas for left and right-side otoliths.

		WAI	CAI	EAI	EGOA	WGOA
Left	WA		0.26	1	0.145	0.005
	CAI			0.803	0.012	0.001
	EAI				0.015	0.001
	EGOA					1
	WGOA					
	Fourier	Psuedo *F* = 4.125; P < 0.001				
	WA		0.672	0.435	0.009	0.001
	CAI			1	0.001	0.001
	EAI				0.001	0.001
	EGOA					0.966
	WGOA					
	Wavelet	Psuedo *F* = 4.654; P < 0.001				
Right	CAI			0.984	0.007	0.030
	EAI				0.005	0.234
	EGOA					1

WGOA				
Fourier	Psuedo *F* = 2.946; P < 0.001			
CAI		1	0.022	0.016
EAI			0.001	0.002
EGOA				0.523
WGOA				
Wavelet	Psuedo *F* = 2.883; P < 0.001			

Figure captions (color for publication)

Figure 1. Map of the study area showing sampling locations and corresponding subareas within the BSAI (WAI = 541, CAI = 542, EAI = 543) and Gulf of Alaska management regions (WGOA = 610, 620, 630; EGOA = 640, 650). Dashed line corresponds to the line of separation between management regions.

Figure 2. Example of a left-side otolith used in this study (A); and its corresponding ShapeR otolith reconstruction outline (b). Scale bar = 1 mm.

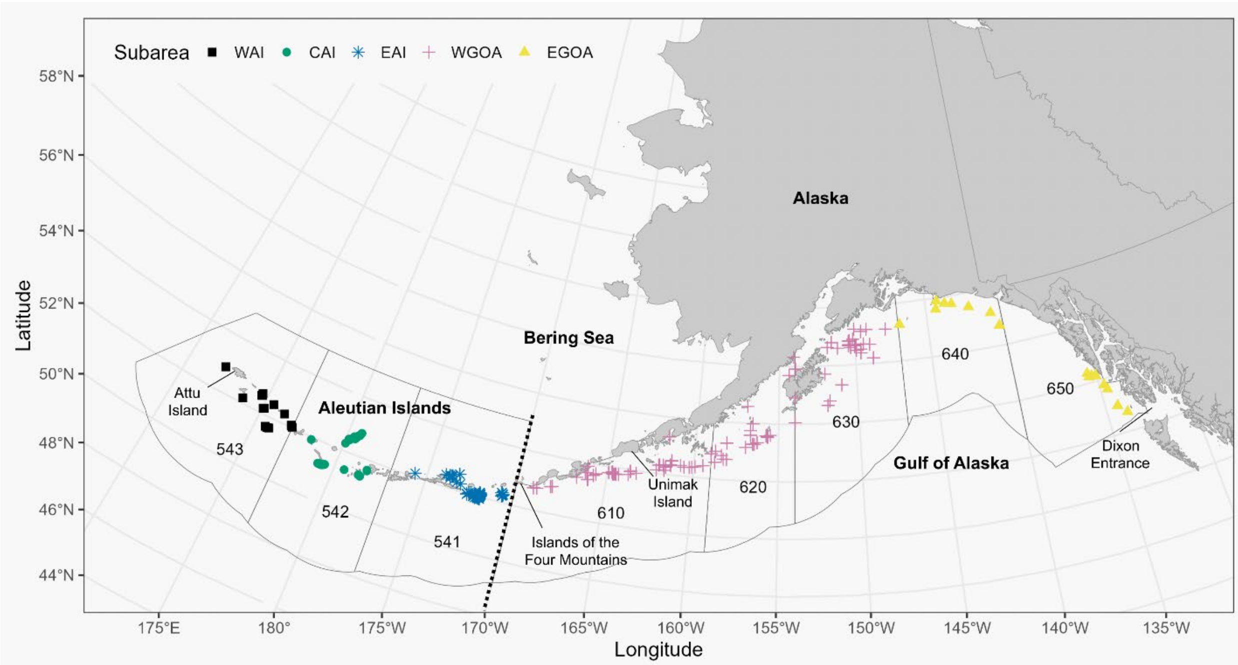
Figure 3. Size and shape indices from otolith analysis of dusky rockfish (*Sebastes variabilis*) within subareas of the Bering Sea and Aleutian Islands (blue) and Gulf of Alaska management regions (orange). Box plots show the median and inter-quartile (IQR) range, maximum and minimum values (±1.5 × IQR), mean (closed circles) and outliers (open circles).

Figure 4. Mean otolith shape of dusky rockfish (*Sebastes variabilis*) based on wavelet reconstruction for each subarea for the left-side otolith (top) and right-side otolith (bottom).

Figure 5. Canonical analysis of principal coordinates (CAP) plot of otolith shapes from standardized wavelet and elliptic Fourier coefficients. Labeled subareas represent the mean canonical coordinates surrounded by two standard errors (SEs).

Figure 6. Classification matrix of the linear discriminant analysis (LDA) for subarea classification of dusky rockfish (*Sebastes variabilis*) based on best performing model for left and right-side otoliths. The cell values indicate the number of otoliths classified by subarea, with correctly classified percentages in the highlighted cells. Shading represents the percent of otoliths classified by observed subarea, with higher classification rates in green.

Figure 1.


Subarea WAI CAI EAI WGOA EGOA
Alaska
Bering Sea
Aleutian Islands
Gulf of Alaska
Attu Island
Unimak Island
Islands of the Four Mountains
Dixon Entrance
543
542
541
610
620
630
640
650
Latitude
Longitude
58°N
56°N
54°N
52°N
50°N
48°N
46°N
44°N
175°E
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155°W
150°W
145°W
140°W
135°W

Figure 2.

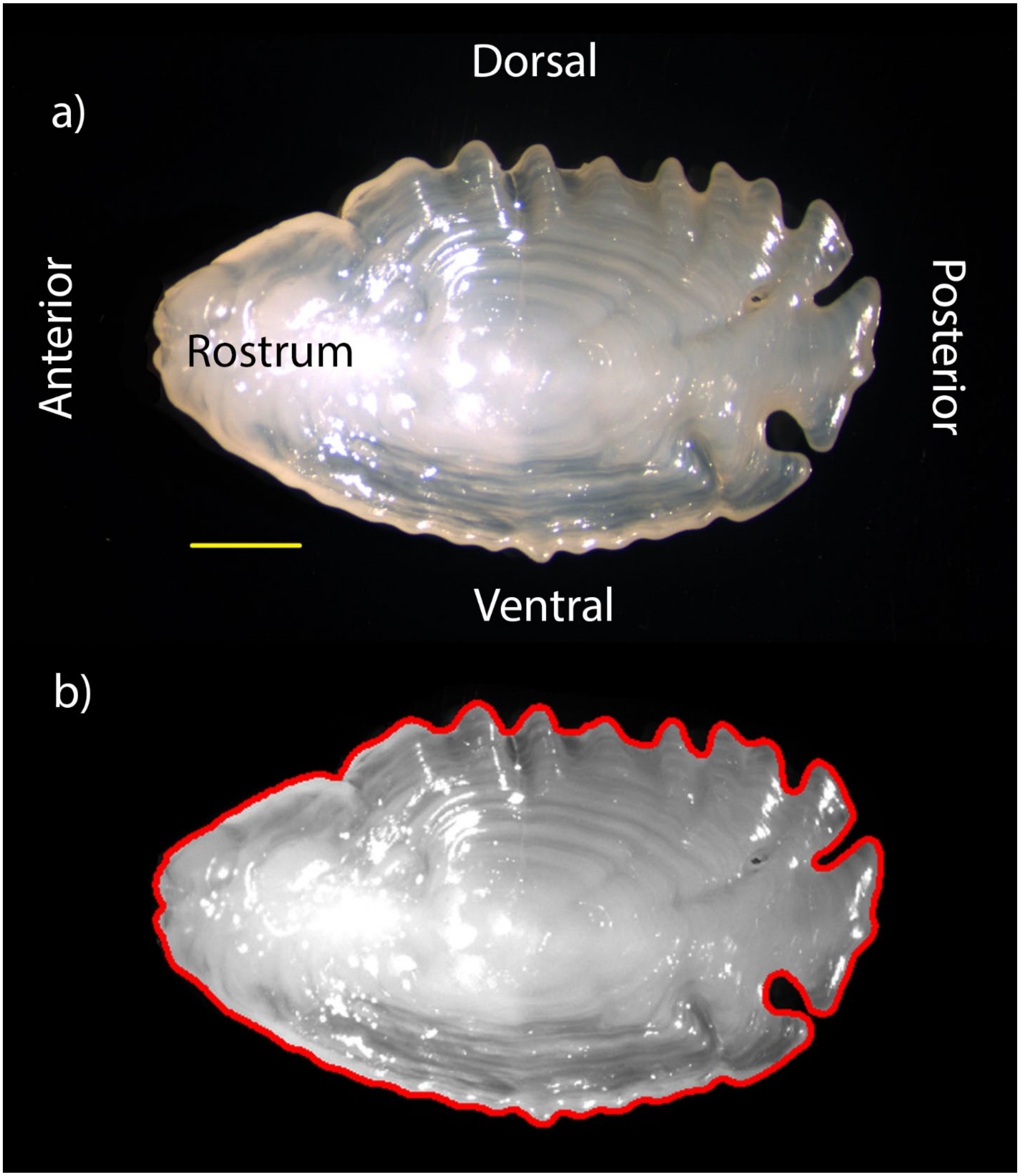
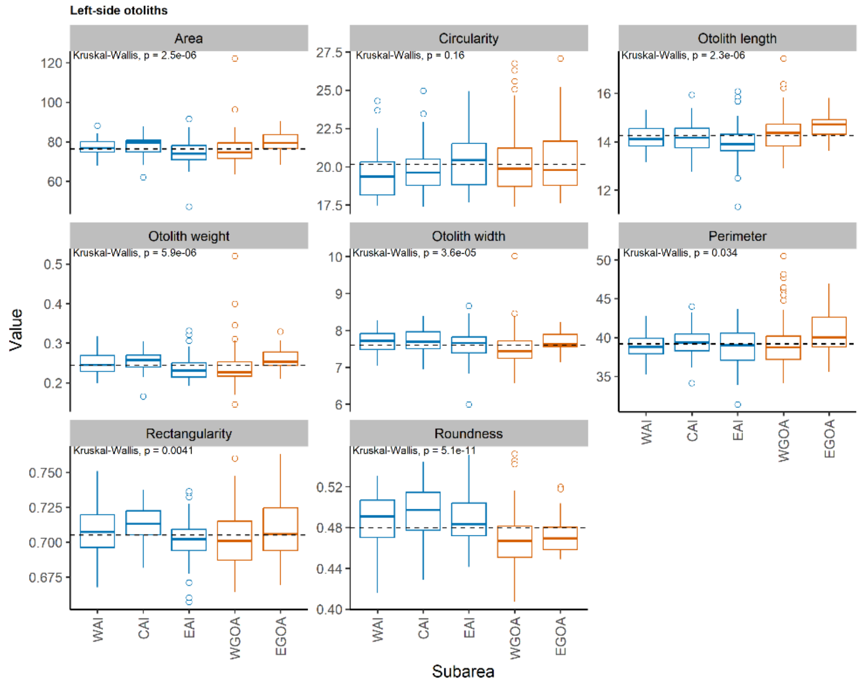


Figure 3.


Left-side otoliths
Area
Kruskal-Wallis, p = 2.5e-06
Circularity
Kruskal-Wallis, p = 0.16
Otolith length
Kruskal-Wallis, p = 2.3e-06
Otolith weight
Kruskal-Wallis, p = 5.9e-06
Otolith width
Kruskal-Wallis, p = 3.6e-05
Perimeter
Kruskal-Wallis, p = 0.034
Rectangularity
Kruskal-Wallis, p = 0.0041
Roundness
Kruskal-Wallis, p = 5.1e-11
Value
WAI
CAI
EAI
WGOA
EGOA
Subarea

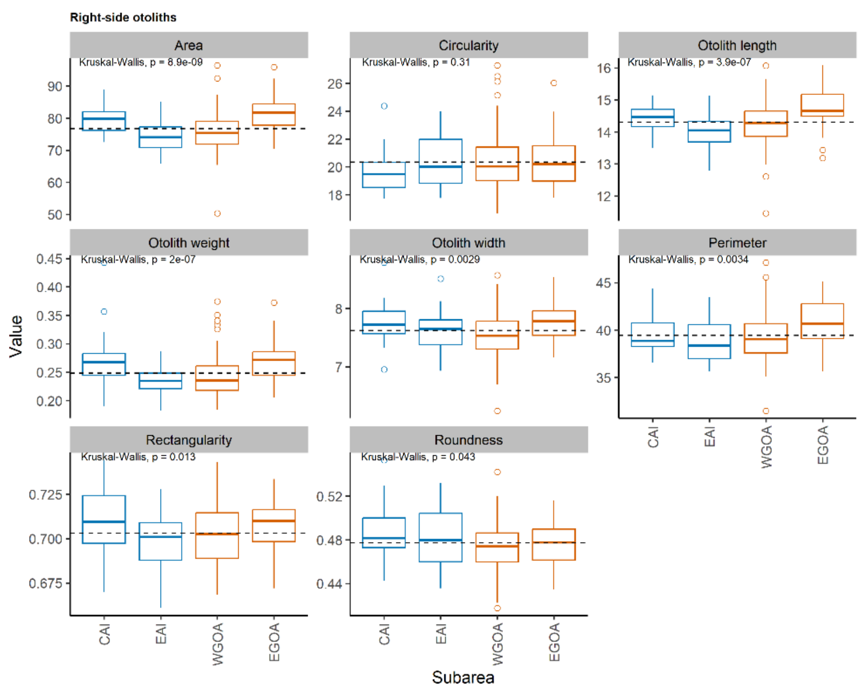
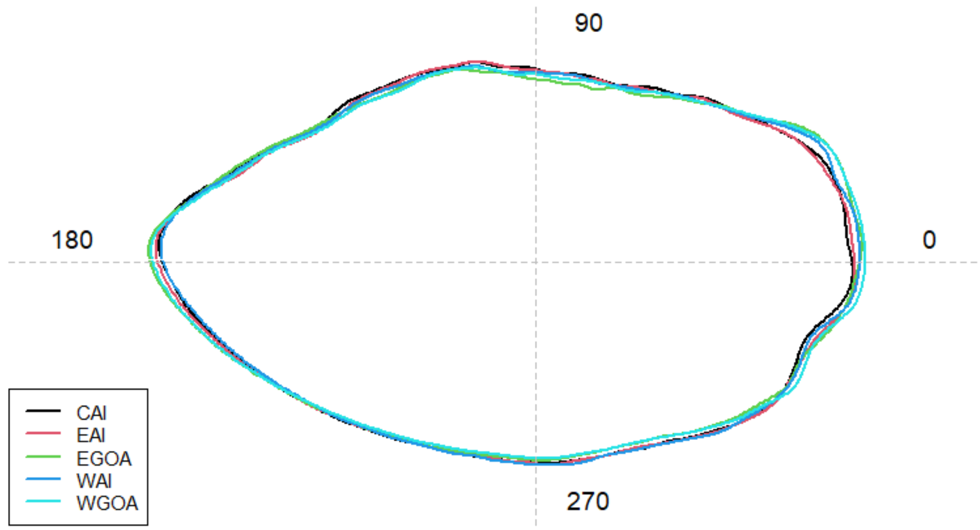

Right-side otoliths
Area
Kruskal-Wallis, p = 8.9e-09
Circularity
Kruskal-Wallis, p = 0.31
Otolith length
Kruskal-Wallis, p = 3.9e-07
Otolith weight
Kruskal-Wallis, p = 2e-07
Otolith width
Kruskal-Wallis, p = 0.0029
Perimeter
Kruskal-Wallis, p = 0.0034
Rectangularity
Kruskal-Wallis, p = 0.013
Roundness
Kruskal-Wallis, p = 0.043
Value
CAI
EAI
WGOA
EGOA
Subarea

Figure 4.



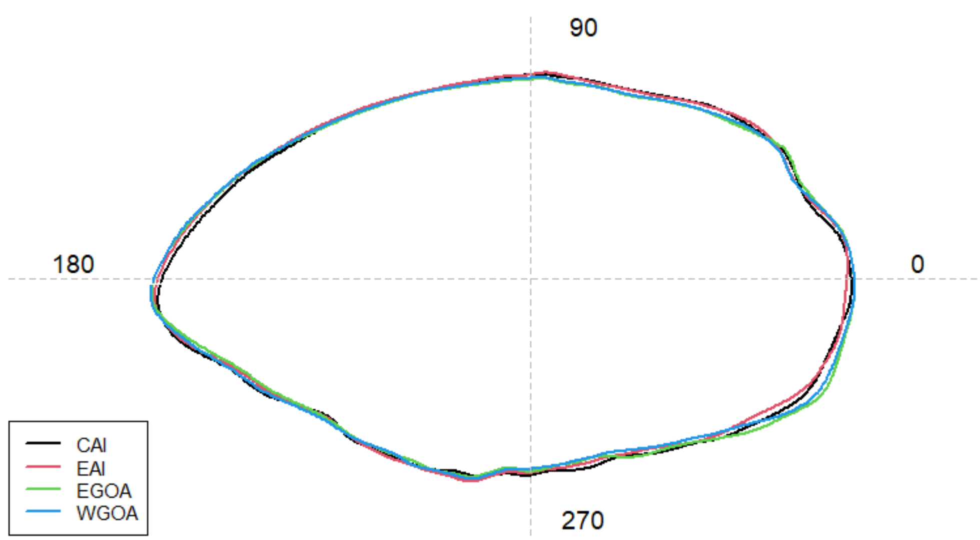
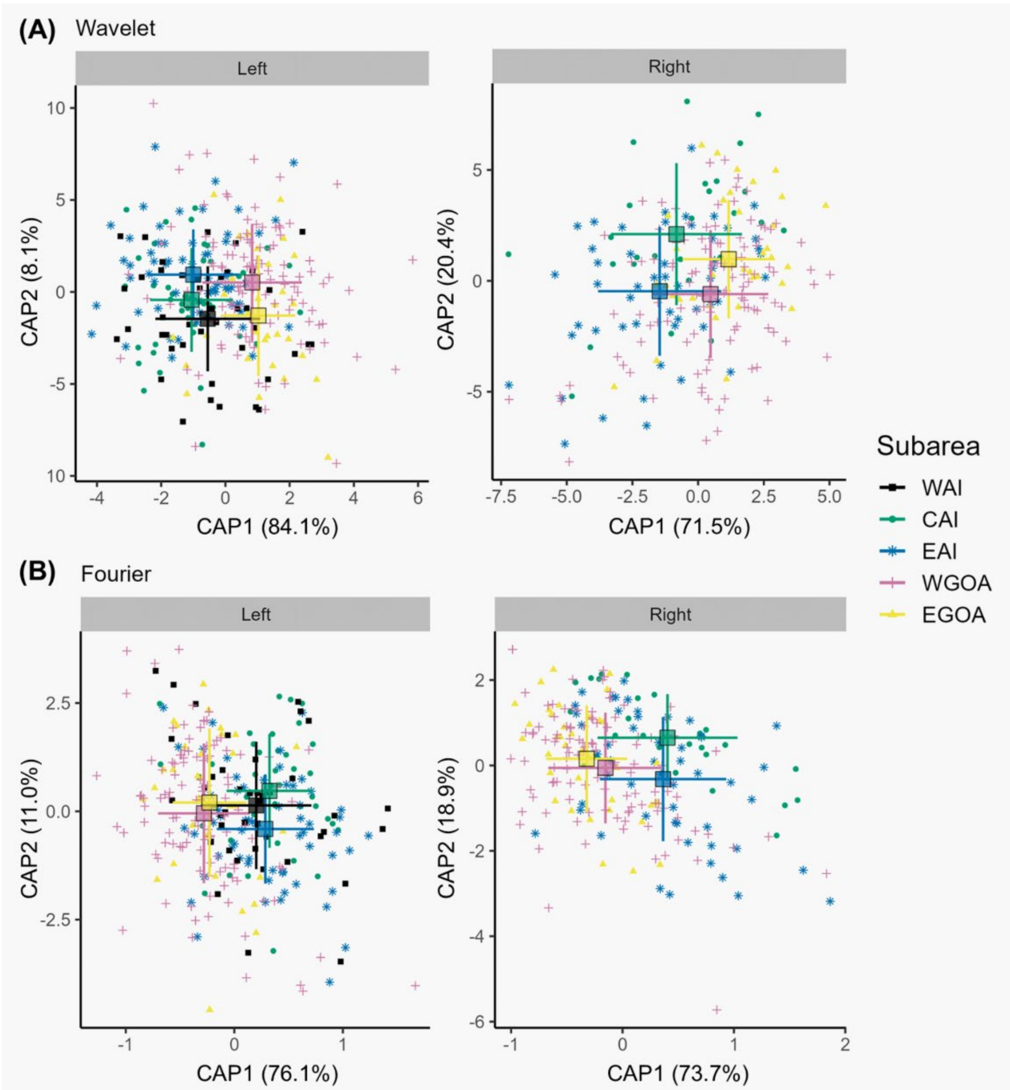
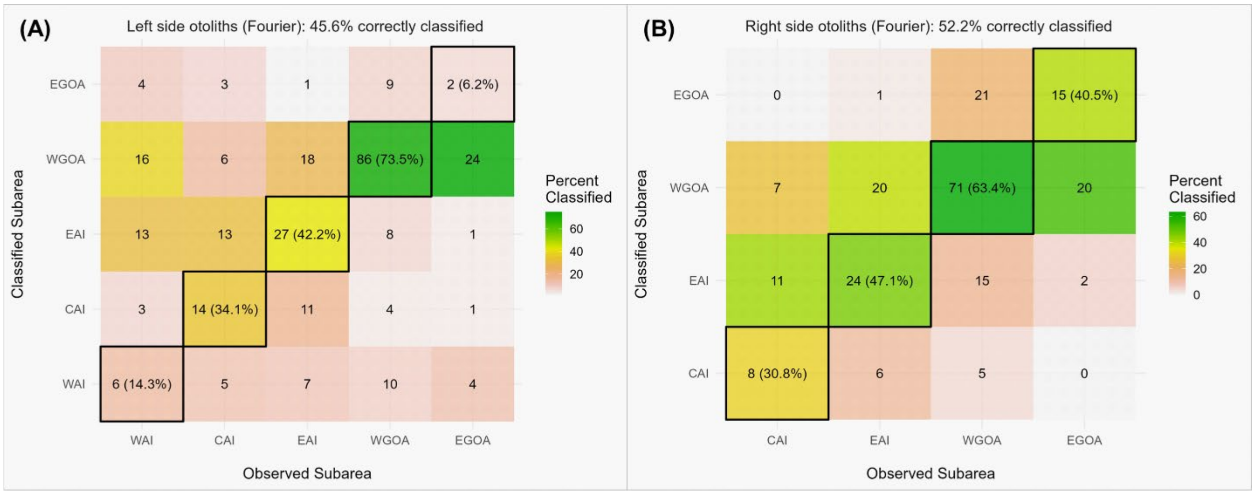


Figure 5.



(A) Wavelet
Left
Right
CAP2 (8.1%)
CAP1 (84.1%)
CAP2 (20.4%)
CAP1 (71.5%)
(B) Fourier
Left
Right
CAP2 (11.0%)
CAP1 (76.1%)
CAP2 (18.9%)
CAP1 (73.7%)
Subarea
WAI
CAI
EAI
WGOA
EGOA

Figure 6.


(A)
Left side otoliths (Fourier): 45.6% correctly classified
Classified Subarea
EGOA
WGOA
EAI
CAI
WAI
4
3
1
9
2 (6.2%)
16
6
18
86 (73.5%)
24
13
13
27 (42.2%)
8
1
3
14 (34.1%)
11
4
1
6 (14.3%)
5
7
10
4
WAI
CAI
EAI
WGOA
EGOA
Observed Subarea
Percent Classified
60
40
20
(B)
Right side otoliths (Fourier): 52.2% correctly classified
Classified Subarea
EGOA
WGOA
EAI
CAI
0
1
21
15 (40.5%)
7
20
71 (63.4%)
20
11
24 (47.1%)
15
2
8 (30.8%)
6
5
0
CAI
EAI
WGOA
EGOA
Observed Subarea
Percent Classified
60
40
20
0