Use of otolith shape analysis to separate blackspotted (*Sebastes melanostictus*) and rougheye rockfish (*Sebastes aleutianus*)

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Introduction

The purpose of the study is to develop a statistical procedure using otolith morphometrics and age that is capable of distinguishing between the blackspotted rockfish (*Sebastes melanostictus*) and rougheye rockfish (*Sebastes aleutianus*). They are large, long-lived rockfish found in the North pacific with a contiguous distribution along the West coast from California northward to the Gulf of Alaska and across the Aleutian Islands and eastern Bering sea. Rougheye and blackspotted rockfishes are caught together and can be commonly misidentified in the field. Blackspotted rockfish can sometimes be distinguished by the presence of black spots or blotches (Fig. 1), but this characteristic is too variable for use in data intended for stock assessments. In order to properly manage these species, a better method is needed to identify survey and fishery samples.

Otoliths are calcium carbonate structures located in the inner ears of fishes that allow them to orient and balance in the water. The stagittae are the largest paired otoliths in most saltwater teleosts, and are used in age determination. Otoliths from different species have unique shapes that can be used for species and stock discrimination. This study develops a model that separates the two species. This model will be used to identify specimens collected in the AFSC fishery observer program, archived in AFSC collections, which were not separated by species until 2006, and as a quality control for current RACE survey collections.

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Methods

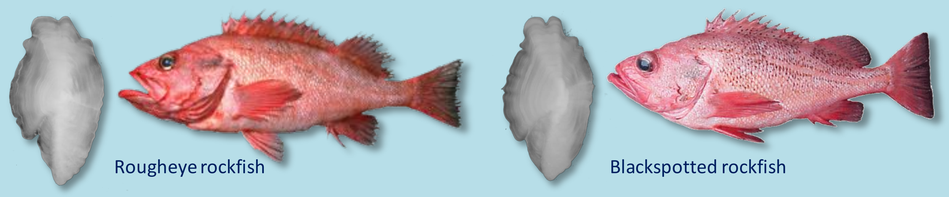

Figure 1. Shows the distal side of whole otoliths along with fish images of the two species.

Otoliths were collected from the Gulf of Alaska in the summer of 2009 and 2013 during the AFSC survey cruises on contracted fishing vessels. AFSC scientists collected otoliths from field identified rougheye and blackspotted rockfishes and placed the otoliths in vials containing 50% glycerol/thymol solution. Fin clips were taken for genetic verification of field identification and placed in separate marked vials. They were preserved in DNA buffer (NaCl2 saturated EDTA solution with 20% DMSO) and sent to NMFS genetics lab in Juneau, AK for analysis. Species were identified with fragment analysis of a single diagnostic microsatellite marker, *Sma6.*

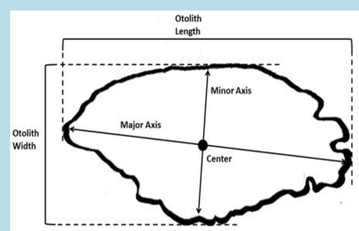

Figure 2. Illustrated diagram of a rockfish otolith.

We measured the shape of each otolith using several metrics: otolith's area, perimeter, Feret length, Feret width, major axis, and minor axis (Fig. 2). All measurements were taken from digitally photographed otoliths using a dissecting microscope and ImagePro 7. Otolith weight was taken using a Santorius milligram balance to the nearest thousandth of a gram. The otoliths were aged by NFMS age readeras part of routine age determination for stock assessment.

Initial exploration of the data suggested potential differences between species in terms of otolith size vs age. The significance of these trends in otolith shape were compared in each species via ANCOVA. A log transformation was applied to otolith ages to render these comparisons as a linear relationship.

We used logistic regression on the 657 otoliths in the model data set (Table 1) to assign classification probabilities to specimens based on their otolith parameters, fork length, and age. This function calculated a probability between zero and one that corresponded to the likelihood that a given specimen was a rougheye rockfish. Otolith measurements were normalized by including age as an interaction effect. Unnecessary parameters were removed using a stepwise backwards elimination algorithm with Aikake's Information Criterion (AIC) used to determine if a variable could be safely eliminated from the model.

Then, the best model was used to predict the identity of the new specimens in the test data set as an independent test of its validity and accuracy. The software R was used to carry out all statistical analyses.

Species	Year	Model Data	Test Data	Totals
Blackspotted	2009	441	40	481
	2013		62	62
Rougheye	2009	216	40	256
	2013		59	59
	Totals	657	201	858

Table 1. Depicts number of otoliths from 2009 and 2013 and how they were used in this study. Otoliths in the "model data" set were used to construct and fit the logistic regression model, whereas the "test data" set were used as an independent assessment of the model's accuracy.

Results

Analysis of the otoliths revealed several notable differences in size and shape between the two species. ANCOVA showed that otolith morphometrics from each species reacted differently to increasing age (Fig 3). Specifically, rougheye rockfish had larger otoliths across several dimensions for a given age. These differences were significant for all 7 otolith parameters.

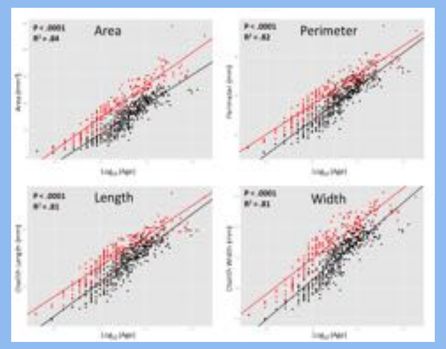

Figure 3. Plots of blackspotted (black dots) and rougheye (red dots) rockfish characteristics vs age. Regression lines were obtained via linear regression of each characteristic versus age and species. P values are the null hypothesis for no difference between species.

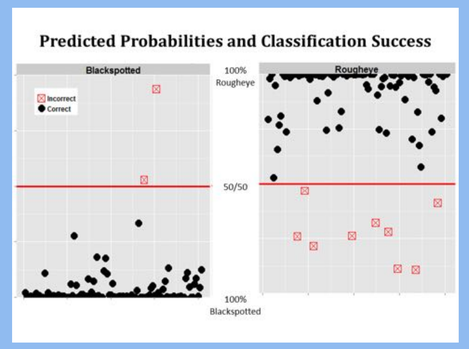

Figure 4. Depicts probability of otolith being either rougheye or black spotted rockfish based on meristic parameters.

The results of the ANCOVA informed the construction of a logistic regression which sought to capture the difference in size at age by including age as an interaction effect with each otolith parameter. The resulting model correctly predicted the identity of 95.4 % of all the otoliths used in building the model (Table 2). The model also performed well when given new data from the test data set, correctly classifying 94.5 % of the specimens correctly (Fig. 4). Overall, this model improved upon the effectiveness of field identifications which generally were correct less than 90% of the time, even when carried out by rockfish experts (Table 2).

Year	Genetic ID	Predicted ID		Total	Correct%
		rougheye	blackspotted		
2009	Blackspotted	181	381	562	67.8
	Rougheye	285	18	303	94.1
2013	Blackspotted	66	378	444	85.1
	Rougheye	363	46	409	88.8
	Model Data				
2009	Blackspotted	14	427	441	96.9
2009	Rougheye	200	16	216	92.6
	Test Data				
2009	Blackspotted	0	40	40	100
	Rougheye	37	3	40	92.5
2013	Blackspotted	2	60	62	96.8
	Rougheye	53	6	59	89.9

Table 2. Depicts the accuracy of field identifications and the logistic model developed in this study. Identifications made using otolith morphometrics and age agree with genetic identity more often than those made based on coloration or field characteristics. The improvement is strongest for blackspotted rockfishes.

Conclusions

- Rougheye and blackspotted rockfishes differed in their otolith morphometrics, with rougheye rockfish having larger dimensions for a given age.
- The model developed in this study can predict the correct species identity in approximately 94% of specimens.

Future work

- Archived rougheye/blackspotted rockfish mixed collections can be separated into respective species to provide a better understanding of past populations.
- The NMFS observer mixed fishery collections can be separated into respective species after being aged, which will potentially allow for improved management plans for each species.