PRELIMINARY EVALUATION OF THE USE OF CALCIFIED STRUCTURES FOR SEPARATING SPOTTED SEATROUT STOCKS

by

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ABSTRACT

Shape and growth characteristics of spotted seatrout (*Cynoscion nebulosus*) scales and otoliths were examined with the assistance of a computer software program to determine if the characteristics would be useful for separating stocks from individual bay systems. Scales and otoliths of spotted seatrout were collected from the lower Laguna Madre (50), Matagorda (34) and Galveston (32) Bays, Texas. Measurements were made from the focus to each of the first 20 circuli on each scale and means calculated for each bay system. Fourier series shape descriptors were used to analyze outlines of otolith silhouettes. A mean of the amplitude of each series (harmonic) was calculated for each bay system. Analysis of variance was used to identify distances to circuli and amplitudes of harmonics which differed significantly among bays. Discriminant analysis, using distances to circuli and amplitudes of harmonics which differed among bays as potential discriminators, was used to develop classification arrays. Fourier series shape analysis of otoliths using harmonics 3, 4 and 6 demonstrated better resolving power than did scale data. Sixty-four, 74 and 69% of the fish were correctly assigned to the lower Laguna Madre, Matagorda and Galveston Bays, respectively, using Fourier series shape analysis. By combining all data and using distances from the focus to the first, seventh and eleventh circuli and the third, fourth and sixth harmonics 72, 71 and 66 % of the fish were correctly assigned to the lower Laguna Madre, Matagorda and Galveston Bays, respectively. Fish were assigned to the correct bay system at a greater rate than could be expected if placement was random indicating analyses of scale and otolith shape and growth characteristics have potential as a tool for identifying Texas spotted seatrout stocks.

INTRODUCTION

The spotted seatrout (Cynoscion nebulosus) is a common species present in all Texas bay systems. Life history information suggests the fish seldom migrate from one bay system to another and spotted seatrout in each bay system within the Gulf may represent distinct stocks (Moffet 1961, Iverson and Tabb 1962, Weinstein and Yerger 1976). Furthermore, recent studies suggest more than one stock may be present within large bay systems (Baker et al. 1986). Confirmation of the existence of separate stocks within or among Texas bay systems could improve development of management plans for this species.

Stocks of fish have been defined as an intraspecific group of randomly mating individuals with temporal or spatial integrity (Ihssen et al. 1981). Stocks have been separated by genotype and/or phenotype. Neither separation by genotype nor phenotype have been found to be less valid (Ihssen et al. 1981). Biochemical methods (eg. electrophoresis) are employed to separate stocks by genotype. Analysis of morphometrics and meristics are used to separate stocks based on phenotype, with varying degress of success. Most recently computer assisted analysis of calcified structures has been used to successfully separate stocks of fishes (Ihssen et al. 1981, Riley and Magraf 1983, Bird et al. 1986). Characters in calcified structures are strongly influenced by environmental conditions, but a genetic basis exists, although it is more difficult to document (Ihssen et al. 1981). King and Pate (In press) have attempted to identify stocks of Texas spotted seatrout using electrophoresis but, to date, have been unsuccessful. No attempt to separate stocks of Texas spotted seatrout based on phenotype has been reported.

The purpose of this study was to provide a preliminary evaluation of computer assisted analysis of shape and growth patterns of calcified structures of spotted seatrout collected from the lower Laguna Madre, Matagorda and Galveston Bays. Specific study objectives were to: 1) familiarize personnel with the Optical Pattern Recognition System (OPRS) software and hardware (BioSonics, Inc., Seattle, Washington) which was used for data collection and analysis and; 2) evaluate the potential of circuli spacing of scales and Fourier series shape analysis of otliths as methods for separating stocks of Texas spotted seatrout.

MATERIALS AND METHODS

Spotted seatrout, 300-450 mm TL, were collected from Galveston Bay (50 fish) Matagorda Bay (50 fish) and the lower Laguna Madre (72 fish) between February and November 1988. Otoliths and scales were collected from each fish. Scales were collected immediately posterior to the left pectoral fin and below the lateral line (Colura et al. 1984). At least three scales from each fish were cleaned in fresh water and mounted between two glass slides (Ambrose 1983).

Data were acquired using the OPRS system (Optical Pattern Recognition System 1987). The system consists of data acquisition software, a microcomputer, video digitizing circuit board, video camera and monitor. The camera was mounted on either a compound or a steroscopic microscope, the image of the scale or otolith displayed on the monitor, and a series of measurements of distance or shape extracted and saved to a computer file for later analysis.

Scales were examined at 100X using the compound microscope. A reference line was drawn from the focus to the left radius of the scale. Measurements were then collected along a line 45° counter clockwise from the reference line. Distance from the focus to minimum luminance values, which correspond to scale circuli distances, were measured for the first 20 circuli of one scale on each slide. For each bay system the mean distance to each of the first 20 minimum luminance values were calculated. Regenerated scales were not used in the analysis.

Otoliths were examined at 4X using the compound microscope. The left otolith of each fish was placed on it's proximal side and the perimeter of the otolith automatically traced using an edge detection algorithm (Roberts 1965, Optical Pattern Recognition System 1987). A series of 128 X, Y coordinates were then taken at equal distances along the boundary and a Fast Fourier Transform (Brigham 1974) taken of the sequence to generate a Fourier series which mathematically describes the shape. Each term of the series (harmonic) consists of a cosine and sine coefficient and describes a shape component. The amplitude of each harmonic represents its contribution to the overall shape of the otolith. The mean amplitude of the first 15 harmonics used to describe the shape of the otoliths was calculated. Fractured otoliths were not used in the analysis.

Analysis of variance was used to identify harmonics and mean distances to circuli which were significantly different among bay systems and might be potential discriminators. A linear discriminant analysis was then performed using the potential discriminators to develop a classification array (percentage of fish correctly assigned to their bay system of origin) using Pattern Recognition II software (OPRS 1989). The program uses the "leaving-one-out" procedure (Lachenbruch 1967) which allows all known-origin scales to be used as both the training and test set for evaluating the performance of the discriminant analyses without introducing bias. Scale and otolith features providing the best discrimination were determined iteratively. Statgraphics 2.6 (Statistical Graphics Corporation 1987) was used to perform analysis of variance.

RESULTS

Fracturing of the anterior edge of otoliths and collection of only regenerated scales resulted in 22 (31%), 16 (32%) and 18 (36%) of the samples collected from the lower Laguna Madre, Matagorda and Galveston Bays respectively, being discarded.

Mean distances from the scale focus to circuli 1, 7, 8, 9, 11, 12, 15, 18 and 19 were significantly different among the three bays. Best classification of fish into their respective bay systems was obtained using the mean distance to circuli 1, 7, 11, and 19. Sixty-four, 59 and 41% of the fish from the lower Laguna Madre, Matagorda and Galveston Bays, respectively, were correctly classified (Table 1). All fish were correctly assigned at a rate greater than could be expected by chance alone (33%). Use of all 9 of the mean distances as discriminators resulted in loss of 9-19% resolving power.

Examination of the Fourier descriptors of otoliths demonstrated that the mean amplitudes of harmonics 3, 4, 6, 7, 9, 10, 12, 13, 14 and 15 were significantly different among the three bays. Best classification of fish into their respective bay systems was obtained using the means of harmonics 3, 4 and 6 to perform the discriminant analysis with 64, 74, and 69% of the fish correctly assigned to the lower Laguna Madre, Matagorda and Galveston Bay, respectively. Fish were correctly assigned to all three bays at a rate greater than would be expected by chance alone (Table 1). As with analysis of scale data use of all 10 harmonics as discriminators reduced resolving power by 10-15%.

Classification of lower Laguna Madre fish improved when linear discriminant analysis was performed on the combined scale and otolith data but resolving power for the other bays decreased slightly. Using distances to the first, seventh and eleventh circuli and third, fourth, and sixth harmonics 72, 71 and 66% of the fish were correctly assigned to the lower Laguna Madre, Matagorda and Galveston Bays, respectively. Although the percentage of fish correctly assigned to Matagorda and Galveston Bay's declined, fish were correctly classified to all three bays at least twice the rate that would be expected by chance alone.

DISCUSSION

Fourier series shape analysis of spotted seatrout otoliths and spacing of circuli show potential as methods for identifying stocks of spotted seatrout. Fish were assigned to the appropriate group using both data types singularly and in combination at a greater rate than could be expected if placement was random. Results, however, represent only data that were collected to establish standards (classification coefficients) and reliability of the standards for classifying test fish (fish of known origin but not used to develop standards) has yet to be determined.

Assignment of fish to the proper group might be improved by the addition or substitution of other data types, such as Fourier shape analysis of scales. This method was not employed in this study but has been shown to be useful for correctly assigning striped bass (Morone saxitilis) to their region of origin (Riley and Margraf 1983). Analysis of the data by sex might also increase the percentage of fish correctly assigned to their bay of origin, since growth of spotted seatrout is reported to differ between sexes (Colura et al. 1984). Increasing sample size may also increase the percentage of fish correctly assigned to their bay of origin. A sample size of 50 fish (preferably 100) is viewed as a minimum for the most reliable use of the OPRS software (J.

Condiotty, BioSonics Inc., Seattle, Washington, personal communication). Although initial sample size collected for the study was ≥ 50 fish per bay system the presence of regenerated scales and fractured otoliths rendered approximately 30% of the samples useless. As such, sample size of future collections should be increased to insure at least 50 fish per bay system are available for analysis.

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Table 1. Percentage of spotted seatrout correctly assigned to their bays system of origin using linear discrimination analysis.

Bay System	n	Circuli distances		Fourier harmonics		Scale and Otolith Combined	*a priori* probability
		Best[a]	All[b]	Best[c]	All[d]		
Lower Laguna Madre	50	64	50	64	50	72	33
Matagorda	34	59	50	74	59	71	33
Galveston	32	41	22	69	59	66	33

a Percentage of fish correctly assigned using distances to circuli 1, 7, 11 and 19 as discriminators.
b Percentage of fish correctly assigned using all circuli distances which differed significantly among bays as discriminators.
c Percentage of fish correctly assigned using harmonics 3, 4 and 6 as discriminators.
d Percentage of fish correctly assigned using all harmonics which differed significantly among bays as discriminators.

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