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RESEARCH ARTICLE

Differences in the otolith shape between the Marmara and Northern Aegean Sea stocks of blotched picarel (*Spicara maena* Linnaeus, 1758)

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Abstract

In this study, the differences between the otolith morphometry of *Spicara maena* individuals in the North Aegean Sea and Marmara Sea have been investigated. The samples were obtained with set nets and line fishing in the North Aegean Sea: Dikili, Altınoluk, and Küçükkuyu, and Marmara Sea: Karabiga, Bandırma, and Tekirdağ shores between February and October 2013. In total, 155 individuals from the North Aegean Sea and 168 individuals from the Marmara Sea were sampled. The coordinates and distance of the points on otolith were determined with image analysis program Image J 1.47v. The otolith shapes of the stocks were compared by the discriminant analysis method. The differences between the otoliths of the individuals sampled from North Aegean Sea and Marmara Sea are statistically significant ($P<0.05$). The discriminant analysis successfully explained the difference between the two stocks at 73.5%. The results showed that the *S.maena* stocks obtained from the North Aegean Sea and the Marmara Sea might be different.

Keywords: Blotched picarel, *Spicara maena*, Marmara Sea, North Aegean Sea, otolith morphometry

Introduction

Spicara maena (Linnaeus 1758), which is a species of the picarel fish, is found in herds generally in *Posidonia oceanica* meadows, areas of rocky and muddy bottom types (Miller and Loates 1997; Imsiridou *et al.* 2011). It is commonly observed in the Mediterranean Sea, Black Sea, as well as waters off Portugal, Morocco and Canary Islands (Jardas 1996). Conversion into male (*protogynous hermaphrodites*) is observed in *S.maena* female individuals (Breder and Rosen 1966; Imsiridou *et al.* 2011). The picarel fish catch amount was determined as 349,9 year/tons for 2014 by the Turkish Statistical Institute (TUIK-Turkish

Statistical Institute 2015). The decrease in capture in our seas every year requires regulatory precautions. In order to apply appropriate management strategy, the stocks must be identified and their abundance must be determined. Several methods have been used in fish stock identification studies (Cadrin *et al.* 2005; Canas *et al.* 2012): life history (e.g. growth, reproduction), natural markers (body and otolith morphometrics, meristics, genetics, parasites, otolith elemental composition, and fatty acid profiles), and applied marks (internal and external tags, electronic tags, and otolith thermal marking). Regarding *S.maena*, life history, body morphology, and genetics have previously been used to study the population structure (Arculeo *et al.* 1996; Mater *et al.* 2001; Çiçek *et al.* 2006; Karakulak *et al.* 2006; Çiçek *et al.* 2007; İlkyaz *et al.* 2007; İşmen *et al.* 2007; Sangun *et al.* 2007; Gökçe *et al.* 2010; Soykan *et al.* 2010; Minos *et al.* 2013).

Otolith shape analysis has been used widely with success in stock identification studies of various marine fish species like cod (*Gadus morhua*), redfish (*Sebastes* spp.), sole (*Solea solea*), and herring (*Clupea harengus)*; e.g. (Campana and Casselman 1993; Cadrin and Friedland 2005; Stransky 2005; Me´rigot *et al.* 2007; Burke *et al.* 2008), although this method has never been used for *S.maena*.

In this study, the otolith morphometry of *S.maena* individuals sampled in the North Aegean Sea and Marmara Sea were compared for stock separation.

Materials and methods

Samples of the picarel fish were taken from commercial fishing (gillnet/ trammel net and line fishing) in the North Aegean Sea (Dikili, Altınoluk, Küçükkuyu) and the Marmara Sea (Karabiga, Bandırma, Tekirdağ) between February and October 2013 (Figure 1).

The total length (TL) was measured to the nearest 1 mm. with a measuring board. The otoliths were placed in petri dish filled with glycerol on a black ground and were digitized by using camera-monitor and Kameram Image Analysis Program connected to Olympus SZX 16 brand stereo microscope (Figure 2).

The four points were marked on the obtained images according to Figure 3 (Turan 2000) with the Image J 1.47v, Image Analysis Program, and coordinates of the points were recorded. The distance between the two points was calculated in Microsoft Excel Program, and the Statistical 6 Otolith Lengths were obtained.

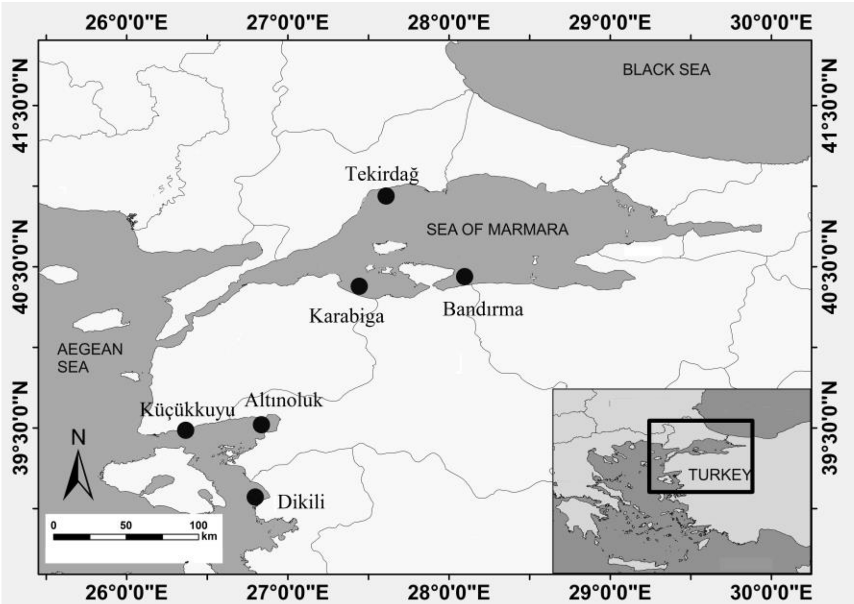
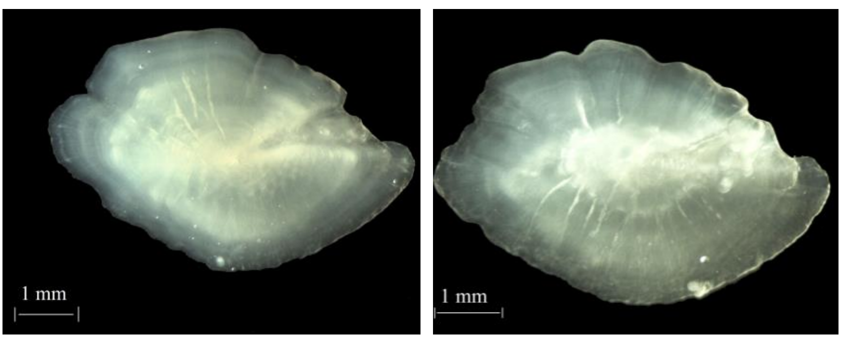


Figure 1. Sampling stations of *Spicara maena* in the Marmara Sea and North Aegean Sea



a. Marmara Sea (TL=14,3 cm) **b.** North Aegean Sea (TL=14,2 cm)

Figure 2. Otolith of Spicara maena from a) the Marmara Sea and b) the North Aegean Sea.

Before the statistical analyses, in order to eliminate the heterogeneity effect stemming from the difference in size among the samples, each measurement was converted by using the formula: $M_{adjusted} = M(L_{SM}/L_S)^b$, where M is the original morphometric measurement, M_{adj} the size adjusted measurement, L_S the standard length of fish, and L_{SM} the overall mean of standard length for all fish from all samples for each variable. The parameter b was estimated for each character from the observed data as the slope of the regression of log M on log

L_S, using all specimens. Correlation coefficients between transformed variables and standard length were calculated to check if the data transformation was effective in removing the effect of size in the data. (Turan 2004; Serpin 2007).

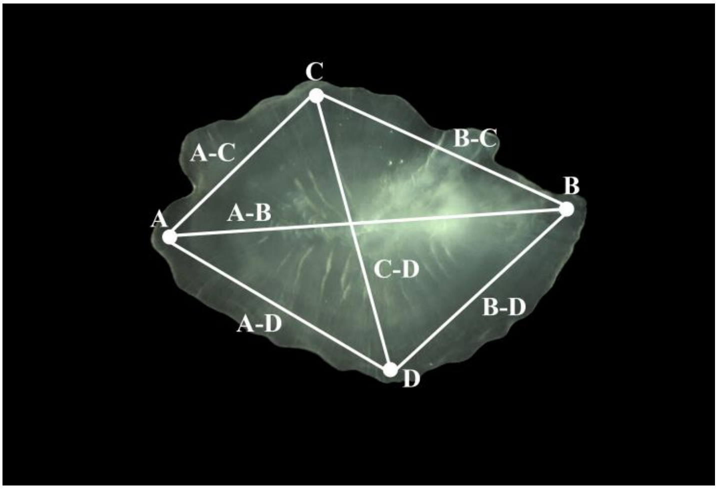


Figure 3. Four points marked on the otolith image (A, B, C, D) and six otolith lengths (A-B, A-C, A-D, B-C, B-D, C-D).

The current status between the stocks in different regions was investigated by forming two groups of the otolith morphometry of the *S.maena* individuals sampled from the North Aegean Sea and Marmara Sea. The mean, standard error, median, standard deviation, the lowest value and the highest values were measured in order to determine the basic structures of the datasets. Kolmogorov-Smirnov Test was used to test for the normality of otolith lengths. The difference between the means was tested with the Independent Two-Sampling T-Test. The Discriminant Analysis was used in order to separate the morphometric data into groups, examine the mutual relation between the data groups and separate the stocks. In order to eliminate the probability of wrong classification, the issues like the multiple normal distribution with discriminant analysis assumptions, the covariance matrices being equal, and multiple linear connection problem being absent were examined before the Discriminant Analysis (Kalaycı 2005). All of the statistical analyses and measurements were made in SPSS 19 program.

Results

Six otolith lengths were measured from four points on the otoliths of *S.maena*. The general characteristics of the measurements are presented in Table 1.

Table 1. Descriptive statistics of the otolith lengths of *S. maena* collected in the North Aegean Sea and Marmara Sea.

Region	Descriptive Statistics	A-B	A-C	A-D	B-C	B-D	C-D
North Aegean Sea	N	155	155	155	155	155	155
	Mean (mm)	5.114	2.723	3.188	3.367	2.847	3.290
	Std error (±)	0.019	0.020	0.018	0.020	0.019	0.021
	Median (mm)	5.109	2.690	3.194	3.381	2.890	3.331
	Std dev. (±)	0.239	0.250	0.227	0.243	0.231	0.260
	Min (mm)	4.347	2.019	2.680	2.835	2.056	2.637
	Max (mm)	5.653	3.494	3.848	3.871	3.430	3.982
Marmara Sea	N	132	132	132	132	132	132
	Mean (mm)	5.234	2.840	3.358	3.440	2.980	3.550
	Std error (±)	0.021	0.018	0.017	0.020	0.021	0.017
	Median (mm)	5.248	2.859	3.364	3.454	3.009	3.560
	Std dev. (±)	0.240	0.211	0.197	0.230	0.246	0.190
	Min (mm)	4.451	2.161	2.299	2.919	2.161	3.000
	Max (mm)	5.849	3.302	3.876	4.004	3.429	4.069

All the datasets showed a normal distribution (Kolmogorov-Smirnov test, $p>0.05$). The difference between the means of the otolith lengths of *S. maena* stocks from the Marmara Sea and North Aegean Sea was examined with the t-test. The difference in the A-B, A-C, A-D, B-D, C-D lengths was found to be statistically significant ($p<0.05$), and as not significant in the B-C length ($p>0.05$) (Table 2).

The relative multivariate kurtosis value of the otolith lengths was measured as 1.321. The relative kurtosis value being lower than 2 shows that the disruption is normal (Kline 1998; Aşkar and Mazman 2013).

Box's M statistic was used to test for homogeneity of covariance matrices. The test result was significant (Box's M=161.621, $p<0.001$). When the number of observations is more, Box'M results can be significant (Kalaycı 2005). In addition the intergroup variances are equal. Therefore it was assumed that covariance matrices of the groups were equal. The correlation between the variables was analysed to search the multiple connection problem between the variables (Table 3), it was determined that the correlation between the variables is lower than 0.70.

Table 2. Comparison of the otolith lengths from the North Aegean Sea and Marmara Sea stocks (t-test; *: $p<0.05$; NS: $p>0.05$)

Otolith lengths	Region	N	M	SD	df	t	p
A-B[a]	Marmara Sea	132	5.234	0.240	285	4.229	0.00*
	Kuzey Ege Sea	155	5.113	0.239			
A-C[a]	Marmara Sea	132	2.840	0.211	285	4.210	0.00*
	Kuzey Ege Sea	155	2.723	0.250			
A-D[a]	Marmara Sea	132	3.358	0.197	285	6.694	0.00*
	Kuzey Ege Sea	155	3.189	0.227			
B-C[a]	Marmara Sea	132	3.440	0.230	285	2.599	0.10[NS]
	Kuzey Ege Sea	155	3.367	0.243			
B-D[a]	Marmara Sea	132	2.980	0.246	285	4.722	0.00*
	Kuzey Ege Sea	155	2.847	0.231			
C-D[a]	Marmara Sea	132	3.550	0.190	285	9.556	0.00*
	Kuzey Ege Sea	155	3.290	0.260			

[a]: Transformed measure to eliminate effect of fish length heterogeneity.

Table 3. Variable cross-correlation values between otolith lengths of *S. maena*

		A-B[a]	A-C[a]	A-D[a]	B-C[a]	B-D[a]
Correlation	A-B[a]	1.000	0.533	-0.683	0.553	-0.606
	A-C[a]	0.533	1.000	-0.369	-0.253	-0.325
	A-D[a]	-0.683	-0.369	1.000	-0.392	0.029
	B-C[a]	0.553	-0.253	-0.392	1.000	-0.267
	B-D[a]	-0.606	-0.325	0.029	-0.267	1.000

[a]: Transformed measure to eliminate effect of fish length heterogeneity.

When the relation between the independent variables and the discriminant functions was examined with “Structure Matrices”, it was observed that the variable separated the highest groups in the 1st Function was “C-D”.

The North Aegean Sea and Marmara Sea stocks were successfully classified at a rate of 73.5% by the discriminant analysis. Based on the results obtained from discriminant analysis of the differences found in the otolith lengths between the North Aegean and Marmara stocks, the Marmara stock was similar to their stock with a rate of 69.8%, and the North Aegean stock with a rate of 31.1%; and the North Aegean stock was similar to their own stock with a rate of 77.4%, and the Marmara stock with a rate of 22.6% (Table 4, Figure 4). The otolith lengths of *S.maena* from the North Aegean Sea and Marmara Sea showed similarity at a lower rate (31.1%-22.6%). In other words, the differences found between the otolith lengths of *S.maena* in the North Aegean and the Marmara Sea support the concept of separate biological stocks.

Table 4. Discriminant analysis grouping results using the otolith lengths of *S. maena* from the Marmara and North Aegean Sea

	Group	Group relations		Total
		a	b	
Quantity	a	91	41	132
	b	35	120	155
%	a	68.9	31.1	100.0
	b	22.6	77.4	100.0

a: Otolith lengths of individuals sampled from the Marmara Sea.
b: Otolith lengths of individuals sampled from the North Aegean Sea.

Discussion

No stock separation had been observed for the *S. maena* individuals with the otolith shape analysis previously, although there have been studies conducted on stock separation for various species. Turan *et al.* (2004) used basic components and discriminant analysis to examine the genetic and morphometric structures of the *Liza abu* populations in the Asi, Fırat and Dicle Rivers. They reported that the Dicle population was different from the Asi and Fırat population. Turan *et al.* (2006) compared the stocks by using the morphometric and meristic properties of *Pomatomus saltatrix* in the Black Sea, Marmara, Aegean Sea and Northeastern Mediterranean Sea. They reported that there were three groups in terms of morphology. Canas *et al.* (2012) conducted a study on the otolith morphometry of *Lophius piscatorius* in the North Atlantic by using covariance analysis, Fourier shape analysis and linear discriminant analysis. They reported that their results supported the stock separation of *L. piscatorius* species (genetic, morphology, migration), and that the stocks were not separated as north and south.

In this study, there was a statistically significant difference in five otolith lengths, except one otolith length (B-C), for *S.maena* specimens obtained from the Marmara and North Aegean Sea. The discriminant analysis was successful at a rate of 73.5% in estimating the group members. At the end of the analysis, the *S. maena* individuals obtained from the Marmara Sea by their otolith lengths were classified in their own group with a rate of 69.8%, and in the North Aegean Sea with a rate of 31.1%; and the ones obtained from the North Aegean Sea were classified in their own group with a rate of 77.4%, and in Marmara Group with a rate of 22.6%. It is possible to say that the otolith measurements of the *S. maena* individuals sampled from North Aegean and Marmara Sea showed lower similarity (31.1%-22.6%). In other words, the differences observed in otolith measurements supported the assumption that the change of individuals between the North Aegean Sea and Marmara Sea populations was limited and was not homogenized in phenotypic terms.

As a conclusion, the comparison of morphometric properties of otoliths implies the separate populations of *S.maena* in the Marmara Sea and North Aegean Sea. However, the results must be supported with further genetic studies for the separation of the *S.maena* stocks in Marmara Sea and North Aegean Sea.

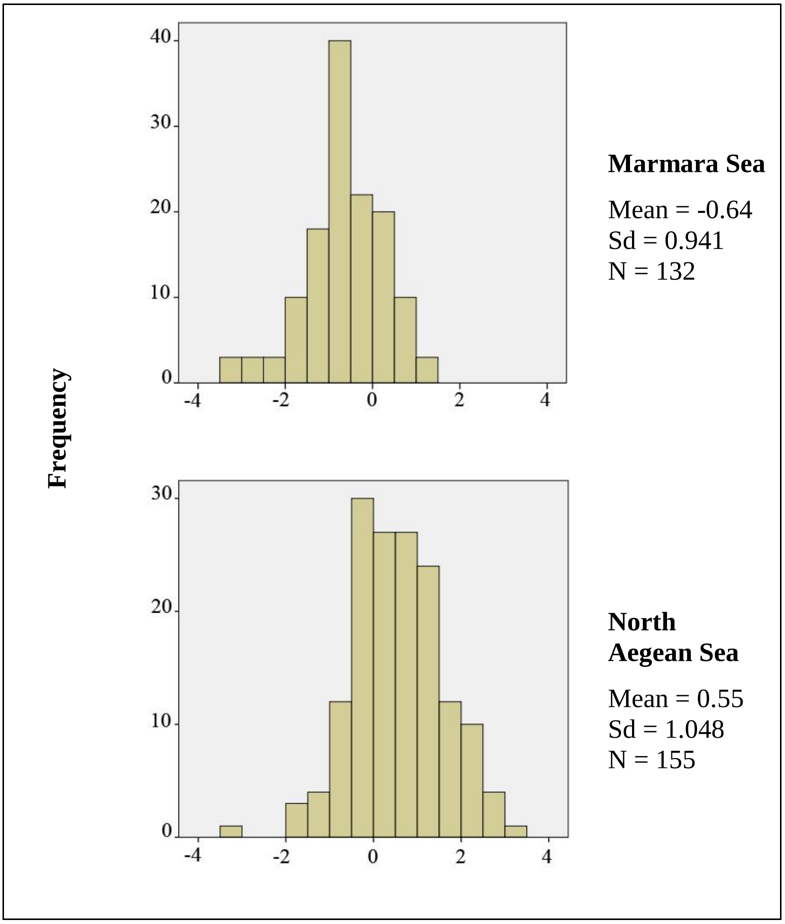


Figure 41. Canonical discriminant function values of the otolith measurements of *S. maena* from the Marmara Sea and North Aegean Sea.

Marmara ve Kuzey Ege Denizlerindeki izmarit balığı (*Spicara maena* Linnaeus, 1758) stoklarının otolit morfometrisi arasındaki farklılıklar

Özet

Bu çalışmada, Kuzey Ege Denizi ve Marmara Denizi'ndeki *Spicara maena* bireylerinin otolit morfometrisi arasındaki farklılıklar incelenmiştir. Örnekler Şubat 2013-Ekim 2013 tarihleri arasında Kuzey Ege Denizi'nde Dikili, Altınoluk, Küçükkuyu ile Marmara Denizi'nde Karabiga, Bandırma, Tekirdağ kıyılarında uzatma ağları ve olta balıkçılığı ile elde edilmiştir. Kuzey Ege Denizi'nden 155 adet, Marmara Denizi'nden 168 adet birey örneklenmiştir. Otolit üzerinde noktaların koordinatı ve noktalar arasındaki uzunluklar görüntü analiz programı ImageJ 1.47v ile belirlenmiştir. Stokların otolit şekil farklılıkları diskriminant analiz yöntemi kullanarak karşılaştırılmıştır. Kuzey Ege Denizi ve Marmara Denizi'nden örneklenen bireylerin otolit uzunlukları arasındaki farklılıkların istatistiksel olarak önemli olduğu tespit edilmiştir ($P<0.05$). Diskriminant analizi iki grup arasındaki farklılığı açıklamada %73.5 oranında başarılı olmuştur. Marmara Denizi'nden örneklenen bireylerin otolit uzunlukları %69.8 oranında, Ege Denizi'nden örneklenen bireylerin otolit uzunlukları ise %77.4 oranında kendi gruplarının özelliklerini taşımaktadır. Bu sonuçlar, Kuzey Ege Denizi ve Marmara Denizi *S.maena* stoklarının farklı olabileceğini ortaya koymuştur.

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