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SEX DETERMINATION OF GREEN TURTLE (*CHELONIA MYDAS*) WITH IMAGE ANALYSIS USING BIOMORPH SOFTWARE

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Abstract

One of parameter of population dynamics in sea turtles is knowledge of sex ratio, which is important for the conservation and management of the species. There are different techniques to determine the sex and sex ratio in sea turtles. In order to developed non-destructive alternative techniques for the sex determination and shorten the complicated processes and to be able to detect on live hatchlings, we aimed to develop sex determination based on image analysis in green turtles (*Chelonia mydas*). Four female and four male dead green turtle hatchlings, of which sex was determined by gonad histology, were collected from Yeniyurt beach (Hatay, Turkey) and photographed. The differences between sexes of green turtle hatchlings was tested with BioMorph software which produced 351 landmark measurements from 27 determined landmarks on the carapace of green turtle hatchlings. Univariate analysis of variance revealed that 4 character located on the upper-right side of the carapace were significantly different between sexes. In Principal component analysis (PCA), 7 principal components were produced of which first and second PCs explained 68 % and 8 % of the variation, respectively. Examination of the contribution of each morphometric character to the first PC indicated that the observed differences were mainly from the height and width measurements, demonstrating these characters to be important in the description of the sex specifications. In discriminant function analysis, overall random assignment of individuals into their original population was 50% that 50% of cross-validated grouped individuals of female and male individuals were classified into their original group, respectively.

Keywords: Sex, Green turtle, morphometric, sex determination, BioMorph

Introduction

The differentiation of an embryo as a male or female brings about significant consequences of its life history, behavior, physiology, morphology and adaptability. Identifying sex, which is the richest source of phenotypic variation within the population, is particularly important in ecology, behavior, conservation and genetic studies. Therefore, the factors governing sex development is an important field of study that is at the focus of scientific interest (Mittwoch, 2000; Warner, 2011). Knowing the sex and the sex ratio in sea turtles is a necessary parameter for determining population dynamics within their life history. However, they may be adversely affected by global climate change due to their temperature-dependent sex within their life cycle, which may affect the existing population as well as the future population. The sex ratio of nesting beaches is important for the prediction of the future of them as well as for effective conservation planning because long-term survival depends on both female and male production (Janzen, 1994). There are some different techniques to determine the sex and sex ratio in sea turtles. These techniques are gonadal histology (Yntema & Mrosovsky, 1980; Godfrey & Mrosovsky, 2006), radioimmunoassay to measure testosterone levels in blood or chorioallantoic fluid laparoscopy on live post-hatchlings (Gross et al., 1995), direct observations of the gonads in situ (van der Heiden et al., 1985) or nest temperature and incubation duration, which are indirect techniques (Kaska et al., 1998). However; these techniques require long laboratory processes or sometimes sacrifice animals, which are endangered, and under protection globally. Therefore, it is need to be developed non-invasive alternative techniques for the sex determination.

A different approach to sex determination is that there may be morphological differences between male and female hatchlings that are not seen with the naked eye (Valenzuela et al., 2004). Typically, linear measurements and statistical differences can be demonstrated with these differences between female and male hatchlings (Valenzuela et al., 2004). On the other hand, some researchers have stated that the main reason for sex discrimination in hatchlings is the lack of morphological differences and the lack of a technique used for this (Lubiana & Ferreira-Junior, 2009). Some researchers have obtained clues suggesting that morphological differences between male and female may occur in both classical and geometric morphometry studies in sea turtle hatchlings (Michel-Morfin et al., 2001; Sönmez et al., 2016; Sönmez et al., 2019).

Therefore, we aimed to develop a technique of sex determination based on image analysis of green turtles hatchlings (*Chelonia mydas*) with BioMorph software.

Material and Methods

The research area is Yeniyurt beach in Dörtyol district of Hatay province, which has been recently found and proposed a new green turtle (*C. mydas*) nesting beach (Turan et al., 2019).

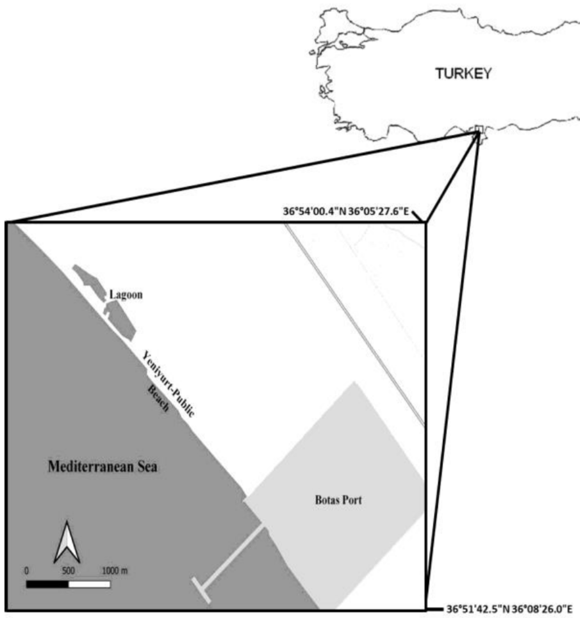

Figure 1. Map of the sampling locations of *Chelonia mydas*

Samples were collected during 2019 nesting season. Dead hatchlings were collected on the way to the sea. Fresh carcasses of hatchlings without any decomposition and bending were chosen in the field. After then, samples were transferred to the laboratory for gonad examine. Firstly, they photographed with a high resolution camera on dorsal view. Later, dead hatchlings were dissected, and their gonads were preserved in 4% buffered para-formaldehyde for gonadal histology. The sex of a hatchling was identified using a microscopic examination of gonad sections by checking the differentiation in gonadal medulla and cortex or the absence of seminiferous tubules (Yntema & Mrosovsky, 1980).

After sex determination with gonad histology, four female and four male dead green turtle hatchlings were used for identification of morphological differences between sexes with BioMorph software (Kutlu & Turan 2018).

Morphological data were generated from each sampled individual photos. Twenty-seven morphometric landmarks were obtained as descriptive on the green turtles' image with the BioMorph (Figure 2).

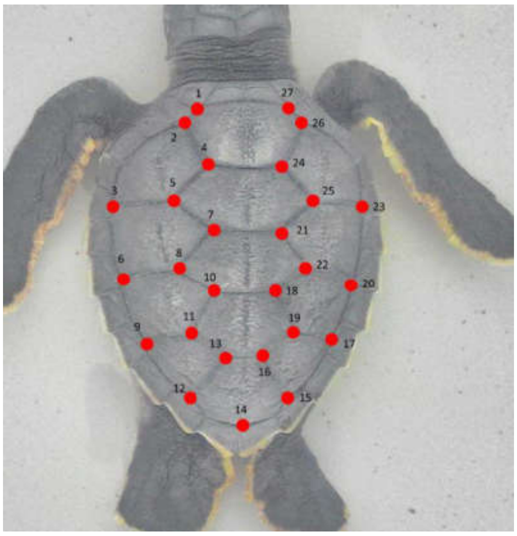

Figure 2. Twenty-seven morphometric landmarks obtained as descriptive on the carapace of *Chelonia mydas.*

In order to determine the biometric measurements, digital photographs from *C. mydas* were taken first, then these images were transferred to the computer from which measurements were made using computer aided software. The landmarks were determined on *C. mydas's* carapace surface on digital photo. In order to eliminate the process of marking manual land marking for each individual, automated predictions of the 27 landmarks were determined by BioMorph (Kutlu & Turan, 2018) for each sample after the first determination of the landmarks on the *C. mydas's* image.

Univariate analysis of variance (ANOVA) was used to test the significance of morphological differences. Created data from the BioMorph were submitted to Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) using SPSS.

Results

BioMorph produced 351 landmark distance measurements from the combination of 27 determined landmarks on the carapace of green turtle hatchlings. In the Variance Analysis Technique using BioMorph, morphometric characters (22-23, 23-25, 20-25, 20-26) revealed significant differences ($P<0.05$) between the sexes (Figure 2).

In PCA, 7 principal components were produced of which the first principal components (PC) explained 77% of morphometric variation and the second principal component explained 5% of morphometric variation (Table 1).

Table 1. Distribution of variance explained for each component by principal components analysis.

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	241.628	68.840	68.840	241.628	68.840	68.840
2	31.059	8.849	77.689	31.059	8.849	77.689
3	26.107	7.438	85.127	26.107	7.438	85.127
4	18.473	5.263	90.390	18.473	5.263	90.390
5	14.047	4.002	94.392	14.047	4.002	94.392
6	12.059	3.436	97.827	12.059	3.436	97.827
7	7.626	2.173	100	7.626	2.173	100

Examination of the contribution of each morphometric character to the first PC indicated that the characters such as 14_27, 6_20, 1_14, 14_26, 5_15, 1_15 have high loadings to the first PC (Table 2).

Table 2. Loadings of first principal component of morphometric characters. Variables were ordered by size of contribution to the differentiation.

Landmark	PC 1	Landmark	PC 1	Landmark	PC 1	Landmark	PC 1	Landmark	PC 1
14_27	0.992	**7_11**	0.966	**16_27**	0.917	**3_9**	0.850	**6_8**	0.686
6_20	0.990	**3_13**	0.966	**13_27**	0.915	**5_8**	0.850	**20_22**	0.684
1_14	0.988	**4_13**	0.966	**2_12**	0.914	**7_17**	0.848	**10_17**	0.683
14_26	0.987	**15_27**	0.965	**8_13**	0.913	**5_18**	0.848	**4_7**	0.681
5_15	0.987	**16_24**	0.962	**13_24**	0.912	**5_10**	0.848	**9_11**	0.681
1_15	0.987	**15_24**	0.962	**1_18**	0.912	**9_14**	0.847	**4_23**	0.681
5_14	0.987	**14_20**	0.961	**14_19**	0.911	**22_24**	0.846	**10_21**	0.677
9_25	0.987	**5_19**	0.960	**10_23**	0.910	**13_17**	0.845	**11_12**	0.672

2_14	0.986	**10_24**	0.960	**1_11**	0.907	**4_22**	0.844	**8_11**	0.668
3_14	0.985	**14_23**	0.959	**3_10**	0.907	**19_21**	0.842	**9_13**	0.667
6_14	0.985	**11_27**	0.958	**18_25**	0.906	**17_18**	0.841	**6_11**	0.665
15_25	0.984	**1_17**	0.958	**15_19**	0.906	**14_15**	0.840	**9_16**	0.662
6_25	0.984	**8_15**	0.957	**15_22**	0.905	**7_27**	0.840	**5_7**	0.659
9_20	0.982	**19_26**	0.956	**6_10**	0.905	**14_17**	0.838	**7_10**	0.651
14_25	0.981	**15_23**	0.955	**6_24**	0.904	**17_21**	0.837	**8_21**	0.642
2_13	0.981	**4_15**	0.955	**1_16**	0.903	**16_23**	0.835	**20_26**	0.637
18_24	0.981	**12_27**	0.954	**16_25**	0.902	**19_23**	0.835	**4_21**	0.636
1_19	0.980	**5_13**	0.954	**7_12**	0.902	**20_24**	0.833	**10_11**	0.630
3_16	0.979	**2_21**	0.953	**5_12**	0.898	**2_4**	0.831	**7_24**	0.628
4_14	0.978	**11_20**	0.953	**5_6**	0.897	**22_27**	0.827	**13_19**	0.627
2_15	0.978	**6_26**	0.951	**10_26**	0.896	**4_18**	0.827	**10_12**	0.624
3_19	0.977	**8_23**	0.951	**12_26**	0.895	**8_9**	0.826	**2_24**	0.623
15_26	0.976	**5_20**	0.951	**10_14**	0.892	**18_21**	0.826	**5_24**	0.621
3_15	0.976	**11_26**	0.950	**2_10**	0.891	**3_4**	0.825	**21_23**	0.617
4_16	0.976	**5_26**	0.949	**6_18**	0.890	**1_8**	0.825	**1_4**	0.614
6_23	0.975	**2_23**	0.948	**7_19**	0.889	**4_8**	0.821	**1_24**	0.612
3_26	0.975	**9_10**	0.948	**1_23**	0.888	**7_25**	0.820	**25_27**	0.574
9_27	0.975	**15_20**	0.948	**2_9**	0.888	**3_6**	0.818	**12_14**	0.567
3_20	0.975	**7_13**	0.947	**3_12**	0.887	**6_12**	0.816	**23_25**	0.562
6_19	0.974	**5_9**	0.946	**12_25**	0.886	**17_20**	0.815	**2_5**	0.561
6_16	0.972	**9_26**	0.945	**13_25**	0.885	**4_10**	0.814	**8_18**	0.557
6_15	0.971	**9_22**	0.945	**3_18**	0.885	**5_22**	0.814	**11_16**	0.547
6_13	0.971	**6_22**	0.943	**2_7**	0.884	**4_20**	0.811	**4_27**	0.520
9_23	0.970	**14_21**	0.942	**9_19**	0.883	**11_17**	0.811	**13_16**	0.211
7_14	0.970	**3_22**	0.942	**12_20**	0.883	**8_19**	0.805	**10_22**	0.541
2_16	0.970	**2_20**	0.942	**14_18**	0.882	**1_10**	0.799	**2_27**	0.472
6_27	0.970	**2_17**	0.942	**13_21**	0.882	**11_21**	0.798	**1_27**	0.506

2_19	0.968	**14_22**	0.940	**23_24**	0.881	**17_23**	0.797	**20_23**	0.503
3_23	0.968	**10_20**	0.940	**8_27**	0.881	**5_27**	0.796	**8_22**	0.541
14_24	0.967	**7_15**	0.939	**9_17**	0.881	**12_24**	0.796	**13_18**	0.595
7_11	0.966	**8_20**	0.939	**20_21**	0.881	**1_21**	0.795	**16_18**	0.482
3_13	0.966	**8_26**	0.939	**3_7**	0.879	**2_25**	0.794	**1_3**	0.317
4_13	0.966	**19_24**	0.938	**12_23**	0.878	**5_25**	0.789	**9_12**	0.452
15_27	0.965	**11_25**	0.938	**5_21**	0.877	**2_3**	0.788	**26_27**	0.553
16_24	0.962	**8_14**	0.937	**2_22**	0.876	**16_22**	0.786	**12_18**	0.441
15_24	0.962	**7_23**	0.937	**6_21**	0.875	**7_8**	0.785	**1_5**	0.272
14_20	0.961	**11_23**	0.936	**14_16**	0.875	**8_24**	0.780	**10_18**	-0.13
5_19	0.960	**10_25**	0.935	**16_20**	0.875	**1_22**	0.773	**24_27**	0.448
10_24	0.960	**16_21**	0.935	**2_8**	0.874	**1_6**	0.771	**12_13**	0.022
14_23	0.959	**6_17**	0.935	**4_6**	0.874	**12_17**	0.770	**11_18**	0.404
11_27	0.958	**3_25**	0.934	**7_16**	0.874	**20_27**	0.769	**21_22**	0.499
1_17	0.958	**8_17**	0.934	**4_12**	0.872	**18_19**	0.769	**21_25**	0.427
8_15	0.957	**13_15**	0.933	**13_20**	0.872	**20_25**	0.761	**10_19**	0.631
19_26	0.956	**5_17**	0.932	**18_23**	0.872	**11_19**	0.758	**24_26**	0.399
15_23	0.955	**9_24**	0.932	**17_27**	0.872	**9_18**	0.757	**10_16**	0.414
4_15	0.955	**19_25**	0.932	**3_27**	0.870	**13_14**	0.755	**16_19**	0.558
12_27	0.954	**1_20**	0.931	**22_25**	0.870	**22_26**	0.755	**15_17**	0.405
5_13	0.954	**2_18**	0.931	**4_9**	0.870	**3_8**	0.751	**21_26**	0.513
2_21	0.953	**19_27**	0.929	**18_26**	0.870	**11_13**	0.750	**4_5**	0.443
11_20	0.953	**17_24**	0.928	**3_24**	0.869	**1_7**	0.750	**24_25**	0.465
6_26	0.951	**5_23**	0.928	**10_27**	0.868	**2_26**	0.748	**4_24**	-0.03
8_23	0.951	**18_20**	0.926	**7_21**	0.868	**7_22**	0.745	**4_26**	0.502
5_20	0.951	**9_15**	0.925	**3_11**	0.867	**22_23**	0.741	**17_19**	0.541
11_26	0.950	**3_21**	0.925	**10_13**	0.866	**12_21**	0.735	**8_10**	0.506
5_26	0.949	**2_6**	0.924	**13_23**	0.866	**16_17**	0.734	**1_2**	-0.41
2_23	0.948	**2_11**	0.924	**10_15**	0.865	**11_22**	0.724	**12_15**	0.603

9_10	0.948	**3_17**	0.923	**7_26**	0.864	**13_22**	0.718	**12_19**	0.513
15_20	0.948	**16_26**	0.922	**3_5**	0.864	**1_26**	0.716	**4_25**	0.412
7_13	0.947	**4_11**	0.922	**1_9**	0.863	**17_22**	0.715	**12_16**	0.237
5_9	0.946	**7_20**	0.921	**9_21**	0.862	**21_24**	0.710	**25_26**	0.093
9_26	0.945	**5_16**	0.921	**6_7**	0.862	**8_12**	0.710	**23_26**	0.540
9_22	0.945	**15_21**	0.920	**7_9**	0.861	**23_27**	0.709		
6_22	0.943	**11_15**	0.919	**4_17**	0.861	**21_27**	0.705		
14_21	0.942	**1_13**	0.919	**17_25**	0.860	**7_18**	0.704		
3_22	0.942	**5_11**	0.919	**17_26**	0.859	**18_22**	0.703		
2_20	0.942	**15_18**	0.918	**11_24**	0.859	**8_16**	0.696		
2_17	0.942	**13_26**	0.918	**19_20**	0.859	**19_22**	0.695		
14_22	0.940	**1_12**	0.917	**18_27**	0.857	**6_9**	0.693		
10_20	0.940	**4_19**	0.917	**15_16**	0.857	**12_22**	0.690		
7_15	0.939	**11_14**	0.917	**8_25**	0.851	**1_25**	0.690		

In discriminant function analysis, overall random assignment of individuals into their original population was 50% that 50% of cross-validated grouped individuals of female and male were classified into their original group.

Table 3. Cross-validated grouped individuals of populations classified into its original group.

			Predicted Group Membership		
		Gender	**Female**	**Male**	**Total**
Cross-validated	Count	Female	2	2	4
		Male	2	2	4
	%	Female	50	50	100
		Male	50	50	100

Discussion

Traditional classical morphology and geometric morphology tests have been used to determine the morphological differences between female and male turtles (Michel-Morfin et al., 2001; Valenzuela et al., 2004; Lubiana & Ferreira-Junior, 2009; Delgado et al., 2010; Türkecan, 2010; Ceballos & Valenzuela, 2011;

Ferreira Junior et al., 2011; Sönmez et al., 2016; 2019). The geometric morphometry test, which gives more accurate results than traditional classical morphology, has been successful in determining the sex among non-sea turtle species (Valenzuela et al., 2004; Lubiana & Ferreira-Junior, 2009; Ceballos & Valenzuela, 2011). However, this test was tried on green turtle and it was failed results (Sönmez et al., 2019). On the other hand, in the *Caretta caretta*, the difference between the sexes was demonstrated by geometric morphometry (Türkecan, 2010; Ferreira Junior et al. 2011). However, in these studies, sex was indirectly defined by using incubation time and distance from nest to the sea to differentiate between male and female hatchlings. In the present study, the sexes were determined directly by gonad histology and the possible differences between male and female hatchlings were first revealed by image analysis in BioMorph software.

Significant morphometric variation was detected between individuals of female and male *C. mydas* from the Yeniyurt beach in the present study. The detected pattern of morphometric differences gives promises for further analysis with high number of individuals. Examination of the contribution of each morphometric character to the first PC indicates that the observed differences were mainly from the height and width measurements, demonstrating these characters to be important in the description of the sex specifications. Variance analysis techniques revealed that 4 character located on the upper-right side of the carapace were significantly different between sexes which also gives promises for further analyses with high sample size.

In conclusion, the landmark based morphometric measurements with highly increased number of measurements give promises on the sex determination based on image analysis of the carapace of green turtle hatchlings with BioMorph software.

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LENGTH-WEIGHT RELATIONSHIP AND FULTON'S CONDITION FACTOR OF ALBURNOIDES COSKUNCELEBII IN ASARSUYU STREAM (DÜZCE, TURKEY)

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Abstract

The length-weight relationship (LWR) is an important tool in fish biology, ecology and fisheries management, while the condition factor is a quantitative parameter of the state of well-being of a fish. The aim of the present study is to provide information about growth model and condition of *A. coskuncelebii* population living in Asarsuyu Stream which is moderately polluted stream in Düzce. A total of 295 fish specimens were caught by electrofishing from July 2018 to June 2019. The coefficient b of the LWR was 3.35 in females and 3.18 in males. These values suggest positive allometric growth for both males and females. There was a statistically significant difference in the slope of LWR regressions between the sexes (Student's t-test, t = 12.63, $P < 0.05$). Fulton's condition factors were estimated as 1.055 for females, 1.039 for males. This results provides information which can be useful for fishery biologists in developing conservation plan of the species through habitat protection.

Keywords: *Alburnoides coskuncelebii*, spirlin, length-weigh relationship, condition factor, Asarsuyu Stream, Büyük Melen River Basin

Introduction

The length-weight relationship (LWR) is an important tool in fish biology, ecology and fisheries management, while the condition factor (K) is a quantitative parameter of the state of well-being of a fish (Wooton, 1990). The relationships between body weight and length of fish are important for converting length observations into weight estimates (Froese, 2006), for estimating growth rates and biomass from length frequency data (Petrakis and Stergiou, 1995; Goncalves et al., 1997). Additionally, these relationships contribute to the comparison of life history and morphological aspects of populations between different regions of the