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MORPHOLOGICAL DISCRIMINATION OF FARMED AND WILD GILTHEAD SEA BREAM (*Sparus aurata* L. 1753) POPULATIONS OF NORTH-EAST MEDITERRANEAN

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This study was aimed to understand morphological discrimination of gilthead sea bream (*Sparus aurata,* L, 1758) from four wild populations (WENZ, WDGN, WKYC and WYMR) and two farmed populations (FRMA and FRMB) which were collected along Mediterranean coast of Turkey. After photographing each specimen, 10 landmarks were determined to understand morphological structure of all populations by tpsDig 232 software program. With all landmark measurements, PCA and DFA were used to see the discrimination of wild and farmed gilthead sea bream populations. As a result, significant differences were found on twenty morphological characters and no significant differences were found in two morphological characters of head in wild populations. This could be due to similar feeding habitat and environments (lagoon) which did not let differ wild population on these two characters. However, morphological characters of farmed populations were significantly different than all wild populations. It is known that some of Turkish hatcheries use Atlantic origin gilthead sea bream breeders which show better reproduction quality than Mediterranean origin. Discrimination between farm and wild population might be occurred by this case. On the other hand, selective breeding studies might have affected the phenotypical characters of farmed sea bream. This indicates that phenotype of wild gilthead sea bream populations had not been affected by farmed fish escapees in the main aquaculture region yet.

Keywords: *Sparus aurata*, Truss network, morphology, discriminant function analysis,

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INTRODUCTION

It is known that phenotype of an organism is determined by its genotype and its environment. Besides, genetic structure of a population, feeding habits, environment, reproduction activities and fishing pressure are important factors on morphology. The method of morphometric discrimination has been one of the major methods to understand phenotypic differences or similarities of animal and plant populations. While the wild population of fish stocks might have been affected by environmental or physiological condition, escaped individuals from cages also affect wild populations. It is obvious that farmed individuals escape from farm facilities because of technical and operational mistakes. Since *Sparus aurata* and *Dicentrarchus labrax* have been two of the main farmed species in Mediterranean, researchers have been pondering about the effects of both farmed fish species on the morphology of wild populations. The fish escape from the farm could be present in spawning areas and could mix with wild populations which were seen in the case of salmonids (Naylor *et al.,* 2005) and cod (Uglem *et al.,* 2008; Meager *et al.,* 2009; Arechavala-Lopez *et al.,* 2012). Therefore, finding the morphological differences or similarities of wild and farmed fish population is a big phenomenon.

Escape is not only a problem for the fisheries, but it also affects consumer preference, which is another important issue. In the Mediterranean countries, many consumers still prefer to consume wild fish instead of farmed fish because of their prejudgment on nutritional content, artificial feeding, body shape and health conditions of cultured fish, also drug and chemicals use during farming activities. Because of consumers' beliefs and perceptions on nutritional quality, health, morphological differences or abnormalities of cultured fish, the price of wild fish has become two or three times higher than cultured fish (Rogdakis *et al.,* 2011; Grigorakis *et al.,* 2002).

S. aurata is one of the important species for Mediterranean aquaculture and also for local fisheries. Artificial breeding was successfully achieved in Italy in the beginning of 80's and a large-scale production of gilthead seabream juveniles was definitively achieved in 1988-1989 in Spain, Italy Greece and Turkey (Alpbaz, 1996; Chavanne *et al.,* 2016). In the last fifteen years, gilthead sea bream production increased to 51,844 ton in 2015 from 15,460 ton in 2000 in Turkey (Turkish Statistical Institute, 2017) while it increased to 166,794 in 2015 from 87,303 ton in 2000 in the Mediterranean basin (Food and Agriculture Organization, 2016). At the beginning of Turkish aquaculture industry, this production was mostly dependent on juvenile collection from nature by shore seine and line-fishing in lagoon systems due to the limited number of commercial marine fish hatcheries and lack of knowledge about hatchery techniques (Sahin, 1995). Collecting juveniles from nature was banned in 1997 in Turkey meanwhile the number of commercial hatcheries increased. Chavanne *et al.* (2016) indicated that Turkey is the second biggest gilthead sea bream juvenile producer (185 million) after Greece (245 million).

Since the consumer preference and escapee are important factors on wild fish populations, there are many researches carried out to find out its effects on nature, genetically and morphologically (Friedland *et al.*, 1994; Loy *et al.*, 1999; Sara *et al.*, 1999; Loy *et al.*, 2000; Rogdakis *et al.*, 2011; Arechavala-Lopez *et al.*, 2012; Segvic-Bubic *et al.*, 2014). Arechavala-Lopez *et al.* (2012) indicated that local fisheries play a major role on the removal of individuals escaped from farms (except Marine Protected Areas). Segvic-Bubic *et al.* (2014) revealed that non-fishing pressure on MPAs leads to an increase in the survival rates of escapees combined with the ability of *S. aurata* to adapt to the wild environment. In order to understand the effects of escapes on wild population, it is necessary to study with genetics tools.

Besides genetic structure information, investigation on morphological aspects helps to understand the effect of escapees on wild population. In previous studies, it was shown that significant differences provided through morphometry evidence of seabream and seabass are different regarding their farm or wild origin at different scales in France, Spain, Greek and Croatian waters (Loy *et al.*, 1999; Rogdakis *et al.*, 2011; Arechavala-Lopez *et al.*, 2012; Segvic-Bubic *et al.*, 2014). In one of the studies from Turkey, there were no differences found between farmed and wild population of gilthead sea bream (Coban *et al.*, 2008). The main reason of differences on morphological structure of farmed fish could be related to hatchery techniques and feeding habits. Loy *et al.* (1999) investigated the importance of larval and post-larval rearing of gilthead seabream in different culture conditions from two different countries (France and Spain). There are other studies carried out on other Sparidae members which are economically important for Mediterranean fisheries such as *Diplodus sargus* (Kaouèche *et al.*, 2017) *Diplodus puntazzo, Lithognathus mormyrus* (Palma and Andrade, 2002; Hammami *et al.*, 2011).

Even though several studies showed no morphological or genetic differences on wild and farmed populations along the Turkish coasts, large scale studies are required to better understand the morphological relation of wild and farmed populations of gilthead sea bream along the North-East Mediterranean coast to give an insight for future studies on environment and/or escapee. The aim of this study was to evaluate the morphological discriminations of wild and farmed gilthead sea bream in North-East Mediterranean coast (East Aegean and Levantine Coast of Turkey).

MATERIALS AND METHODS

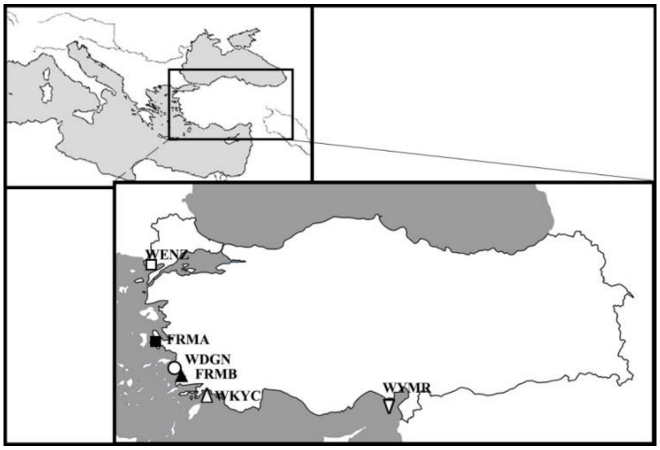
Specimens: The sampling areas of wild populations were chosen considering where the commercial hatcheries have been collecting wild broodstock candidates. Farm populations were selected from different cage units where the juveniles come from different hatcheries. A total of 221 individuals (Table 1) were collected from four wild populations from local fishermen or lagoon cooperative in July-December 2016. Body deformation was not observed in any wild individuals and also same sizes of the specimen were selected in order to avoid generation differences and size-dependent variation. Total 100 individual samples were supplied from two different cage units of one commercial fish farm in February 2017 but 85 of them were used in analyses. All specimens were transferred to the laboratory of Fisheries Faculty at Akdeniz University in an ice box and they were weighted by digital scale (0.01 gr precision) and the total length was measured by fish measuring board (1 mm precision) (Table 1). Left side of each specimen was photographed by digital camera (NIKON D60). A scale (mm) was used for photography to make correct calibration during the image processing.

Landmarks and image processing: Ten landmarks were determined on the gilthead seabream and 22 truss network measurements were collected from each specimen (Çoban *et al.*, 2008; Segvic-Bubic *et al.*, 2014). For digitizing landmarks tpsDig2 software (Rohlf, 2005) was used. All the images of specimen processed X and Y coordinates were saved by software. The landmarks are shown, and measurements are described in Figure 2. Distances between landmarks were calculated by Pythagorean Theorem in MS Excel.

Table 1. Description of sampling data (mean ± SD).

Location	Abbreviation	Origin	N	Total length (cm)	Total weight (g)
Enez	WENZ	Lagoon	61	19.54±0.12	109.16±1.86
Doganbey	WDGN	Open sea	49	18.54±0.15	86.65±2.89
Koycegiz	WKYC	Lagoon	63	19.58±0.17	110.65±3.28
Yumurtalık	WYMR	Lagoon	48	18.75±0.11	97.74±1.91
Izmir	FRMA	Cage	45	23.17±0.18	203.01±5.70
Akbuk	FRMB	Cage	40	15.33±0.19	59.10±2.38

Statistics**:** Before performing statistical analysis, size-dependent variation was removed using an allometric approach given by Reist (1985): $M_{trans} = \log M - \beta (\log SL - \log SL_{mean})$, where M_{trans} is the transformed measurement, M is the original measurement of the distance between landmarks, β is the within-group slope regressions of log M vs. log SL, SL is the standard length of the fish and SL_{mean} is the overall mean of the standard length.



(□: WENZ; ○: WDGN; △: WKYC; ▽: WYMR; ■: FRMA; ▲: FRMB)

Figure 1. Sampling locations of gilthead sea bream *Sparus aurata* along the north-eastern Mediterranean coast.

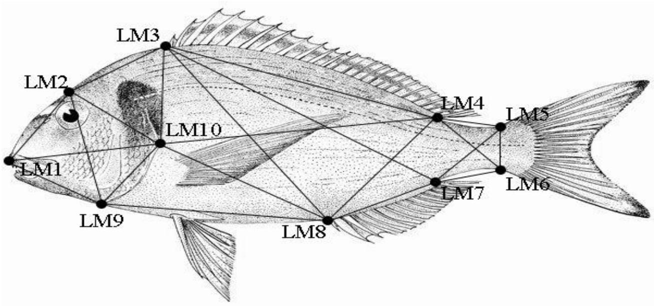


Figure 2. Schematic drawing of gilthead sea bream (*Sparus aurata*) body with measured distances and landmarks (LM: Landmark).

All statistical analyses were done in between wild populations and also between wild and farmed populations separately in order to understand morphological differences of wild population more efficiently. After normality test applied showed no deviation between the individuals in each population, one-way analysis of variance was used to check the significant differences on all the morphometric measurements among the populations. In addition, the t-test was applied to understand the averages of variable, which are significantly different between wild and farm population. Principal component analysis (PCA) was used to examine the contribution of each of the 22 morphometric measurements in the shape of fish with varimax rotation. All eigenvalues of PCAs bigger than 1 were accepted as important (Segvic-Bubic *et al.*, 2014). The discriminant function analysis (DFA) (Kaouèche *et al.,* 2011) was used to understand the pattern of morphological discrimination between populations and to disclose the degree of similarities and/or differences between the populations. Wilk's λ is used to test for significant differences of on the individual predictor variables in between the samples. It tells which variables contribute a significant amount of prediction to help separate the groups.

RESULTS

PCA analysis showed that 81.07% of the total variation associated with the 22 morphometric characters was accounted for the first seven principal components (Fig. 3A) for wild populations. However, in farm populations 86.21% of total variation was accounted for the first three principal components (Fig. 3B). That means while 7 variables of 22 characters could explain enough variance in wild population, only 3 variables were enough to explain the variance which has a strong separation with few amounts of characters.

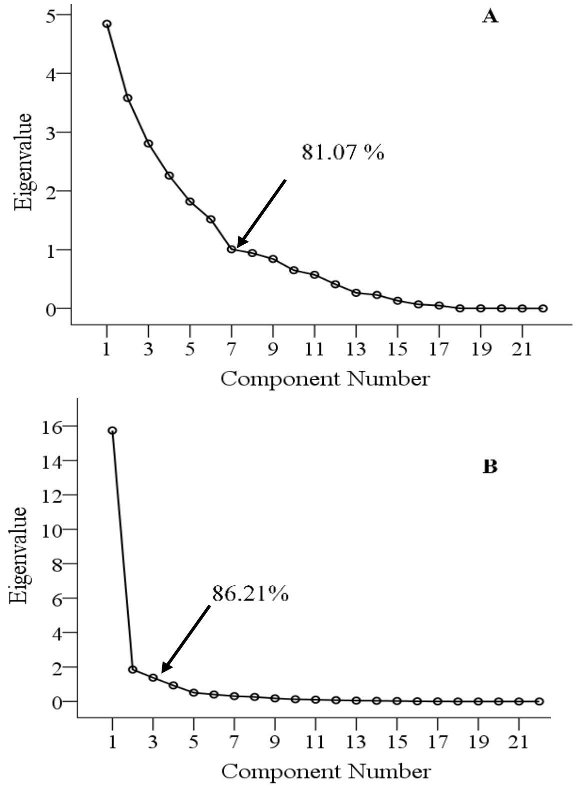


Figure 3. Plots of Eigenvalue of PCA components. A: wild populations, B: all populations

Table 2. Tests of Equality of Group Means. Wilks' λ , F, and Significance value of each characters.

	Wild Populations			All Populations		
Characters	**Wilks' λ**	**F**	**Significance**	**Wilks' λ**	**F**	**Significance**
LM1-2	0.959	3.114	0.027	0.401	89.660	0.000
LM1-10	**0.977**	**1.705**	**0.167**	0.379	98.269	0.000
LM1-9	0.883	9.608	0.000	0.372	101.324	0.000
LM2-3	0.941	4.506	0.004	0.317	129.447	0.000
LM2-10	0.926	5.821	0.001	0.325	124.646	0.000
LM2-9	0.924	5.963	0.001	0.297	142.166	0.000
LM3-4	0.868	11.040	0.000	0.269	162.861	0.000
LM3-7	0.850	12.758	0.000	0.247	182.437	0.000
LM3-8	0.864	11.412	0.000	0.268	163.760	0.000
LM3-10	0.811	16.826	0.000	0.291	145.844	0.000
LM4-5	0.845	13.255	0.000	0.419	83.182	0.000
LM4-6	0.908	7.343	0.000	0.315	130.604	0.000
LM4-7	0.806	17.407	0.000	0.288	148.067	0.000
LM4-8	0.807	17.316	0.000	0.293	144.629	0.000
LM4-10	0.824	15.499	0.000	0.237	193.696	0.000
LM5-6	0.915	6.691	0.000	0.319	128.351	0.000
LM5-7	0.803	17.741	0.000	0.339	117.057	0.000
LM6-7	0.922	6.104	0.001	0.551	48.827	0.000
LM7-8	0.826	15.190	0.000	0.354	109.605	0.000
LM8-9	0.822	15.672	0.000	0.249	181.094	0.000
LM8-10	0.833	14.541	0.000	0.244	185.884	0.000
LM9-10	**0.987**	**0.984**	**0.401**	0.401	89.527	0.000

Value in bold didn't show significant differences. P>0.05,

Table 3. Classification results for the discriminant analysis (Predicted Group Membership).

Population	**WENZ**	**WDGN**	**WKYC**	**WYMR**	**FRMA**	**FRMB**	**Total**
WENZ	52 (85.2)	1 (1.6)	7 (11.5)	1 (1.6)	0	0	61 (100.0)
WDGN	2 (4.2)	37 (77.1)	2 (4.2)	7 (14.6)	0	0	48 (100.0)
WKYC	7 (11.1)	2 (3.2)	51 (81.0)	3 (4.8)	0	0	63 (100.0)
WYMR	1 (2.1)	5 (10.4)	3 (6.3)	39 (81.3)	0	0	48 (100.0)
FRMA	0	0	0	0	45 (100.0)	0	45 (100.0)
FRMB	0	0	0	0	0	40 (100.0)	40 (100.0)

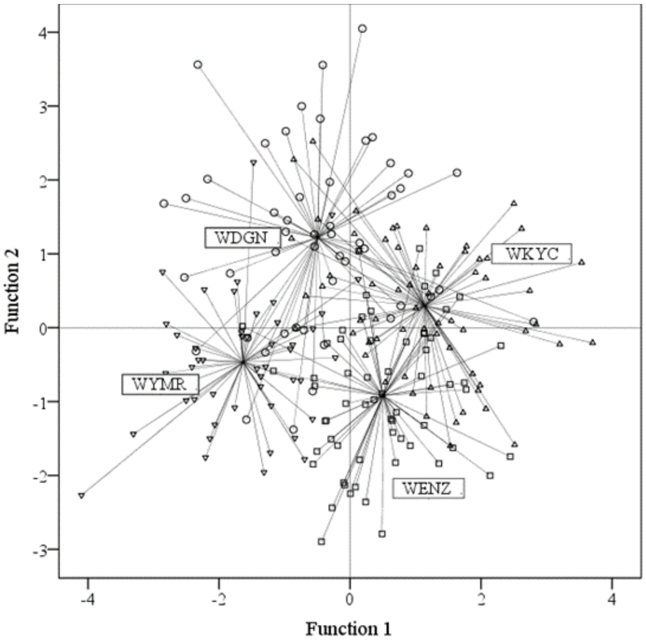
Value in the row indicated the number of individual in each population and value in parenthesis are indicated the percentage in the population.

The predicted group membership shows how many individuals are morphologically classified with which populations and 86.6% of original grouped cases are correctly classified (Table 3). In this table it can clearly be seen that the least corrected classification was in WDGN (77.1%) population because in total 11 out of 48 individuals have common morphometric characters with other wild populations. WENZ (85.2%) population was the most correctly classified by morphological characters in wild populations. FRMA and FRMB populations which come from two different hatcheries were mostly isolated populations with 100.0% membership rate (Table 3).

The scatter plot of the two canonical variables shows all wild populations separated by morphometric characteristics (Fig. 4). When wild and farm populations were analyzed together, it was observed that wild populations were gathered in one location and farm populations separated from wild and from each other (Fig. 5A) but when details of wild population are examined, it is seen that WDGN and WYMR populations have more morphological similarities than the other two wild populations (Fig. 5B).

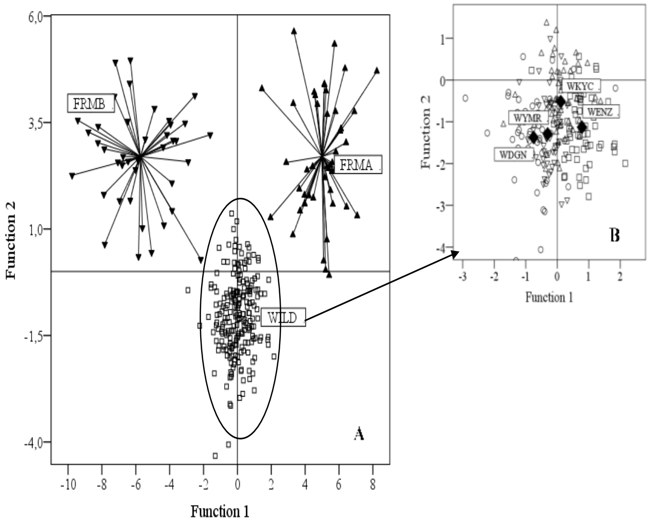
This might be the results of their similar feeding habitat and behaviors in the lagoon system. When farmed population were added in analysis, this showed two of the farmed population significantly discriminated from wild populations. This case might be the reason of different feeding habitat of farmed fish and swimming performance than wild fish and/or can be explained by initial selective breeding studies which had been started in commercial hatcheries in Turkey.

According to Wilk's λ value, it was determined that when excluding LM1-10 (P>0,05), LM9-10 (P>0,05) characters from head out of 22 investigated characters, all other 20 morphological parameters belong to body and caudal region showed significant important differences ($P<0.05$) which explained the discriminations between four wild populations (Table 2).



(□: WENZ; ○: WDGN; △: WKYC; ▽WYMR)

Figure 4. Canonical discriminant functions of the discriminant analysis of wild populations.



A (□: WILD ■: FRMA; ▲: FRMB) and detailed view of wild population B (□: WENZ; ○: WDGN; △: WKYC; ▽: WYMR; ◆: centroids)

Figure 5. Canonical discriminant functions of the discriminant analysis of wild and farm populations.

The similarities of head region and caudal pentacle might be the reason of similar feeding and swimming behavior in similar lagoon environments of wild population as these five characters were related to the head region. Significant differences on body length, depth and fin length of wild fish showed the strong effect of food availability in their habitat because most of these characters are related to condition factor of the samples.

Farmed populations added in analysis showed that two of the farmed populations were significantly discriminated from wild populations by all the morphological characters. This might be the reason of different feeding habitat of farmed fish and swimming performance than wild fish.

DISCUSSION

As *Sparus aurata* is one of the pioneer species in Mediterranean aquaculture, in the last decade, researchers have intended to see the environmental effects of hatchery reared population on wild population due to escape. Morphological characters are influenced by habitat, sampling time, rearing environment, broodstock origin, or selective breeding programs applied in aquaculture (Rogdakis *et al.*, 2011; Segvic-Bubic *et al.*, 2014). In this study, it is investigated clearly that wild and farmed population of gilthead sea bream morphologically discriminate from each other. Although the environment of the lagoon systems seems similar to culture environment, farmed fish are stocked in the cages or tanks with a periodic and sufficient feeding rate, which allows them to get available food easily (Merigoux and Ponton, 1998) and these cases discriminate the morphological shapes from wild fish (Arechavala-Lopez *et al.*, 2012). In addition to that knowledge, this kind of differences might be partly explained by dietary shifts, which induce changes on the body shape (Keast, 1978). Moreover, farmed fish species shape anomalies have been widely reported under culture conditions (Divanach *et al.*, 1996).

In current study it is investigated that head morphology, fin lengths and body depth of wild and farmed fish were significantly different ($P<0.05$) while two of the head morphology was not significantly different in between wild populations (Table 2). It is thought that larval rearing conditions (Loy *et al.*, 1999), physico-chemical conditions (Kaoueche *et al.*, 2017) and environmental characteristics seemed to be related to the discrimination between wild and farmed fish. Besides, similar lower chin formations in wild population might relate to the feeding behavior of wild populations in lagoon system.

Although WYMR is the most distant population, when all the samples were analyzed together, DF analysis showed similarities between WYMR and WDGN populations (Fig. 5B), which may result from the juvenile transfer from WYMR area to WDGN area (Fig. 1) for early aquaculture in

Turkey. Bilgen (2002) examined genetic similarities of *Sparus aurata* broodstock from south east and west coast of Turkey (nearby WYMR and WDGN populations in current study, respectively) by RADP method and found limited genetic separation between two populations, which indicated a gen exchange between two populations. This might explain the morphological similarities between WYMR and WDGN populations in findings.

Similar studies were carried out on sea bass along the Turkish coasts and similarities with our results were found where the population separation between Aegean and Levantine Sea are clearer (Erguden and Turan 2005; Bodur *et al.*, 2017). Bodur *et al.* (2017) found the geographical barrier at Gulf of Fethiye which goes 4000 m depth hadal zone limits the gen flow between neighboring wild populations of sea bass. This could be a reason of barely differences in four wild populations.

The clear morphological separation of two farmed populations from each other and from wild population might be the reason of several cases. Significant differences of morphological characters could be cause of the selective breeding programs, genetic drift following founding generations, or the different origin of fish used as broodstocks (Rogdakis *et al.*, 2011; Arechavala-Lopez *et al.*, 2012; Segvic-Bubic *et al.*, 2014). It is known most of the gilthead sea bream hatcheries supply the breeders (especially females) from Atlantic (De Innocentiis *et al.*, 2004) in many Mediterranean countries and in Turkey (B. Okçelik, personal communication). In this study the origin of broodstock might be one of the important results of morphological separation. Another possible reason for this separation level could be selective breeding studies and/or wrong broodstock management. Also, it is indicated that some of the Turkish hatcheries use same breeders for several years (Çoban *et al.*, 2004) and *S. aurata* is protandric hermaphrodite which may cause inbreeding depression in the stocks (Alarcòn *et al.*, 2004) if same breeder would be used as male and later female and this might lead an affect the body shape of individuals. To understand the main reason of these morphological differentiations of farmed population a well design genetic analysis should be done. On the other hand, in a study, it was investigated that hatchery released *S. aurata* has been changing its shape to wild-like shape after a 6-7 months in a wild habitat (Rogdakis *et al.*, 2011).

Canonical discriminant functions of the discriminant analysis of wild and farmed populations supported the predicted classification correction as both farmed populations are significantly unlike compared to the wild populations. Loy *et al.* (1999) found the evidence of discrimination between *S. aurata* from two different hatcheries and explained this differentiation with different larval and post-larval rearing conditions. Current study has similar results as two hatchery origins' samples are morphologically well discriminated. This might also be due to the broodstock management techniques of different hatcheries since it has been known that they sometime mix different wild origin populations.

In a previous study on morphometric comparison of wild and farmed *S. aurata* populations, morphological similarities were found (Çoban *et al.*, 2008) in contrast to the results of current study. This might be the reason of broodstock origin of cultured populations, which is again an important issue for these similarities in previous study.

In conclusion, in this study wild and farmed populations of seabream are morphologically separated. This shows morphological characters of wild populations of gilthead sea bream did not affect by farm escape. Genetic tools are needed to better understand this difference affecting the genetic characteristics of populations which are located at the most preferred broodstock collection regions of hatcheries. On the other hand, results showed that the image analysis is a satisfying tool to understand the discrimination of morphological characters of *S. aurata* along the North-East Mediterranean Coasts.

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