

RESEARCH ARTICLE

The bio-ecological and genetic characteristics of sand steenbras (*Lithognathus mormyrus*) in the Black Sea

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Abstract

The sand steenbras (*Lithognathus mormyrus* L., 1758) are generally distributed in the Mediterranean Sea, and it is not yet commercially crucial for the Black Sea fisheries. Although *L. mormyrus* are caught by fishermen with increasing amounts in recent years, there is a lack of information on the subject of biological features of the Black Sea species. In this study, length-weight distribution, morphologic and meristic features, age, sex distribution (according to histological examination), and genetic characteristics of *L. mormyrus* in the Black Sea were examined. Besides, the genetic analysis showed a significant level of genetic differentiation between three populations (Black Sea, Mediterranean and Aegean Sea) and populations.

Keywords: *Lithognathus mormyrus*, sand steenbras, Black Sea, growth, genetic

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Introduction

The sand steenbras (*Lithognathus mormyrus* L., 1758) distributes in the eastern Atlantic, Mediterranean Sea, Aegean Sea, Red Sea, and southwestern Indian Ocean (Bauchot and Hureau 1990). This species lives in littoral waters with sandy, rocky or muddy bottoms and seagrass beds from the surface to 50 m depth (Bauchot and Hureau 1986; Pajuelo *et al.* 2002). *Lithognathus mormyrus* feeds

on crustaceans, worms, molluscs, sea urchins, and small fishes. The reproduction period of this species occurs during the months of spring and summer. This species has gonadal sex reversal as a protandrous hermaphrodite fish species (Whitehead *et al.* 1986; Kraljevic *et al.* 1995; Turkmen and Akyurt 2003). Several studies were carried on the age, growth, reproduction, geographical distributions, length-weight relationships of *L. mormyrus* in the Mediterranean Sea and the Aegean Sea (Suau 1970; Kraljevic *et al.* 1995; 1996; Lorenzo *et al.* 2002; Palma and Andrade 2002; Kallianiotis *et al.* 2005; Hammami *et al.* 2007; Emre *et al.* 2010). It is commercially important for the coastal and lagoon fisheries in the Mediterranean and the Aegean coast of Turkey (Emre *et al.* 2010). Although this species had been seen in the Black Sea coastal zone in the past (Slastenenko 1955), it was not seen for a long time. However, it is frequently found in the gillnets nowadays (Engin *et al.* 2015; Kasapoglu *et al.* 2016; Aydin 2017a, 2017b; Guchmanidze and Boltachev 2017) because of the Mediterranzation of the Black Sea waters (Turan *et al.* 2009). For this reason, we investigated the reproduction, meristic, and morphometric characteristics of *L. mormyrus* and genetic differences among the Mediterranean Sea, Aegean Sea, and the Black Sea populations.

Materials and Methods

The sampling was done by gillnets (mesh size 20 mm) from 20 m depth in the coast of Faroz, Trabzon (41° 0'42.74 "N 39° 42'32.53 "E and 41° 0'43.41 "N 39° 42'35.27 "E) (Figure 1) on 19 October 2015 to determine morphological and meristic features of *L. mormyrus*. The specimens were weighed and measured following the measuring scheme in Figure 2.

All length measurements were made with a digital caliper, which is the nearest 1 mm precision. The specimens were weighed within 0.01 g precision. Both left and right otoliths were analyzed with a stereomicroscope (Leica MZ75) on a black background for age determination. A total of 97 samples were used in this study. Forty-one individuals were caught in the Black Sea, and they used for growth, morphometric and meristic measurements, while 56 specimens were used for genetic analyses in order to difference level of the populations (12 specimens from 12 m depth for the Black Sea, 41° 00'37.89 "N 39° 42'19.82 "E and 41° 00'38.15 "N 39° 42'21.00 "E, 20 specimens from 41 m depth for the Mediterranean Sea, 36° 50'33.57 "N 30° 38'38.22 "E and 36° 50'38.40 "N 30° 38'52.83 "E, and 24 specimens from 16 m depth for the Aegean Sea, 38° 31'47.31 "N 26° 40'40.36 "E and 38° 32'07.94 "N 26° 40'50.99 "E).

For the histological examination, stained sections were examined under the light microscope via NIS-Elements Advanced Research software (Harris *et al.* 2001; Ünal *et al.* 2014). According to MEDITS Protocol (2017) and Follesa and Carbonara (2019), the maturity stages were determined.

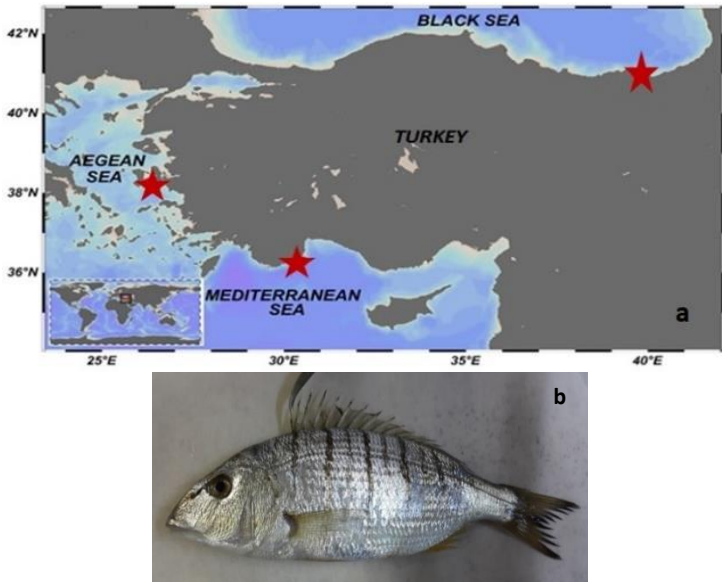


Figure 1. a. Sampling stations of this study (Schlitzer, R. (2014) Ocean Data View), b. *Lithognathus mormyrus* (Photo taken by Kasapoglu and Çankırlılıgil, 2015).

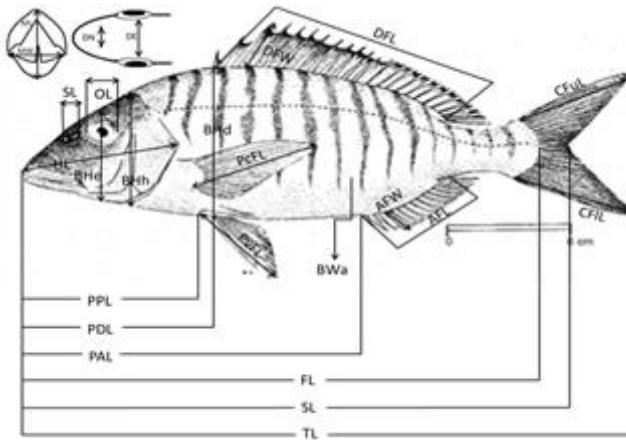


Figure 2. Morphometric measurements of *Lithognathus mormyrus* (modified from Fischer *et al.* 1987). All measurements are point to point.
 (TL, total length; W, weight; SL, standard length; FL, fork length; PPL, pre-pelvic length; PDL, pre-dorsal length; PAL, pre-anal length; BHd, dorsal fin based body height; BWa, anal fin based body width; DFL, dorsal fin length; DFW, dorsal fin width; PpFL, pectoral fin length; PvFL, pelvic fin length; AFW, anal fin width; AFL, anal fin length; CFuL, caudal fin upper lobe length; CFIL, caudal fin lower lobe length; HL, head length; SL, snout length; OL, orbital length; BHh, head based body height; BHh, Eye Based Head Height; DN, distance between nostrils; DE, distance between eyes; ML, mouth length; MW, mouth width)

In the determination of genetic characteristics, approximately 1.0x1.0 cm sized tissue was collected from the anal fin of the fish and stored in 98% ethanol. Total DNA isolation was made using commercial kits. The purity estimation and DNA concentration of the samples were specified by UV/visible spectrophotometer (BIO-RAD, The SmartSpec Plus) in 260 and 280 nm wavelengths. COI, Cyt-b, 16S, and 12S mitochondrial gene regions were amplified with PCR from the total DNA of the sand steenbras for genetic characterization. The samples were purified for the sequence of all gene regions, and the DNA sequence was done by ABI 3500 device. Raw data of mtDNA, COI, 12s, 16s, and Cyt-b regions were evaluated and converted into FASTA format. Ultimately, achieved data were aligned BioEdit software version 7.2.5 (Hall 1997). ARLEQUIN (v.5) software was used for FST pairwise values and genetic heterogeneity (Schneider *et al.* 2000). Molecular variance analysis was used for determining hierarchical population structure (AMOVA; Excofier *et al.* 1992), and Tajima D statistics were used for populations by DnaSP (v.5.10.01) (Rozas *et al.* 2003). Finally, phylogenetic analyses were carried out with popArt software to construct a phylogenetic tree.

Results

The mean length and standard deviation were calculated as 19.31±2.11 cm, 20.65±2.99 cm, and 20.08±2.25 cm, while the mean weight was 106.42±37.91 g, 125.17±40.62 g, and 115.80±39.81 g for female, male, and the pooled samples, respectively. The maximum age was observed as three years for females and two years for males. The length-weight relationship was determined as $W=0.0246L^{2.807}$ for all specimens. Figure 3 shows that 54.76% of the samples were found between 18 and 20 cm. According to the meristic measurements, the count of fin rays was found as XI+13, V+3, and XI+3 for dorsal, pectoral, and anal fins, respectively. Scale count was counted as 62 in the linea lateralis.

The gonadal sections were examined for male, female and hermaphrodite individuals, and they were determined in the III stage of the maturity of each sex (Figure 4). It is proved that the species can reproduce in the Black Sea habitat. Two hermaphrodite individuals were observed in the sampling, which was measured as 21.8 and 23.8 cm. For the first time, the hermaphroditism was observed with histological sections in this species for the Black Sea. The sex ratio was calculated as 52.38% for males, 38.10% for females, and 9.52% for hermaphrodites. The morphometric measurements were realized according to gender in Table 1 after excluding the hermaphrodite individuals.

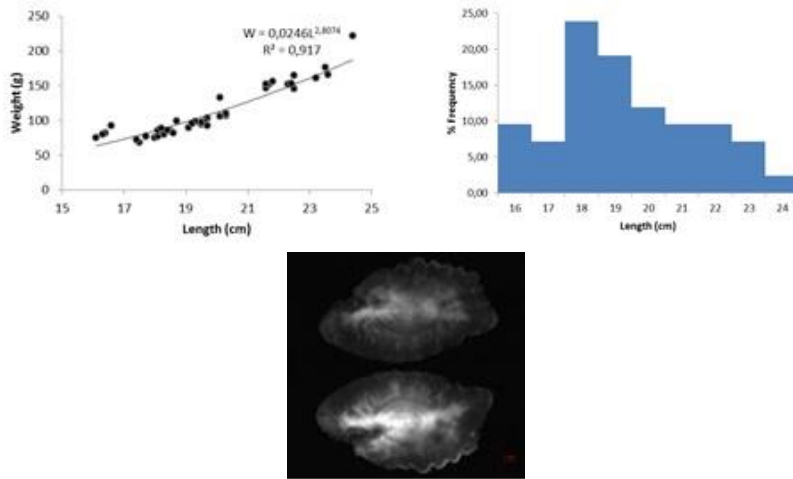


Figure 3. Length-weight relationship and length-frequency and otolith of the sand steenbras

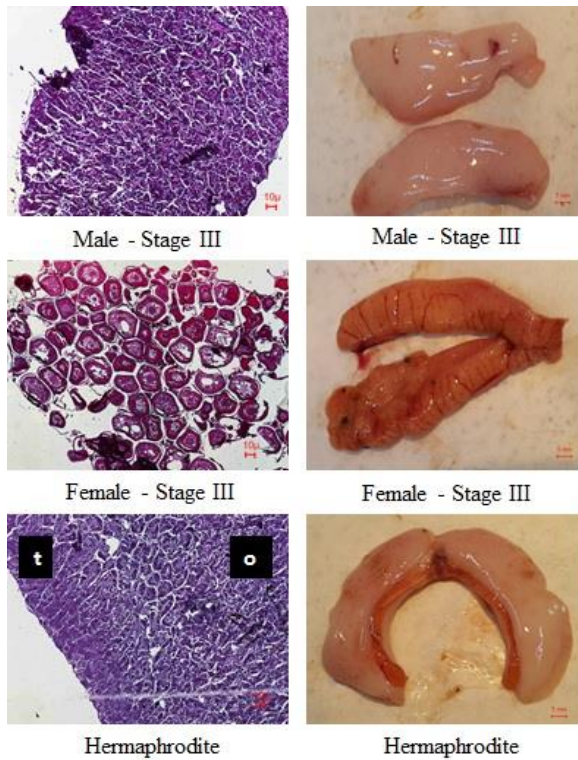


Figure 4. The gonad sections of the samples of *Lithognathus mormyrus* (Hermafrodite individuals: 22.6 cm, 2 year old; o: ovarium; t: testis)

Table 1. Measurements of *Lithognathus mormyrus* (SD: Standart Deviation)

| Measurements | ♀ | | ♂ | |
|------------------------------|--------------|---------------|-------------|---------------|
| | Mean±SD | Range | Mean±SD | Range |
| Total Length | 191.25±9.92 | 161.00-244.00 | 201.55±6.85 | 174.00-235.00 |
| Fork Length | 166.60±10.21 | 125.00-210.46 | 179.69±6.81 | 148.00-207.61 |
| Standard Length | 154.55±9.09 | 119.00-196.75 | 166.58±5.08 | 145.00-190.01 |
| Pre-pelvic Length | 55.23±3.06 | 45.75-68.23 | 58.28±2.00 | 48.93-65.92 |
| Pre-dorsal Length | 63.95±5.28 | 52.17-97.13 | 64.44±2.36 | 54.78-74.26 |
| Pre-anal Length | 97.95±4.23 | 82.56-117.40 | 98.56±2.71 | 85.03-108.66 |
| Dorsal Fin Based Body Height | 52.37±4.02 | 41.65-76.11 | 55.29±2.39 | 44.18-67.34 |
| Anal Fin Based Body Height | 46.05±2.63 | 39.68-61.88 | 47.18±1.54 | 40.21-55.36 |
| Dorsal Fin Length | 79.02±3.43 | 69.63-97.05 | 81.78±3.21 | 71.51-99.05 |
| Dorsal Fin Width | 14.72±0.90 | 12.27-20.26 | 15.06±0.48 | 13.21-18.41 |
| Pectoral Fin Length | 33.29±1.38 | 29.81-41.87 | 35.48±1.06 | 31.68-41.39 |
| Pelvic Fin Length | 22.73±0.98 | 19.53-28.64 | 24.16±0.85 | 19.84-29.08 |
| Anal Fin Width | 12.12±1.28 | 6.87-16.68 | 12.73±1.11 | 8.01-18.64 |
| Anal Fin Length | 27.99±4.25 | 22.14-35.16 | 29.86±1.22 | 24.79-36.90 |
| Caudal Fin Upper Lobe Length | 39.40±1.79 | 34.79-50.76 | 41.49±1.14 | 36.04-47.83 |
| Caudal Fin Lower Lobe Length | 34.91±1.25 | 31.04-41.67 | 37.39±1.09 | 32.85-42.47 |
| Head Length | 47.66±2.69 | 37.89-60.05 | 50.23±1.68 | 43.58-60.1 |
| Snout Length | 20.69±1.66 | 14.09-29.60 | 23.04±0.93 | 18.65-28.26 |
| Orbital Length | 8.90±0.43 | 6.84-9.98 | 8.88±0.27 | 7.58-10.19 |
| Head Based Body Height | 47.11±2.93 | 40.04-63.86 | 47.85±1.35 | 41.57-55.09 |
| Eye Based Head Height | 35.02±1.33 | 30.96-42.05 | 35.03±0.78 | 31.65-40.72 |
| Distance Between Nostrils | 9.96±0.89 | 6.14-13.56 | 10.68±0.51 | 8.07-13.67 |
| Distance Between Eyes | 15.19±1.19 | 10.01-19.43 | 15.82±0.69 | 12.25-20.34 |
| Mouth Length | 17.19±1.54 | 11.36-25.26 | 17.99±0.89 | 14.14-22.91 |
| Mouth Width | 14.62±1.11 | 11.81-19.68 | 15.25±0.52 | 12.27-18.31 |

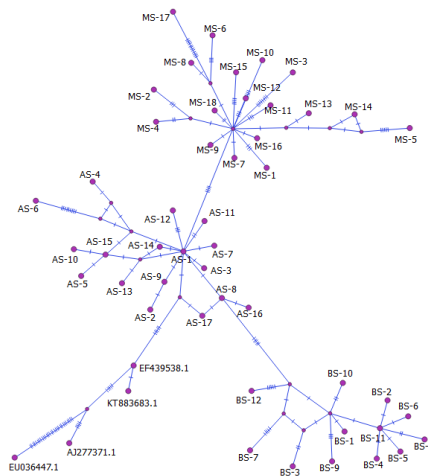


Figure 5. Median-joining tree of nucleotide divergence between *Lithognathus mormyrus* populations analyzed according to haplotype frequency distributions and haplotype nucleotidic divergence (EU036447.1: the northern Aegean Sea-Greek coast; KT883683.1: the western Mediterranean; AJ277371.1: the northwestern Mediterranean-French coast; EF439538.1: the western Mediterranean-Spanish coast; MS: Mediterranean Sea; AS: Aegean Sea; BS: Black Sea)

Table 2. Distribution of mtDNA (COI, 16s, 12s, Cyt-b) haplotype among *Lithognathus mormyrus* populations (MS: Mediterranean Sea; AS: Aegean Sea; BS: Black Sea)

| HAPLOTYPES | TOTAL | MS | AS | BS |
|--------------|-----------|-----------|-----------|-----------|
| HAP-1 | 1 | 1 | | |
| HAP-2 | 1 | 1 | | |
| HAP-3 | 1 | 1 | | |
| HAP-4 | 3 | 3 | | |
| HAP-5 | 1 | 1 | | |
| HAP-6 | 1 | 1 | | |
| HAP-7 | 1 | 1 | | |
| HAP-8 | 1 | 1 | | |
| HAP-9 | 1 | 1 | | |
| HAP-10 | 1 | 1 | | |
| HAP-11 | 1 | 1 | | |
| HAP-12 | 1 | 1 | | |
| HAP-13 | 1 | 1 | | |
| HAP-14 | 1 | 1 | | |
| HAP-15 | 1 | 1 | | |
| HAP-16 | 1 | 1 | | |
| HAP-17 | 1 | 1 | | |
| HAP-18 | 1 | 1 | | |
| HAP-19 | 8 | | 8 | |
| HAP-20 | 1 | | 1 | |
| HAP-21 | 1 | | 1 | |
| HAP-22 | 1 | | 1 | |
| HAP-23 | 1 | | 1 | |
| HAP-24 | 1 | | 1 | |
| HAP-25 | 1 | | 1 | |
| HAP-26 | 1 | | 1 | |
| HAP-27 | 1 | | 1 | |
| HAP-28 | 1 | | 1 | |
| HAP-29 | 1 | | 1 | |
| HAP-30 | 1 | | 1 | |
| HAP-31 | 1 | | 1 | |
| HAP-32 | 1 | | 1 | |
| HAP-33 | 1 | | 1 | |
| HAP-34 | 1 | | 1 | |
| HAP-35 | 1 | | 1 | |
| HAP-36 | 1 | | | 1 |
| HAP-37 | 1 | | | 1 |
| HAP-38 | 1 | | | 1 |
| HAP-39 | 1 | | | 1 |
| HAP-40 | 1 | | | 1 |
| HAP-41 | 1 | | | 1 |
| HAP-42 | 1 | | | 1 |
| HAP-43 | 1 | | | 1 |
| HAP-44 | 1 | | | 1 |
| HAP-45 | 1 | | | 1 |
| HAP-46 | 1 | | | 1 |
| HAP-47 | 1 | | | 1 |
| TOTAL | 56 | 20 | 24 | 12 |

In the genetic studies, 56 individuals were genotyped, and 47 haplotypes were found in samples belong to three populations (Figure 5). Haplotype samples were

shown in Table 2. The pairwise F_{ST} values were ranged between 0.45497 and 0.64018. F_{ST} values of populations were found as statistically significant and shown in Table 3. According to AMOVA, a significant level of genetic differentiation was found between three populations as well as within populations (Table 4). Tajima's D test, which indicates neutrality, showed statistical significance, and the population has started to expand after a bottleneck (Table 5).

Table 3. Pairwise F_{ST} values between three populations of *Lithognathus mormyrus* (* $p < 0.05$)

| Locality | Mediterranean Sea | Black Sea | Aegean Sea |
|-------------------|-------------------|-----------|------------|
| Mediterranean Sea | 0.00000 | | |
| Black Sea | 0.61832* | 0.00000 | |
| Aegean Sea | 0.45497* | 0.64018* | 0.00000 |

Table 4. Analysis of molecular variance (AMOVA) of three populations of *Lithognathus mormyrus* based on mtDNA (COI, 16s, 12s, Cyt-b) gene sequences.

| Source of variation | Sum of squares | Variance components | Percentage of variation | Fixation Indices | p-value |
|---------------------|----------------|---------------------|-------------------------|------------------|---------|
| Among populations | 121.768 | 3.24106 | 56.01682 | | |
| Within populations | 134.875 | 2.54481 | 43.98318 | FST: 0.56017 | 0.00000 |
| Total | 256.643 | 5.78587 | | | |

Table 5. Neutrality statistics of *Lithognathus mormyrus* populations

| | |
|---------------------------------------|------------|
| Nucleotide diversity | 0.00613642 |
| Number of segregating sites | 146 |
| Number of parsimony-informative sites | 43 |
| Tajima's D statistic D | -2.28501 |
| p (D \geq -2.28501) | 0.997341 |

Discussion

The maximum length of *L. mormyrus* was 24.4 cm, and this length was similar to Guchmanidze and Boltachev (2017) and Vasil'eva (2007). The meristic and morphometric measurements were found similar to the findings of Engin *et al.* (2015), Guchmanidze and Boltachev (2017), and Aydin (2017a, b).

The maximum age was observed as three years old for this study, whereas Emre *et al.* (2010) reported four years for the Mediterranean population as the maximum length they found was 27.4 cm.

Male samples comprised the majority of the studied samples in this study, which was similar to several previous studies such as Türkmen (2000), Lorenzo *et al.* (2002), Türkmen and Akyurt (2003), Emre *et al.* (2010), and Vitale *et al.* (2011), despite that these studies were conducted in different locations. In this study, the proportion of hermaphrodite individuals was found as approximately 10% for the Black Sea. This value was found higher than Iskenderun Bay (Türkmen 2000), while it was found lower than France (Besseau and Brusle-Sicard 1995). Different habitats may cause differences, as mentioned above.

Since there are limited genetic studies related to *L. mormyrus*, the results of this study were compared to other Sparidae species (*Dentex dentex*, *Pagrus pagrus*, and *Spondylisoma cantharus*) along with *L. mormyrus*. According to alloenzyme studies, significant genetic differences existed between the *L. mormyrus* in the Atlantic Ocean and the Mediterranean Sea. This result was shown sharpened phylogeographical breakage (*sensu* Avise) for the mentioned species between the Atlantic Ocean and the Mediterranean Sea. Besides, it was found that ecological and paleogeomorphologic factors affecting geographical variation was not affected by some genetic variation (Bargelloni *et al.* 2003).

Arculeo *et al.* (2003), based on alloenzyme of *L. mormyrus*, found no difference within the Mediterranean Sea and found that the Adriatic and the Mediterranean populations were different. Regarding the mtDNA data, it was determined that sand steenbras populations in the Mediterranean Sea and the coast of Portugal were found different, considering that two haplotype groups (Hammami *et al.* 2007). In Bargelloni *et al.* (2003), mtDNA of the *L. mormyrus* is compatible with dividing geographic samples into two separate phylogenetic groups. Mutual monopoly was specified in the mtDNA haplotype between the Atlantic Ocean and the Mediterranean Sea of this species. This adaptation proves that similar historical biogeographic factors are likely to affect genetic differentiation in this species. In previous studies (Düzgüneş *et al.* 2018; Gordina *et al.* 1997), it was proved that the straits of Turkey such as the Çanakkale Strait (Dardanelles) and İstanbul Strait (Bosphorus) prevent gene flows between the Aegean Sea and Black Sea populations for many species. Similarly, the Strait of Gibraltar causes two different biogeographic regions, namely the Mediterranean Sea and the Atlantic Ocean, for many species to result in intraspecific genetic variation (Borsa *et al.* 1997; Pannacciulli *et al.* 1997; Naciri *et al.* 1999; Perez-Losada *et al.* 1999; Zane *et al.* 2000). Based on our genetic analysis, it is considered that the İstanbul Strait acts as a barrier for *L. mormyrus* genetic variation (Borsa *et al.* 1997; Pannacciulli *et al.* 1997; Naciri *et al.* 1999; Perez-Losada *et al.* 1999; Zane *et al.* 2000).

In the last two decades, Sparidae Family representatives were observed in the Black Sea coast in increasing amounts due to the Mediterranization effect of the Black Sea ichthyofauna (Pusanov 1967; Boltachev and Yurakhno 2002). This

study reveals that *Lithognathus mormyrus* has been established in the Black Sea conditions because they reproduce in this habitat.

Acknowledgment

The authors would like to thank Dalida Bedikoğlu, a marine biologist, for her contribution to the preparation of ODV maps.

Karadeniz'de mirmır balığının (*Lithognathus mormyrus*) biyo-ekolojik ve genetik karakterizasyonu

Öz

Lithognathus mormyrus (L., 1758) özellikle Akdeniz sularında dağılım gösterir ve Karadeniz balıkçılığı için çok bilinmemekle birlikte ticari olarak önemli türler arasında değildir. *Lithognathus mormyrus* son yıllarda balıkçılar tarafından artan miktarlarda yakalanmakta olmasına rağmen bu türün Karadeniz'deki biyolojik özellikleri ile ilgili bilgi eksikliği bulunmaktadır. Bu çalışmada, Karadeniz'deki *Lithognathus mormyrus*'un boy-ağırlık dağılımı, morfolojik ve meristik özellikleri, yaş, cinsiyet dağılımı (histolojik incelemeye göre) ve genetik karakterizasyonu incelenmiştir. Genetik sonuçlar, üç popülasyon (Karadeniz, Akdeniz ve Ege Denizi) arasında ve popülasyonlar içinde önemli düzeyde genetik farklılaşma bulunduğunu göstermiştir.

Anahtar kelimeler: *Lithognathus mormyrus*, mirmır, Karadeniz, büyüme, genetik

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