

Genetic and Bio-Ecologic Characteristics of Common Pandora *Pagellus erythrinus* from the Eastern Mediterranean Coast of Turkey for the Ecosystem-Based Fishery Management

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Abstract

Genetic structure and growth characteristics of common pandora *Pagellus erythrinus* populations collected from the Iskenderun, Mersin and Antalya Bays were revealed through D-loop sequencing of mtDNA and length-weight and age-length relationships. While the highest value of genetic diversity was obtained in the Mersin population (0.0024), the lowest value was in Iskenderun population (0.0006). A total of 9 haplotypes was found and mean haplotype diversity was found 0.7485. The highest genetic divergence was observed between the Mersin and Iskenderun Bay populations (0.0206), whereas the Iskenderun and Antalya Bay populations had the least genetic divergence (0.0165). BioMorphv3 was used for determining the length-weight and age-length relationships. The length-weight relationships for all individuals of Iskenderun, Mersin and Antalya populations were estimated as $W=0.0693 \times L^{2.3887}$ ($r=0.9456$), $W=0.0786 \times L^{2.3338}$ ($r=0.9473$) and $W=0.0693 \times L^{2.3887}$ ($r=0.9542$), respectively. The von Bertalanffy growth parameters for all individuals of Iskenderun, Mersin and Antalya populations were calculated as $L_t=39.22 [1 - e^{0.210(t+0.32)}]$, $L_t=50.48 [1 - e^{0.120(t+2.76)}]$, $L_t=38.08 [1 - e^{0.158(t+0.48)}]$, respectively.

Introduction

The common pandora *Pagellus erythrinus* (Linnaeus, 1758) is a commercially important demersal fish species which belongs to Sparidae family, distributed in the coastal waters of the Mediterranean, the western Black Sea and the eastern Atlantic Ocean from Norway to Angola (Whitehead, Bauchot, Hureau, Nielsen & Tortones, 1986; Froese & Pauly, 2019) and lives at depths of 20 to 300 m on sandy-muddy zones (Santos, Monteiro, & Erzini, 1995). The common pandora have the feature of protogynous hermaphroditism (Girardin & Quignard, 1985; Papaconstantinou, Mytilineou, & Panos, 1988; Livadas, 1989). Females usually turn into males when they are at the ages of two or three and reach a total length of 170-180 mm (Coelho *et al.*, 2010). Reproduction occurs

during late spring and summer, and eggs and larvae are pelagic (Spedicato *et al.*, 2002). The main foods of the common pandora consist of Decapoda, Bivalvia, Polychaeta, Euphausiacea, Teleostei, Mysidacea, and Cephalopoda (Šantić, Paladin, & Rađa, 2011). It is also known as one of the most promising new candidate species in Mediterranean aquaculture (Micale, Garaffo, Genovese, Spedicato, & Muglia, 2006). The maximum total length is reported as 60 cm (Froese & Pauly, 2019) and weight as 3.2 kg (IGFA, 2001).

Fisheries pressure on the common pandora has increased further in the last 30 years, especially on the North African coasts of the Mediterranean. (FAO, 2018). This species is also considered as an important fishery resource in the Mediterranean and Atlantic waters (Erzini, Gonçalves, Bentes, Lino, & Ribeiro, 1998). The signs of the over-exploitation have been reported in the

existing stocks of the species in various Mediterranean geographical sub-areas (GSAs) (Abella, Colloca, Sartor, & Mannini, 2010). Despite the increasing of the catch rates, the common pandora is reported as the least worried species in the IUCN Threatened Species Red List (Russell, 2014). In the current conservation legislation for the fisheries in the Mediterranean Sea, the minimum total length limit for this species has been identified as 15 cm (European Commission, 2006). Common pandora is captured by gills or trammel nets, longline and trawl. The total production amount is 980 tones, which relates to 0.37% of the quantity of caught sea fish in the Turkish Seas (TURKSTAT, 2016).

Several studies about genetic structure of the common pandora were conducted by Fassatoui, Mdelgi, and Romdhane (2009); Fassatoui, Mdelgi, and Romdhane (2011); Apostolidis, Moutou, Stamatis, and Mamuris, (2009); Fassatoui and Romdhane (2010); Angiulli, Sola, Ardizzone, Fassatoui, and Rossi (2016); Rossi *et al.* (2019). On the other hand, growth parameters and biological characteristics of the common pandora were investigated in various areas of the Mediterranean such as, the southeast coasts of Spain (Valdes *et al.*, 2004), the Tyrrhenian Sea (Busalacchi, Bottari, Giordano, Profeta, & Rinelli, 2014), the Adriatic Sea (Šantić *et al.*, 2011), the Cretan shelf (Somarakis & Machias, 2002), the Ionian Sea (Caragitsou & Papaconstantinou, 1988), the Libyan coasts (Hashem & Gassim, 1981), the Algerian coasts (Mahdi, Talet, & Boutiba, 2018) and the Tunisian coasts (Ben Smida & Hadhri, 2014; Fassatoui, Hmida, Jenhani, & Romdhane, 2019). In Turkish marine waters, the population characteristics were studied by Özyayın (1997); Tosunoğlu, Akyol, Metin, Tokaç, and Ünsal (1997); Stergiou and Moutopoulos (2001); Hoşsucu and Çakır (2003); Gökçe, Aydın, and Metin (2007); Metin, İlkyaz, Soykan, and Kınacıgil (2011); Gurbet, Akyol, and Yalçın (2012); Akalın, İlhan, and Özyayın (2015); Öztekin, Özekinci, and Daban (2016); Ayyıldız and Altın (2018); Yapıcı and Filiz (2019) in the Aegean Sea; Sangün, Akamca, and Akar (2007); Gökçe, Çekic, and Filiz (2010); Özvarol (2014) in eastern Mediterranean Sea.

The assessment of gen flow levels among populations and population dynamics parameters is vital for management of marine stocks (Palsbøll, Berube, & Allendorf, 2007). Mitochondrial DNA (mtDNA) is

widely used as a marker for population studies due to its compact size, rapid evolution rate, and exclusive maternal inheritance mode (Harrison, 1989). The mtDNA control region is also known as displacement loop (D-loop) region evolves much faster than average due to reduced functional constraints (Brown, 1985).

According to our knowledge, the genetic variations between *P. erythrinus* populations in the marine waters of Turkey was still unknown in detail. Thus, genetic structure of *P. erythrinus* populations from the Iskenderun, Mersin and Antalya Bays were revealed based on mitochondrial D-loop sequence data in this study. In order to contribute to a better knowledge of the population dynamic studies, we investigated growth parameters of the common pandora from Iskenderun, Mersin and Antalya Bays.

Materials & Methods

Sample Collection

P. erythrinus specimens were sampled by collecting from commercial trawl fishing from three fishing areas of the eastern Mediterranean, comprising the Antalya Bay (ANT), Mersin Bay (MER) and Iskenderun Bay (ISK) between 2017-2019 (Figure 1). 20 common pandora samples were collected from each sites for population genetic analysis. The specimens were delivered to the laboratory and stored in a deepfreeze at -30°C until DNA extraction. In the length-weight and age-length relationships, overall 958 specimens, of which 346 ISK, 310 MER and 302 ANT populations, were measured for the length- weight relationships.

Genetic Analysis

The standard phenol: chloroform: isoamyl alcohol procedure (Sambrook, Fritsch, & Maniatis, 1989) was used to extract DNA from muscle of all fish samples. The mitochondrial D-loop gene was amplified through PCR with universal primers.

F: 5'-ATC ATC GGC CAA ATC GCA TC-3'

R: 5'-GAA CTG TAG GGC ATT CTC AC-3'



Figure 1. Sampling locations of *P. erythrinus*: ANT (Antalya Bay); MER (Mersin Bay); ISK (Iskenderun Bay).

Polymerase chain reactions were at using a reaction volume of 25 μ l containing 5 units of *Taq* polymerase (Thermo scientific), 2 mM of each primer, 10 mM dNTPs (Thermo scientific), 25 mM MgCl₂ (Thermo scientific), 10 mM Tris-HCl pH 8.8, 50 mM KCl and 1 μ l template DNA (~10-25 ng). The amplification was performed with a profile of 1 cycle of denaturation at 94°C for 5 min, followed by 40 cycles of strand denaturation at 94°C for 1 min, annealing at 50°C for 1 min and primer extensions 72°C for 1 min 30 s, and 1 cycle of final elongation at 72°C for 5 min. After PCR amplification, a 3 μ l sample of each PCR product was controlled in 1.5% agarose gels.

Finally, sequencing process of the PCR products were carried out in the forward direction with an automated sequencing machine.

Sequence Alignment

Clustal W (Thompson, Higgins, & Gibson, 1994) was used to align the partial sequences of D-loop, and BioEdit (Hall, 1999) was used to perform manual final alignment. MEGA 5 (Tamura *et al.*, 2011) was used to analyze mtDNA sequence data and to interpret values of pairwise nucleotide variation and to find out the nucleotide composition of populations individually.

Statistical Analysis

After sequence alignment, MEGA v5 was used to determine the genetic diversity and sequence divergences, and to construct the phylogenetic tree (Tamura *et al.*, 2011). Genetic diversity was measured within and between populations by the maximum likelihood estimation (Nei, 1987). Jukes-Cantor model (Jukes & Cantor, 1969) was found as best-fitting model of nucleotide substitution and genetic diversity via ModelTest (Posada & Crandall, 1998). Neighbor Joining tree (Saitou & Nei, 1987) was used to demonstrate genetic relationship between populations with consideration of negative branch length. The statistical robustness in the nodes of the resulting tree was determined by 1000 bootstrap replicates. In order to analyze the relevance of the DNA sequence evolution to

neutrality, Tajima's D test (Tajima, 1989) was performed.

Estimating of Population Parameters

The length-weight relationships were separately evaluated for each common pandora populations of all individuals with the formula $W=a \times L^b$, where W is total body weight (g), L is total length (cm), and a and b are coefficients (Ricker, 1973). Growth characteristics was estimated by the von Bertalanffy growth functions (Sparre & Venema, 1998). The function $L_t=L_\infty [1 - e^{-k(t-t_0)}]$ was applied to the data where L_t is the fish length (cm) at the time t (year), L_∞ is the asymptotic length (cm), k is the growth coefficient (year⁻¹), and t_0 (year) is the theoretical time at which the length is equal t_0 zero.

The photographs were taken from all the common pandora samples. Total weight (nearest 0.01 g) of all the samples and the age readings as suggested by Holden and Raitt (1974) from all the sagittal otoliths were recorded. BioMorphv3 was used for the age and growth relationship from the images of fish and recorded weight and age data.

Results

Genetic results

314 bp partial D-loop gene sequences were obtained after alignment. The average nucleotide composition of thymine (T), cytosine (C), adenine (A), and guanine (G) were examined as 26.5%, 26.7%, 26.0% and 20.8%, respectively. The D-loop dataset contained 13 variable sites, 10 of which were parsimony informative. DNA sequence analysis with the D-loop gene revealed 9 different haplotypes (Table 1). Mean haplotype diversity between populations was found to be 0.7485.

The phylogenetic relationships among the identified haplotypes were constructed and consequently a star-like shape, symbolized by a large number of unmatched haplotypes, often related to the central and plenty of haplotypes was obtained with Minimum Spanning Tree (Hap1) (Figure 2).

Table 1. Distribution and frequency of D-loop haplotypes of *P. erythrinus* populations.

Haplotypes	ISK	MER	ANT
Hap_1	-	-	1
Hap_2	5	6	19
Hap_3	15	-	-
Hap_4	-	1	-
Hap_5	-	1	-
Hap_6	-	5	-
Hap_7	-	1	-
Hap_8	-	5	-
Hap_9	-	1	-
Total	20	20	20

Intra-population genetic diversity and inter-population total genetic distance values are presented in Table 2.

Genetic diversity was determined as the lowest in the ISK samples, whereas the highest was in the MER samples.

Inter-population genetic diversity was 0.0127. Mean genetic divergence among populations was 0.0183. For inter-population comparison, the ISK and ANT populations showed the lowest genetic differentiation (0.0165), while the ISK and MER

populations was the highest (0.0206) (Table 2). Pairwise comparisons of genetic distance indicated that all the three populations statistically differed from each other ($P < 0.05$).

In NJ tree analysis, the MER populations was differ ($P < 0.05$) from the other populations, whereas the ISK and ANT were clustered together (Figure 3).

Tajima's D (Tajima, 1989) for the *P. erythrinus* populations was found to 1.3631, indicating that the observed heterozygosity is higher than that expected (Table 3).

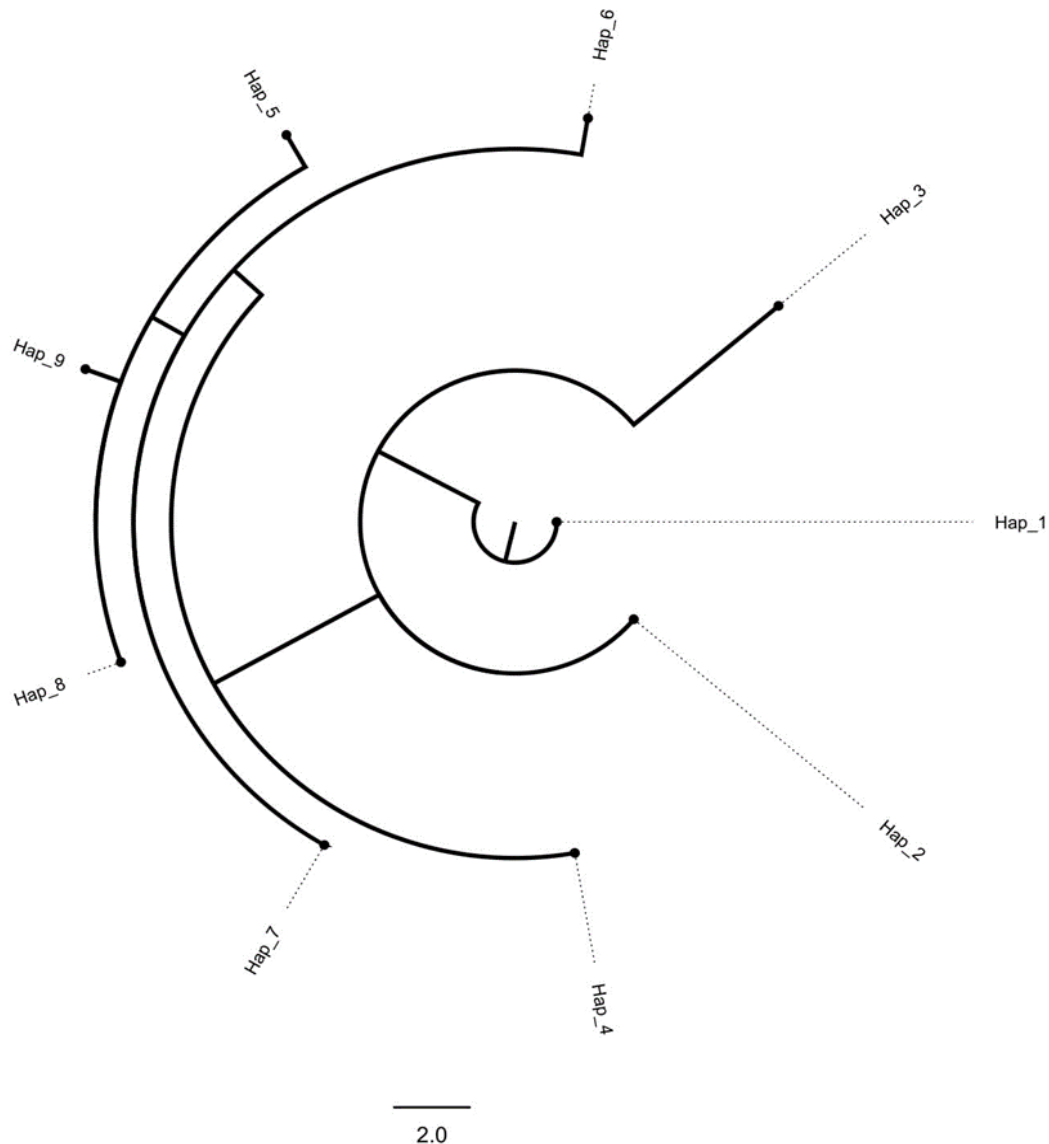


Figure 2. Minimum Spanning Tree of the mtDNA D-loop haplotypes of *P. erythrinus* reconstructed with neighbor joining method.

Table 2. Pairwise genetic distance between populations (below the diagonal). Bold numbers in Triangular line represent mean genetic diversity within populations. *, $P < 0.05$.

Populations	ISK	MER	ANT
ISK	0.0006		
MER	0.0206*	0.0024	
ANT	0.0165*	0.0178*	0.0008

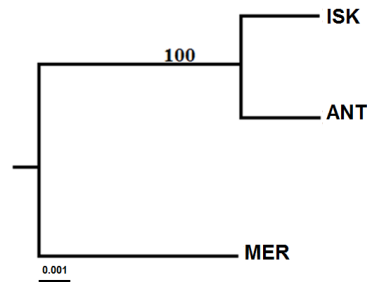


Figure 3. Mitochondrial DNA D-loop neighbor joining tree of *P. erythrinus* samples.

Table 3. Neutrality tests and the estimated parameters of mismatch distribution for populations of *P. erythrinus* from Turkish waters.

m	S	P _s	Θ	π	D
75	13	0.041401	0.008517	0.012632	1.363177

m, number of sequences; S, number of segregating sites; P_s, polymorphic site rate; Θ, population mutation rate; π, average pairwise distance; D, Tajima's D.

Length-Weight Relationship

The length-weight relationships were separately evaluated for each populations of all individuals, and were given in Figure 4. The exponent *b* demonstrated a negative allometric growth. The length-weight relationships were calculated for ISK, MER and ANT populations of all individuals as $W=0.1219 \times L^{2.1543}$ ($r=0.9456$), $W=0.0786 \times L^{2.3338}$ ($r=0.9473$) and $W=0.0693 \times L^{2.3887}$ ($r=0.9542$), respectively.

Age-Total Length Relationship

The estimated von Bertalanffy growth parameters of all individuals of common pandora $L_{\infty}=39.22$ cm, $K=0.210$ and $t_0=0.32$ for ISK samples; $L_{\infty}=50.48$ cm, $K=0.120$ and $t_0=2.76$ for MER samples and $L_{\infty}=38.08$ cm, $K=0.158$ and $t_0=0.48$ for ANT samples.

Discussion

Genetic structure and bio-ecological characteristics of *P. erythrinus* populations were analyzed together for the first time in this study. The Mersin population of *P. erythrinus* showed higher degree of genetic diversity than the other populations in Turkish marine waters. At the same time, the three populations were genetically different from each other ($P<0.05$). Furthermore, the lowest *b* value was estimated in the Iskenderun population, whereas the highest was in the Antalya population. The highest asymptotic length (L_{∞}) was determined in the Mersin population.

In the mtDNA sequencing analysis of D-loop region, nine different haplotypes were detected and the mean haplotype diversity of *P. erythrinus* was found to be 0.7485 at the present study which was lower than to the other studies by Tabata and Mizuta (1997); Ball, Beal, Chapman, and Sedberry (2007); Ashton (2013); Angiulli *et al.* (2016); Viret *et al.* (2018), whereas higher than the study by Stockley, Menezes, Pinho, and Rogers (2005). Tabata and Mizuta (1997) found haplotype diversity of

Pagrus major populations based on D-loop gene to be 0.892. Ball *et al.* (2007) studied population genetic analysis of *Pagrus pagrus* using D-loop gene and determined haplotype diversity as 0.998. Ashton (2013) conducted population genetic analysis of *Pagrus auratus* based on D-loop sequence and detect haplotype diversity as 0.858. Angiulli *et al.* (2016) carried out phylogeographical analysis of *Pagellus erythrinus* populations and found haplotype diversity inferred from D-loop sequence data 1.000. Viret *et al.* (2018) observed haplotype diversity of *Dentex dentex* populations as 0.985. Conversely, Stockley *et al.* (2005) found lower haplotype diversity of *Pagellus bogaraveo* populations to be 0.5919. Hence, low haplotype diversity may be associated with the properties of the marine species (Palumbi, 1994) and the specific features of the D-loop gene as well as ecological and human-originated factors.

The mean genetic diversity among *P. erythrinus* populations was also low (0.0127) when compared to the studies on the same species using D-loop gene such as Apostolidis *et al.* (2009); Fassatoui, Chenoui, and Romdhane (2012); whereas higher than the study by Angiulli *et al.* (2016). When we compare to the mean genetic diversity findings of other sea bream species on the basis of D-loop sequence data, we found lower than the studies by Ball *et al.* (2007); Ashton (2013); Viret *et al.* (2018), whereas higher than the studies by Jean, Lee, Chen, and Hui (1998); Stockley *et al.* (2005). Apostolidis *et al.* (2009) analyzed mitochondrial DNA sequences of *P. erythrinus* populations using D-loop gene and found mean genetic diversity as 1.12. Fassatoui *et al.* (2012) investigated genetic structure of *P. erythrinus* populations inferred from mtDNA sequence analysis of D-loop gene and found mean genetic diversity to be 0.483. Angiulli *et al.* (2016) investigated the genetic variation of *P. erythrinus* populations based on the geographical distribution and found genetic diversity value between 0.008 and 0.012. Ball *et al.* (2007) found mean genetic diversity on the studied population structure of the red porgy *Pagrus pagrus* as 0.014. Ashton (2013) investigated genetic structure of the silver sea bream *Pagrus auratus* and determined mean

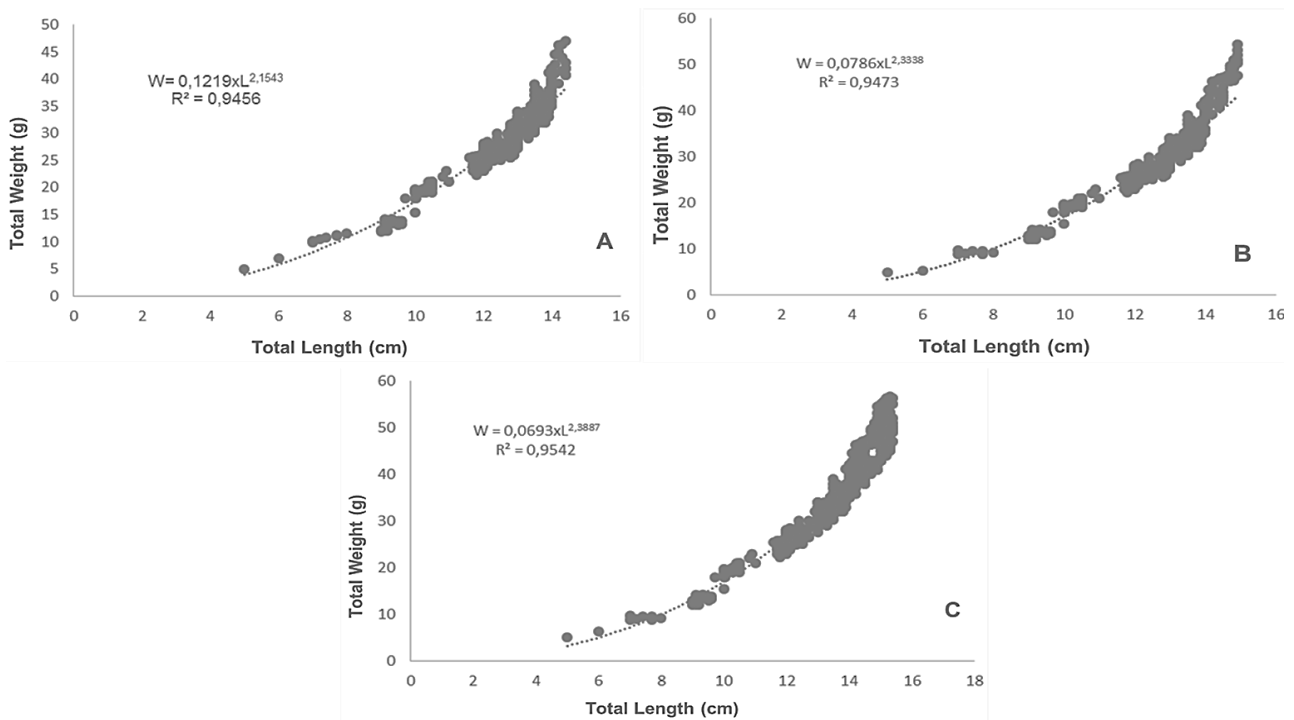


Figure 4. Length-weight relationships (A) ISK, (B) MER and (C) ANT populations of all individuals.

genetic diversity value as 0.031. Viret *et al.* (2018) assessed the genetic structure and phylogeography of the common dentex *Dentex dentex* and found genetic diversity to be 0.02104. On the other hand, Jean *et al.* (1998) studied genetic structure of the black porgy *Acanthopagrus schlegeli* populations and found genetic diversity 0.00275. Stockley *et al.* (2005) found genetic diversity value to be 0.002111 in the blackspot seabream *Pagellus bogaraveo* populations. Fisheries pressure in Turkish marine waters might lead to a decrease in genetic diversity and a reduction in evolutionary potential and adaptability (Allendorf, England, Luikart, Ritchie, & Ryman, 2008).

Intra-population genetic diversity was observed as the lowest (0.0006) in the Iskenderun Bay population. The low genetic diversity observed in the Iskenderun Bay population may relate to the potential excessive fisheries activities and other environmental factors reported by Koçak, Kubilay, Tuğrul, and Mihalopoulos (2010); Yemişken, Dalyan, and Eryılmaz (2014); Turan, Ergüden, and Gürlek (2016). Koçak *et al.* (2010) reported that the potential of the chemical industry units and municipal wastes could be origin of the pollution in the Iskenderun Bay. Yemişken *et al.* (2014) noted that regional fish stocks have been exposed to heavy fisheries activities in Iskenderun Bay and are abused every year by numerous trawler vessels. Turan *et al.* (2016) stated that the temperature has increased progressively in the last 40 years in the Iskenderun Bay and this situation affects biodiversity in this region. Tajima's D (Tajima, 1989) was found to be 1.363177 for *P. erythrinus* populations which indicate that the observed heterozygosity is higher than that expected. In point of biological explanation, the positive Tajima's D indicates low levels of polymorphisms, indicating an

inclination or sign regarding population contraction.

The overall genetic divergence among *P. erythrinus* populations was found low (0.0183). Tabata and Mizuta (1997) investigated genetic structure of the red sea bream *Pagrus major* populations from four locations of Western Japanese coasts by using D-loop and found mean genetic divergence as 0.0158. When we compare the present finding with other sea bream species *P. major*, genetic divergence is higher at *P. erythrinus* in the present study. Generally, marine species exhibit low genetic variation owing to the absence of large geographic barriers to distribution and gene flow (Avisé, Reeb, & Saunders, 1987).

In the length-weight relationships, the *b* value in the length-weight relationship showed that negative allometric growth type was obtained from all the sampled three populations. The lowest *b* value was observed in the Iskenderun population (2.1543). In terms of negative allometric growth, previous studies carried out in both the Mediterranean and Aegean Seas showed almost same results with (Rijavec & Lupanovic, 1965; Andaloro & Giarritta, 1985; Vassilipoulo *et al.*, 1986; Livadas, 1989; Özaydın, 1997; Hoşsucu & Çakır, 2003; Metin *et al.*, 2011; Busalacchi *et al.*, 2014; Özvarol, 2014; Akalin *et al.*, 2015; Fassatoui *et al.*, 2019; Yapıcı & Filiz, 2019). The *b* values in each fish population may vary based on the species, sex, age, sexual maturity of the fish as well as the season and fish feeding (Ricker, 1975).

In the age-length relationships, the asymptotic length (L_{∞}) was determined as 50.48 cm total length in the Mersin population. In the other coasts of Turkish marine waters, this value was found to be 35.7 cm by Tosunoğlu *et al.* (1997) in Gülbahçe Bay, Hoşsucu and Çakır (2003) reported as 23.99 cm in Edremit Bay, Metin

et al. (2011) found to be 30.7 cm, Yapıcı and Filiz (2019) calculated as 38.29 cm in the Gökova Bay. In different coasts of Mediterranean, Andaloro and Giarritta (1985) found to be 36.7 cm in Sicily, Girardin and Quignard (1985) determined as 34.5 cm in the Lyon Bay, Papaconstantinou *et al.* (1988) calculated to be 32.6 cm in Greek coasts, Livadas (1989) found 30 cm for Cyprus, Somarakis and Machias (2002) reported 27.8 cm on the Cretan Shelf, Busalacchi *et al.* (2014) estimated at 45.4 cm in the southern Tyrrhenian Sea, Fassatoui *et al.* (2019) calculated as 34.07 cm and 28.01 cm for the northern and southern Tunisian waters, respectively. These findings reveal the difference in the growth rate of *P. erythrinus* in the regions between the Western Mediterranean and Eastern Mediterranean, and also Mediterranean and Aegean coasts of Turkey. Differences in estimated asymptotic length for both sites can be attributed to phylogeographic variation of each population in such as fisheries, climate and pollutants. The detected differences may be resulting from genetic factors, environmental variables and/or their combination.

Conclusion

This study supplies first ecosystem-based fishery information about the genetic structure and biological characteristics of *P. erythrinus* populations from eastern Mediterranean coasts of Turkey. The results obtained from genetic analysis and biological parameters support each other. The detected lowest genetic diversity and *b* value from Iskenderun population prove that preventions should be taken for fisheries management aspect in the face of this situation.

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