

Genetic, morphologic and bio-ecological perspective of Randall's Threadfin Bream, *Nemipterus randalli*, in the Eastern Mediterranean Coast, Turkey

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Original Article

Abstract

Nemipterus randalli Russell, 1986 was first recorded from the Israel coast in 2006. Since then, the species has successfully been established in the Eastern Mediterranean seas and is commercially important in the Mediterranean Sea. In this study, the genetic structure of *N. randalli* populations was investigated with the COI gene region with population parameters from the Iskenderun, Mersin and Antalya Bays on the coast of Turkey. A total of 582 bp of partial COI sequences were obtained after alignment. The average nucleotide composition of thymine (T), cytosine (C), adenine (A), and guanine (G) were 33.2%, 26.6%, 22.6% and 17.6%, respectively. The analysis of the COI region revealed 14 different haplotypes, and haplotype diversity within populations was found to Antalya, 0.4833, Mersin, 0.6733 and Iskenderun, 0.5533. The mean haplotype diversity between populations was 0.8310. The highest genetic differentiation was between Iskenderun and Antalya populations (0.00524). Length-weight relationships were separately evaluated for each population of all individuals. The exponent *b* demonstrated positive allometric growth. The length-weight relationships were calculated for Iskenderun, Mersin and Antalya populations of all individuals as $W=0.0059 \times L^{3.2887}$ ($r^2=0.9780$), $W=0.0054 \times L^{3.3281}$ ($r^2=0.9816$), $W=0.0077 \times L^{3.1975}$ ($r^2=0.9802$), respectively. The *b* value in the length-weight relationship showed that a positive allometric growth type was obtained from all the sampled three populations. This study supplies the first fishery information about the genetic structure and biological characteristics of *N. randalli* populations from the coasts of Turkey. The results obtained from genetic analysis and biological parameters support each other and can support future studies.

Keywords: *Randall's threadfin bream, Nemipterus randalli, mtDNA, COI gene, length-weight relationships, Turkey*

Introduction

Randall's threadfin bream *N. randalli* has a natural distribution in the western Indian Ocean, including the east and west coasts of India, the Persian Gulf, the Red Sea and the eastern African coast. In the Mediterranean Sea, *N. randalli*, first recorded by Golani and Sonin (2006), was collected off the northern Israeli coast and mistakenly identified as the Japanese threadfin bream *Nemipterus japonicus*. Subsequently, this species was reported in Lebanon (Lelli *et al.*, 2008), Turkey (Bilecenoglu, 2008), Syria (Ali *et al.*, 2013), and Cyprus (Iglesias and Frotte, 2015). Within 13 years of its first record, *N. randalli* showed high-range extension on the coast of Turkey (Turan *et al.*, 2018), and today this species successfully established itself in the eastern Mediterranean waters and is commercially important in many parts of the Mediterranean (Yapıcı and Filiz, 2019). *N. randalli* inhabits open sandy or muddy substrate at depths of 30-100 m in the Mediterranean. It feeds mainly on crustaceans and, to a lesser extent, on small fishes, polychaetes and rarely on shellfish and echinoderms (Gurlek *et al.*, 2010; Gilaad *et al.*, 2017).

Several studies on *N. randalli* about age and growth parameters (Ergüden *et al.*, 2010; Al-Kiyumi *et al.*, 2014; Innal *et al.*, 2015; Uyan *et al.*, 2019; Demirci *et al.*, 2020) and length-weight relationships (Ergüden *et al.*, 2009; Dineshbabu, 2013; Edelist, 2014; Ozvarol, 2014; Ates *et al.*, 2017; Kalhor *et al.*, 2017; Uyan *et al.*, 2019; Demirci *et al.*, 2020) have been reported from Turkey. Besides, some growth parameters and biological characteristics of Randall's threadfin bream were investigated in different areas of the Mediterranean such as (Samuel, 1990; Kimoto and Ibrahim, 1996; Granada *et al.*, 2004; Wu *et al.*,

2008) and also in the Iskenderun Bay (Ergüden *et al.*, 2010). In the Arabian Sea, the growth parameters of the species have been reported by length-frequency data (Kalhor *et al.*, 2017).

The assessment of gene flow levels among populations and population dynamics parameters is vital for the management of marine stocks (Uyan *et al.*, 2020). 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 cytochrome oxidase subunit I (COI) region is sufficiently diverse to allow the specific identification of fish species and populations (Harrison, 1989; Eytan and Hellberg, 2010; Satoh *et al.*, 2016; Turan *et al.*, 2017). To date, there has not been any study on the genetic structure analysis of the populations of *N. randalli* in the Mediterranean. In this study, the genetic structure of *N. randalli* populations was investigated with COI gene region with population parameters from the Iskenderun, Mersin and Antalya Bays on the Mediterranean coast of Turkey for management considerations.

Material and methods

Randall's threadfin bream, *N. randalli*, specimens were sampled from commercial trawlers in three fishing areas of the north-eastern Mediterranean, comprising Antalya Bay (ANT), Mersin Bay (MER) and Iskenderun Bay (ISK) (Fig. 1). Twenty-five samples were collected from each site for population genetic analysis. The specimens were delivered to the laboratory and stored in a deep freeze at -30 °C until DNA extraction. In the length-weight and age-length relationships, overall 900 specimens, of which 300 samples from each site ISK, MER and ANT populations, were measured and examined for the length-weight data and

otolith reading. Total genomic DNA was extracted from the muscle and fin samples using the DNeasy Blood and Tissue Kit (Qiagen, USA). The manufacturer's protocols were used during all steps. The mtDNA COI gene region was amplified through PCR with universal primers (Ward *et al.*, 2005).

Fish_F: 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3'
Fish_R: 5'-ACT TCA GGG TGA CCG AAG AAT CAG AA-3'

The PCRs were conducted in a 50 µl total volume with 0.4 µM of each primer, 0.2 mM of dNTP and 1.25U of Taq DNA polymerase in a PCR buffer that included 20 mM of Tris-HCl (pH 8.0), 1.5 mM of MgCl₂, 15 mM of KCl and 1-2 µl template DNA. The denaturation step was at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s for 30 cycles and followed by a final extension for 7 min at 72 °C. The PCR products were visualized using electrophoresis on 1.5 % agarose gel. DNA sequencing was attempted to determine the order of the nucleotides of the mtDNA COI gene region. The chain termination method by Sanger *et al.* (1977) was applied with Bigdye Cycle Sequencing Kit V3.1 and ABI 3130 XL genetic analyzer. The initial alignments of partial COI sequences were performed with the Clustal W, and the final alignment was completed manually with BioEdit (Hall, 1999).

After sequence alignment, MEGA X was used to determine the genetic diversity and sequence divergences and to construct the phylogenetic tree (Kumar *et al.*, 2018). Genetic diversity was measured within and between populations by the maximum likelihood estimation (Nei, 1987). Jukes-Cantor model (Jukes and Cantor, 1969) was found as the best-fitting model of nucleotide substitution and genetic diversity via the Model Test

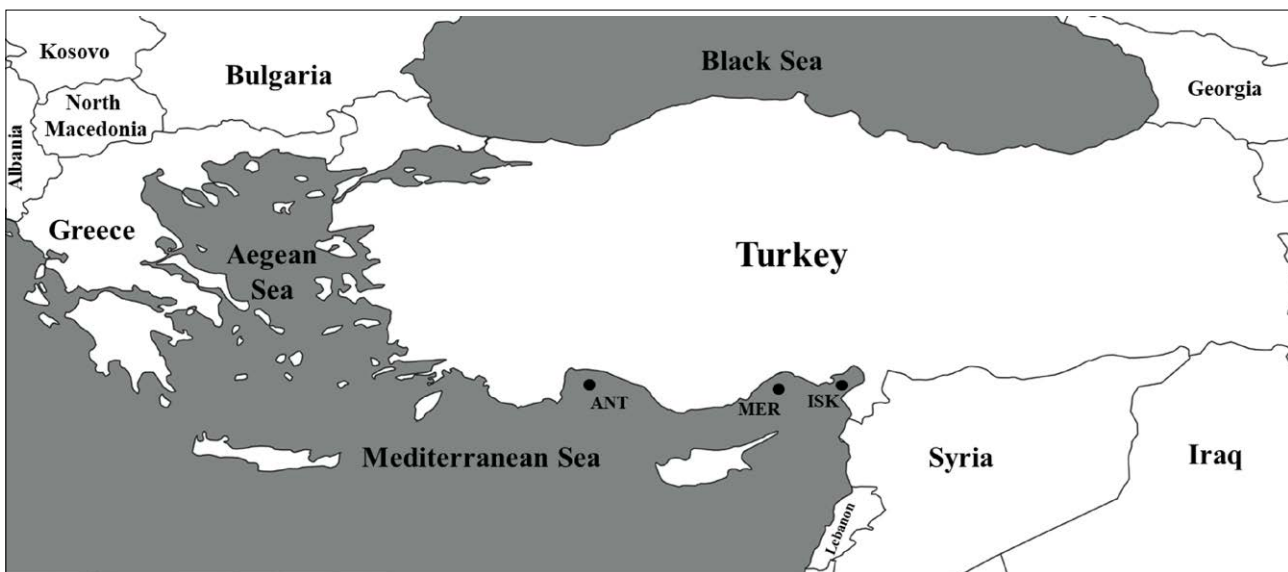


Fig. 1. Sampling location (ANT: Antalya Bay, MER: Mersin Bay, ISK: Iskenderun Bay)

(Posada and Crandall, 1998). A neighbour-joining tree was used to demonstrate a genetic relationship between populations considering negative branch length (Saitou and Nei, 1987). The statistical robustness in the nodes of the resulting tree was determined by 1000 bootstrap replicates. To analyze the relevance of the DNA sequence evolution to neutrality, Tajima's D test was performed (Tajima, 1989).

All samples were collected from İskenderun Bay, Mersin Bay and Antalya Bay. The length-weight relationship model ($W = a \times L^b$) was determined according to Ricker (Ricker, 1975). Where W is weight in g, L is TL in cm, *b* represents the length-weight factor, and *a* is a constant. The total length and weight were measured for all specimens pooled as well as for males and females separately. The student t-test was used to conclude the significance of differences from the isometric growth ($b= 3$) and the estimated *b* value of the equation following Sokal and Rohlf (2012). The von Bertalanffy function $L_t = L_\infty \times (1 - e^{-K(t-t_0)})$ was applied to define the growth in size and weight, where L_t is the total length at time *t*, *K* is a growth constant, L_∞ is the asymptotic length, and t_0 is the theoretical age at length Zero.

Results

After alignment, 582 bp partial COI gene sequences were obtained. The average nucleotide composition of thymine (T), cytosine (C), adenine (A), and guanine (G) were 33.2%, 26.6%, 22.6% and 17.6%, respectively. The result of the COI dataset contained 13 variable sites, 12 of which were parsimony informative. The analysis of the COI gene region revealed 14 different haplotypes (Table 1). Haplotype diversity within populations was found in Antalya, 0.4833, Mersin, 0.6733 and Iskenderun, 0.5533. The

Table 1. Distribution and frequency of COI haplotypes of *N. randalli* populations.

Haplotypes	ISK	MER	ANT
Hap_1	15	-	-
Hap_2	8	-	-
Hap_3	2	14	-
Hap_4	-	1	-
Hap_5	-	2	-
Hap_6	-	1	-
Hap_7	-	3	18
Hap_8	-	3	-
Hap_9	-	1	-
Hap_10	-	-	1
Hap_11	-	-	1
Hap_12	-	-	2
Hap_13	-	-	1
Hap_14	-	-	2
Total	25	25	25

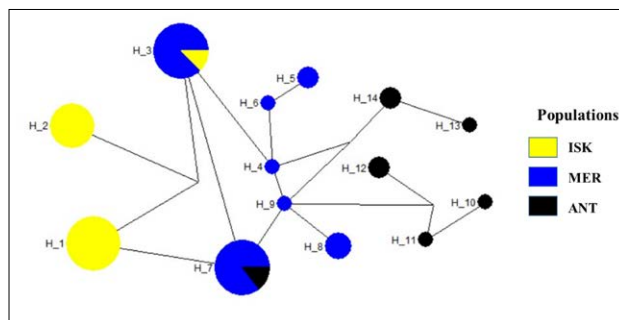


Fig. 2. Median Joining Tree of the mtDNA COI haplotypes of *N. randalli* populations

mean haplotype diversity between populations was found to be 0.8310. All sequence accession numbers were obtained between OK559912-OK559986 from Genbank.

The Median Joining Tree (MJT) method was used to create a tree showing relationships between haplotypes based on nucleotide differences in *N. randalli* populations (Fig. 2).

Genetic differences between populations and genetic diversity inter-population are shown in Table 2. Mean genetic diversity was found at 0.00404. The highest genetic differentiation was found between Iskenderun and Antalya populations (0.00524). The lowest genetic differentiation was found between Iskenderun and Mersin populations (0.00413). The average value of the genetic difference between populations was observed at 0.00864. In the Neighbour-Joining Tree (NJ) analysis, the ANT populations differed ($P < 0.05$) from the other populations, whereas the ISK and MER were clustered together (Fig. 3). Tajima's D (Tajima, 1989) for

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

Populations	ISK	MER	ANT
ISK	0.00191		
MER	0.00413*	0.00246	
ANT	0.00524*	0.00438*	0.00424

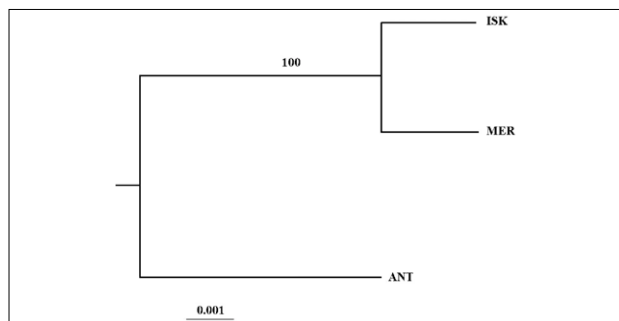


Fig. 3. mtDNA COI neighbour-joining tree (NJ) of *N. randalli* populations

Table 3. The von Bertalanffy functions results of *N. randalli* population

Population	Sex	N	L_{∞}	K	t0	\emptyset	Denklemler
Iskenderun	Female	164	23,93	0,284	-1,468	2,21	$L_t = 23,93 [1-e^{0,284(t+1,468)}]$
	Male	136	21,78	0,371	-1,223	2,24	$L_t = 21,78 [1-e^{0,371(t+1,223)}]$
	Female+Male	300	22,20	0,348	-1,317	2,23	$L_t = 22,20 [1-e^{0,348(t+1,317)}]$
Mersin	Female	108	20,76	0,258	-2,71	2,04	$L_t = 20,76 [1-e^{0,258(t+2,71)}]$
	Male	192	24,86	0,164	-3,13	2,00	$L_t = 24,86 [1-e^{0,164(t+3,13)}]$
	Female+Male	300	25,87	0,142	-3,65	1,97	$L_t = 25,87 [1-e^{0,142(t+3,65)}]$
Antalya	Female	156	31,37	0,120	-2,752	2,07	$L_t = 31,37 [1-e^{0,120(t+2,752)}]$
	Male	142	20,37	0,288	-1,949	2,07	$L_t = 20,37 [1-e^{0,288(t+1,949)}]$
	Female+Male	300	40,33	0,075	-3,522	2,08	$L_t = 40,33 [1-e^{0,075(t+3,522)}]$

the *N. randalli* populations was found to be -0.338999, indicating that the observed heterozygosity is higher than that expected. Results of neutrality tests estimated parameters were calculated P_s ; 0.022337, Θ ; 0.004570, π ; 0.004018 for *N. randalli* populations.

The length-weight relationships were separately evaluated for each population of all individuals and were given in Fig. 4. The exponent b demonstrated positive allometric growth. The length-weight relationships were calculated for ISK, MER and ANT populations of all individuals and observed as $W=0.0059 \times L^{3.2887}$ ($r^2 = 0.9780$), $W=0.0054 \times L^{3.3281}$ ($r^2=0.9816$), $W=0.0077 \times L^{3.1975}$ ($r^2=0.9802$), respectively.

The von Bertalanffy functions were separately evaluated for each population of all individuals and were given in Table 3. In Iskenderun Bay, L_{∞} height values for *N. randalli* individuals; were 23.93 cm for females, 21.78 cm for males and 22.20 cm for all individuals, and growth performances were calculated as 2.21, 2.24 and 2.42, respectively. L_{∞} height values for *N. randalli* individuals were obtained from Mersin Bay; 20.76 cm for females, 24.86 cm for males and 25.87 cm for all individuals, and their growth performance was calculated as 2.03, 2.00 and 1.97 respectively. L_{∞} height values for *N. randalli* individuals were obtained from Antalya Bay; 31.37 cm for females, 21.78 cm for males and 40.33 cm for all individuals, and their growth performance was calculated as 2.07, 2.07 and 2.08, respectively.

Discussion

The genetic structure and bio-ecological characteristics of *N. randalli* populations were analyzed together for the first time in this study. The results of the mtDNA COI gene region were observed at 582 bp, of which 13 variable sites and 12 parsimony informative. Ogwang *et al.* (2021) studied the genetic structure of the *N. randalli* population in the Red Sea and Mediterranean using the COI gene region as 570 bp, which

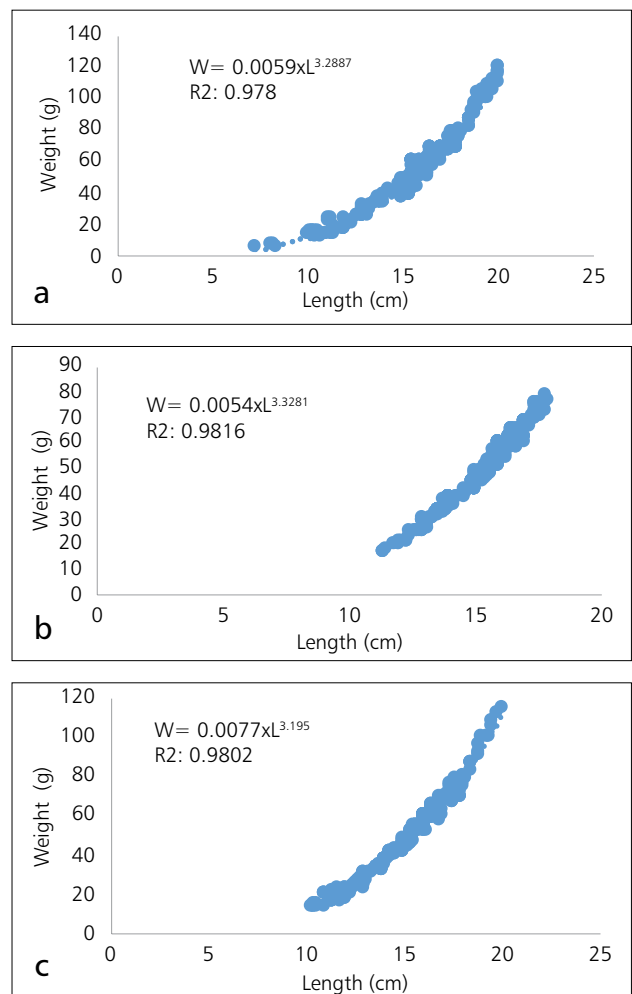


Fig. 4. Length-weight relationships (a) ISK, (b) MER and (c) ANT populations of *N. randalli*

reported 213 bp as variable sites and 176 bp as parsimony informative. Lakra *et al.* (2009) analysis of seven species of the Sciaenidae family in the Indian Sea using the COI gene region as 655 bp, in which 219 bp as variable sites and 217 bp as parsimony was informative. Mohammed-Geba (2015) used the

Table 4. Comparisons of *N. randalli* length-weight relationship parameters

References	N	a	b	r ²	Area
Murty (1981)	1	0.0223	2.877	-	Indian Coast
Erguden <i>et al.</i> (2009)	10	0.0130	2.687	0.979	Iskenderun Bay
Erguden <i>et al.</i> (2010)	379	0.0011	3.061	0.982	Iskenderun Bay
Edelist (2014)	168	0.0101	3.080	0.970	Israel
Al-Kiyumi <i>et al.</i> (2014)	1749	0.0135	3.06	0.94	Gulf of Oman
Özvarol (2014)	143	0.012	2.97	0.93	Antalya Bay
Innal <i>et al.</i> (2015)	175	0.0105	3.04	0.98	Antalya Bay
Ates <i>et al.</i> (2017)	1273	0.0201	2.98	0.96	Gökova Bay
Kalhor <i>et al.</i> (2017)	1141	0.0350	2.740	0.970	Arabian Sea
Uyan <i>et al.</i> (2019)	221	0.0171	2.92	0.96	Gökova Bay
Demirci <i>et al.</i> (2020)	587	0.0106	3.090	0.970	Iskenderun Bay
Present study	300	0.0059	3.2887	0.9780	Iskenderun Bay
	300	0.0054	3.3281	0.9816	Mersin Bay
	300	0.0077	3.1975	0.9802	Antalya Bay

COI gene in their study to examine the population structure of *Epinephelus akaara* species and found 633 bp and 17 bp as variable sites. Chou *et al.* (2015) examined the genetic structure of the *Hirundichthys oxycephalus* stocks using the COI gene, and as a result of the sequence analysis, they found the length of the COI gene as 616 bp, 62 bp as variable sites, and 28 bp as a parsimony-informative region.

The analysis of COI gene sequencing observed 14 different haplotypes. Haplotype diversity within populations was found in Antalya (0.4833), Mersin (0.6733) and Iskenderun (0.5533). The mean haplotype diversity between populations was found to be 0.8310. Tikochinski *et al.* (2019) investigated the genetic structure of *N. randalli* populations in the Red Sea and the Mediterranean Sea using the mtDNA D-loop gene region, which observed haplotype diversity of 0.9451 and 0.7491, respectively. Ogowang *et al.* (2021) studied the genetic structure of four *Nemipterus* species in the Red Sea and Mediterranean using the COI gene region, which reported of haplotype diversity *N. bipunctatus*, *N. japonicas*, *N. randalli* and *N. zysron* as 0.576, 0.333, 0.714 and 1.000, respectively. Turan *et al.* (2020) investigated molecular identification of *Pterois miles* and *P. volitans* in Turkish marine waters using the COI gene region, which reported mean haplotype diversity of 0.9869. In terms of haplotype diversity, there were observed 3 haplotypes in Iskenderun, 5 haplotypes in Mersin and 8 haplotypes in Antalya populations. This relatively low haplotype diversity presents a strong case for the founder effect (Grant and Bowen, 1998; Turan *et al.*, 2017). The reason why these values differ from the value in our study is most probably that the Mediterranean Sea population was represented by a small number of *N. randalli* that originated from the Red Sea. Tikochinski *et al.* (2019) also stated that the reduced haplotype

diversity of the *N. randalli* population in the eastern Mediterranean might be the result of a bottleneck effect.

In the present study, the genetic diversity of *N. randalli* in ISK, MER and ANT populations were observed at 0.00191, 0.00246 and 0.00424, respectively. Mean genetic diversity was found at 0.00404. Ravitchandirane *et al.* (2012) studied phylogenetic relationships with nine *Nemipteridae* species in India using the COI gene region, which reported a mean genetic diversity of 0.1342. Ogowang *et al.* (2020) studied the genetic structure of four *Nemipterus* species in the Red Sea and Mediterranean using the COI gene region, which reported haplotype diversity *N. bipunctatus*, *N. japonicas*, *N. randalli* and *N. zysron* as 0.00110, 0.00217, 0.00303 and 0.02203, respectively. Generally, marine species exhibit low genetic variation owing to the absence of large geographic barriers to distribution and gene flow (Uyan *et al.*, 2020; Avise *et al.*, 1987).

The overall genetic divergence among *N. randalli* populations was found to be low at 0.00864. The lowest values of genetic difference among all populations were found at 0.00413 between ISK and MER. Chen *et al.* (2008) found a genetic difference between 0.006 and 0.017 in their study on *Epinephelus akaara*. Ravitchandirane *et al.* (2012) studied phylogenetic relationships with nine *Nemipteridae* species in India using the COI gene region, which reported the mean genetic divergence among species of 0.109. Ogowang *et al.* (2020) reported the genetic divergence of *N. randalli* between the Mediterranean and Red Sea populations as 0.0053. The currently weak gene flow between populations may be an indicator of the genetic diversification of the Mediterranean (Cayuela *et al.*, 2020).

Tajima's D was found to be -0.33899 for *N. randalli* populations, which indicates that the observed heterozygosity is higher than that expected. Chou *et al.* (2015) found the Tajima (D) value as -2.1052 in their study in which they examined the genetic structure of *Hirundichthys oxycephalus* stocks using the COI gene. The Tajima D value turns out to be negative in cases of selective sweep or population expansion when new mutations are seen in high amounts (Murty, 1981; Innan and Stephan, 2000). This situation complements each other, as seen in low haplotype and genetic diversity.

In the length-weight relationships, the b value in the length-weight relationship showed that a positive allometric growth type was obtained from all the sampled three populations. The lowest b value was observed in the Antalya population (3.1975). Previous studies of length-weight data are shown in Table 4. Some differences in the b exponents could be attributed to the different sampling areas as well as to the differences in age, maturity and sex (Erguden *et al.*, 2010).

According to the von Bertalanffy functions results, L_{∞} values were found to be 23.93 for female individuals, 21.78 for male individuals, and 22.20 for female and male individuals in the Iskenderun population of *N. randalli*. L_{∞} values were found to be 20.76 cm for female individuals, 24.86 cm for male individuals and 25.87 cm for male and female individuals in the Mersin population. In the Antalya population, L_{∞} values were found to be 31.37 for female individuals, 20.37 for male individuals, and 40.33 for male and female individuals. Innal *et al.* (2015) found the L_{∞} value of *N. randalli* individuals from Antalya Bay to be 33.15 cm. Ergüden *et al.* (2009) examined the age and growth parameters of *N. randalli* species and found that the L_{∞} value was 34.96. When the results obtained in our research were compared with other studies, it was seen that the L_{∞} value was low. The low L_{∞} value is thought to be due to increased fishing pressure.

Conclusions

This study reported the first fishery information about the genetic structure and biological characteristics of *N. randalli* populations from the Mediterranean coasts of Turkey. The results obtained from genetic analysis and biological parameters support each other and are thought to be useful for future studies.

Acknowledgements

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