J. Appl. Ichthyol. 25 (2009), 142–147

ISSN 0175–8659

Received: February 18, 2008
Accepted: October 8, 2008
doi: 10.1111/j.1439-0426.2009.01223.x

Genetic differentiation of Mediterranean horse mackerel (*Trachurus mediterraneus*) populations as revealed by mtDNA PCR-RFLP analysis

By C. Turan[1], M. Gurlek[1], D. Yaglioglu[1] and B. Ozturk[2]

[1]*Department of Basic Sciences, Faculty of Fisheries, Fisheries Genetics Laboratory, Mustafa Kemal University, Iskenderun, Hatay, Turkey;* [2]*Department of Basic Sciences, Faculty of Fisheries, Istanbul University, Istanbul, Turkey*

Summary

The genetic population structure of Mediterranean horse mackerel, *Trachurus mediterraneus*, from seven locations throughout the Black, Marmara, Aegean and eastern Mediterranean seas was investigated using restriction fragment length polymorphism (RFLP) analysis of the mtDNA 16S rDNA region. An approximately 2000-bp segment was screened in 280 individuals using six restriction enzymes, resulting in 10 composite haplotypes. The most common haplotype was present in 56.42% individuals; the next most frequent haplotype was present in 22.85% individuals. Average haplotype diversity within samples was moderate (0.38), and nucleotide diversity was low (0.00435). Mean nucleotide divergence for the seven sampling sites was 0.0028. Nucleotide divergence among samples was moderate, with the highest value detected between the Aegean Sea (Izmir) and the eastern Black Sea (Trabzon) populations (0.007055), and the lowest (−0.000043) between the Marmara Sea (Adalar) and the western Black Sea (Sile) populations. In Monte Carlo pairwise comparisons of haplotype frequencies, the Sinop from the middle Black Sea, Trabzon from the eastern Black Sea, and Iskenderun Bay from the north-eastern Mediterranean Sea exhibited highly significant ($P < 0.001$) geographical differentiation from each other and from all other populations. Mantel's test indicated that the nucleotide divergence among populations of *T. mediterraneus* was not significantly associated with their geographical isolation ($r = -0.2963$; $P > 0.05$). Consequently, the mtDNA 16S rDNA region provided evidence for the existence of three distinct *T. mediterraneus* populations (Sinop, Trabzon and Iskenderun Bay) in the Black and north-eastern Mediterranean seas.

Introduction

Diversity within natural species is critical for adaptation and survival in changing environments. For this reason, a reliable management of fish stocks should be based on truthful data for sustainable exploitation of biological marine resources (Carvalho and Hauser, 1994; Hauser and Ward, 1998; Ludwig, 2008). Sustainable utilization of marine biological resources calls for limiting catches at such levels that would ensure stable reproduction of intensively exploited fish species. For a given resource, genetic population analysis can provide basic information on geographic limits of stocks and gene flow among populations, allowing the identification of self-recruiting units (Ryman and Utter, 1987). Data retrieved from population genetic analysis is also important for the conservation of genetic diversity within species.

Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner, 1868) is a principal semi-pelagic carnivore fish species of the commercial fishery, both by weight and value in the Mediterranean Sea (Alheit and Pitcher, 1995). *T. mediterraneus* inhabits the Mediterranean, Aegean, Marmara, and Black seas, and along the eastern Atlantic coast from Morocco to the English Channel (Fischer et al., 1987; Turan et al., 2007).

High fluctuations in total catch of *T. mediterraneus* in Turkish territorial waters have been observed. For example, total catches declined drastically to near-zero in the Black and Marmara seas in 1988 due to over-fishing in previous years (DIE, 2001), recovering to 16 030 tonnes in 2004 (DIE, 2004). Unfortunately there is limited knowledge of its stock structure among fishing areas of the eastern Mediterranean Sea. Turan (2004) analysed the population structure of *T. mediterraneus* in Turkish coastal waters using morphometric and meristic traits and reported on population structuring in three areas, the Black Sea, Marmara Sea and the north-east Mediterranean Sea. Otolith shape and chemistry analyses of *T. mediterraneus* stocks from the same waters (Turan, 2006) revealed a clear discreteness of the middle Black Sea and Aegean Sea samples. Despite this reported phenotypic heterogeneity, Karaiskou et al. (2003) found no significant heterogeneity among *T. mediterraneus* populations from the Aegean, Ionian and west Mediterranean seas using sequence analysis of 16S rDNA and cytochrome b genes. Despite the phenotypic studies described above, little is known about the genetic structure of *T. mediterraneus* population in Turkish seas, which could deter the establishment of adequate conservation measures.

Molecular genetic studies on mtDNA have proven useful for examining hypotheses on the historical distribution of animal taxa at or below the level of the species (Avise, 1994; Meyer, 1994). Restriction fragment length polymorphism (RFLP) of products of the polymerase chain reaction (PCR) analysis of mtDNA regions may be a quick tool to score the polymorphism of the population level (Avise, 1994; Tabata and Taniguchi, 2000). Because different regions of mtDNA evolve at different rates, specific mtDNA regions have been targeted for intraspecific variation (Hauser et al., 2001; Mohindra et al., 2007; Waldman et al., 2008). The 16S rDNA gene has proven to be a valuable evolutionary marker for fishes because it has produced robust phylogenies at various taxonomic levels (Brown et al., 1982; Karaiskou et al., 2003; Turan, 2008).

In the present study, PCR–RFLP analyses on the mtDNA 16S rDNA region were performed on samples of *T. mediterraneus* in order to reveal genetic variability and population

structure over Turkish territorial waters, including the Black, Marmara, Aegean and north-eastern Mediterranean seas.

Materials and methods

Sample collection

Samples were collected separately by commercial fishing vessels from seven fishing ports in the Black, Aegean, Marmara and north-eastern Mediterranean seas from September to October 2005 (Fig. 1). Age of each sample was recorded by identifying and counting annuli of otoliths, as proposed by Waldron and Kerstan (2001). Sex was determined macroscopically from the gonads whenever possible. The sample size and relative information on collected samples are given in Table 1.

Mitochondrial DNA analysis

Total DNA was extracted from muscle using the standard phenol: chloroform: isoamyl alcohol procedure (Sambrook et al., 1989). PCR amplification of the mitochondrial 16S rDNA gene was carried out using the universal primers:

A: 5′-CG (CT) AAG GGA A (ACT) G CTG AAA-3′

B: 5′-CCG GTC TGA ACT CAG ATC ACG TAG-3′

The amplification was performed with a profile of 94°C for 4 min, followed by 35 cycles of 94°C / 30 s strand denaturation, 52°C / 20 s annealing and 72°C / 1 min 30 s primer extensions, and a final 7 min elongation at 72°C. The 16S rDNA amplification conditions were: 1.5 μl 10 × polymerase buffer, 0.5 μl dNTP (10 mM), 0.3 μl Taq DNA polymerase (3 U μl^{-1}), 0.10 μl primers, 1 μl template DNA, and water for a total reaction volume of 25 μl.

The PCR product was restricted with one of six endonuclease enzymes: *BsurI*, *AluI*, *EheI*, *Hin6I*, *RsaI* or *XhoI*. The fragments of the restricted DNA samples were separated on 6% polyacrylamide gels, together with a pGem marker (Promega). A modified silver nitrate staining protocol (Tegelstrom, 1987) was used to visualize the DNA fragments.

Data analysis

PCR-RFLP generated fragment profiles were classified by letters that were then combined to define composite mtDNA haplotype patterns. Sizes of the restriction fragments were estimated from their mobility relative to a standard DNA ladder molecular size marker using the DNA-FRAG version 3.03. Nucleotide sequence diversities and divergence (Nei and Tajima, 1981; Nei, 1987) were determined using the REAP computer package (McElroy et al., 1991). The significance of geographic heterogeneity in haplotype distribution was tested with a Monte Carlo simulation (Roff and Bentzen, 1989) and 1000 randomisations of the data. Where multiple tests were involved, significance levels were adjusted according to the sequential Bonferroni procedure (Rice, 1989). A mismatch analysis was performed using Arlequin v3 (Excoffier and Schneider, 2005) to compare the occurrence of demographic changes of the seven Mediterranean horse mackerel populations (Slatkin and Hudson, 1991; Rogers and Harpending, 1992). This analysis compares the distribution of the frequency of pairs of individuals that differ by a certain number of

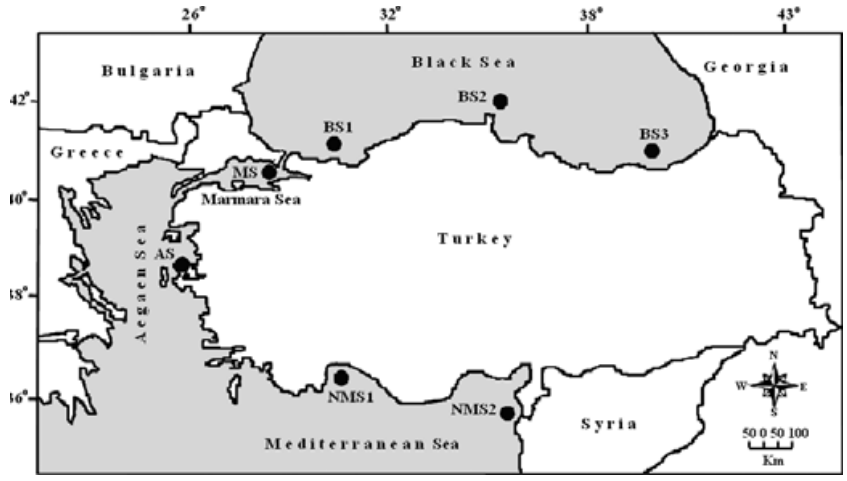

Fig. 1. Sampling sites for *T. mediterraneus*. •: indicates sampling locations

Table 1
Frequency of 10 composite mtDNA haplotypes from RFLP data within studied populations of *T. mediterraneus*

Composite genotype	BS1	BS2	BS3	MS	AS	NMS1	NMS2	Total
1 AAAAAA	32		10	31	38	36	11	158
2 ABAAAA	6	36	10	8	2	2		64
3 BABBBB			20				4	24
4 BBAAAA							9	9
5 AABBBB							8	8
6 AAABAA							4	4
7 AAAABA							4	4
8 ABBAAA		4						4
9 AAAABB	2			1				3
10 AAACAA						2		2
H	0.3436	0.1846	0.6410	0.3679	0.0974	0.1897	0.8244	0.380
N	0.00217	0.00075	0.01174	0.00190	0.00040	0.00078	0.01271	0.00716

Letters reflect individual haplotypes for six restriction enzymes: *Bsur*I, *Alu*I, *Ehe*I, *Hin6*I, *Rsa*I, *Xho*I (left to right). *H*: haplotype diversity; *N*: nucleotide diversity.

nucleotide differences. The distance matrices of pairwise comparisons among haplotypes were used to generate tree with the neighbour joining algorithm in the program neighbour (PHYLIP; Felsenstein, 1985). These latter procedures were carried out using the programs SEQBOOT and CONSENSE. Bootstrapping with replicates encompassing 1000 data sets was performed to investigate the robustness of nodes in each cluster.

Results

PCR amplification resulted in a product of approximately 2000 bp, corresponding to about 12% of the mitochondrial genome (Miya et al., 2001). The restriction enzymes that showed polymorphic patterns, *BsurI*, *AluI*, *EheI*, *Hin6I*, *RsaI* and *XhoI,* were used to define haplotypes. A total of 10 composite haplotypes was found within 280 individuals. The composite haplotypes and their occurrence in each population are given in Table 2. Haplotype 1 (AAAAAA) was the most common, present in 56.42% of individuals, while the next most frequent (haplotype 2) was present in only 22.85%.

Haplotype and nucleotide diversity values for each population were calculated from the restriction fragment data of mtDNA. Average haplotype diversity within populations was moderate (0.38), and nucleotide diversity was low (0.00435). According to both measures, the lowest level of variation was found in the Aegean Sea population (AS) (Table 2), and the highest in the Iskenderun Bay population (NMS2). Mean nucleotide divergence among populations of *T. mediterraneus* was 0.0028. Nucleotide divergence was moderate for between-population differentiation, with the highest value detected between the Aegean Sea and the eastern Black Sea (BS3) populations (0.007055), and the lowest (−0.000043) between the Marmara Sea (MS) and western Black Sea (BS1) populations.

Tests for genetic heterogeneity in haplotype frequencies revealed overall highly significant heterogeneity in haplotype frequencies among *T. mediterraneus* populations, indicating genetic structuring within the species. In Monte Carlo pairwise comparisons of haplotype frequencies, the Sinop, Trabzon and Iskenderun Bay populations exhibited highly significant ($P < 0.001$) geographical differentiation from each other and from all other populations (Table 3). The Marmara population also showed significant differences from the Izmir and Antalya Bay populations ($P < 0.05$), but non-significant separation after Bonferroni correction.

In the mismatch analyses, the parameters of the model of sudden expansion (Rogers and Harpending, 1992) and the goodness-of-fit test to the model are given in Table 3. All populations were fitted to an expansion model. Estimated τ (time in number of generations) values were 16.3 and 18.2 for BS3 and NMS2, respectively. Lower τ values (3) were detected for the other populations, indicating that population expansion in these groups may date back to about the same historical period.

Table 3
Parameters of sudden expansion model and goodness-of-fit test to the model, with respective significance for each population

Population							
Parameters	BS1	BS2	BS3	MS	AS	NMS1	NMS2
S	18	12	18	18	18	18	18
θ_0	0	0	0	0	0	0	0
θ_1	0.30	0.12	2.69	0.34	0.05	0.12	9.94
τ	3	3	16.3	3	3	3	18.2
Hri	0.5710	0.7330	0.4247*	0.6066	0.8336	0.6906	0.2943*
Goodness-of-fit test							
SSD	0.11609*	0.04934*	0.18364	0.15494*	0.01399*	0.03290*	0.18364

S, number of polymorphic sites; θ_0, pre-expansion population size; θ_1, post-expansion population size; τ, time in number of generations; SSD, sum of squared deviations; Hri, Harpening's Raggedness Index.
*Significant at $P < 0.05$.

The nucleotide divergence between haplotypes is summarized in the form of a Neighbour-Joining algorithm (Fig. 2), which showed that the two most distinct populations, Trabzon and Iskenderun Bay, were highly distinct from each other and from the other populations. The Antalya Bay and Aegean Sea populations clustered together and were the sister group to the Marmara Sea and Sile populations.

Mantel's test, employing 999 random permutations, showed that the nucleotide divergences among seven populations were not significantly associated with their geographical distances ($r = -0.2963$; $P > 0.05$).

Discussion

The present RFLP analysis of mitochondrial 16S rDNA gene provided evidence for the existence of population-specific haplotypes within each study location. Thus the presented data and results thereof demonstrate the genetic identity of each population. The cluster analysis supports three groups of haplotypes; these statistically significant groups follow a geographic east-west distribution around Turkey, but no north-south distribution divided by Turkey. The Iskenderun Bay population (NMS2) in the easternmost part of the Mediterranean Sea showed the highest genetic heterogeneity of all populations. The consistent differentiation of these populations in phenotypic analyses (Turan, 2004) may suggest lack of intermingling of Iskenderun Bay populations with other populations. Another easternmost population (BS3) was

Table 2
Pairwise estimates of nucleotide divergence (below diagonal) and diversity (above diagonal) among *T. mediterraneus* populations

Sample	BS1	BS2	BS3	MS	AS	NMS1	NMS2
BS1	–	0.004109	0.012954	0.001994	0.001370	0.001570	0.009920
BS2	0.002644***	–	0.010627	0.003814	0.004349	0.004555	0.010113
BS3	0.005994***	0.004375***	–	0.012785	0.013130	0.013106	0.013440
MS	−0.000043	0.002485***	0.005961***	–	0.001255	0.001454	0.009800
AS	0.000081	0.003768***	0.007055***	0.000103[a]	–	0.000590	0.009640
NMS1	0.000089	0.003783***	0.006839***	0.000110[a]	−0.000005	–	0.002962
NMS2	0.002477***	0.003377***	0.001211***	0.002493***	0.003081***	0.002962***	–

Significance value of Monte Carlo χ^2 tests including all samples; ***$P < 0.001$, [a]$P < 0.05$ (but non-significant when corrected by sequential Bonferroni procedure).

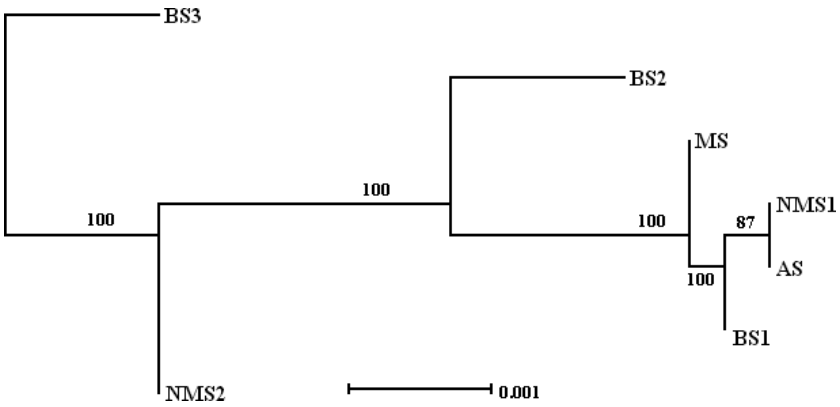

Fig. 2. Neighbour-joining tree based on nucleotide divergence. Numbers above branches represent bootstrap values obtained from 1000 replicates of restriction fragment data

also distinct from the other populations. The observed high genetic differentiation of this population from the others suggests that there is also a self-recruiting population in the western Black Sea. This is in contrast to the lack of differentiation found in the previous morphometric and meristic study (Turan, 2004), and the otolith shape and chemistry study on the same sample set (Turan, 2006). The lack of phenotypic differentiation of this population in contrast to genetic differentiation may be attributed to migration of this population between similar habitats for feeding and high isolation in the spawning place. The middle Black Sea sample (BS2) also showed significant heterogeneity from all other populations. Concordant phenotypic (otolith shape and chemistry) discreteness of the middle Black Sea population from the adjacent waters on the same sample set had also been reported (Turan, 2006). Interestingly, this population seems to be characterised by the absence of common haplotype (ABAAAA). All haplotypes are more or less homogeneously distributed among populations, and four of them are potentially population-specific. Only the common haplotype (ABAAAA) is not found within the Sinop population. Therefore some environmental factors such as selective fishing, overfishing or bottleneck effects on the Sinop population may play a role in the genetic structuring.

Unlike freshwater species, marine species usually show low genetic differentiation due to the lack of major geographical barriers in dispersal and gene flow (Ward et al., 1994a). Species with high dispersal and large population size, such as pelagic fish, often result in low or no genetic structuring across a large geographic scale. Genetic divergence among populations of *T. mediterraneus* is found to be considerably high in comparison to other marine species. Mean nucleotide divergence was found to be 0.0028. Mean nucleotide divergence among populations of marine species was 0.00055 for striped red mullet *M. surmuletus* (Mamuris et al., 2001) and −0.00027 for long-tailed hake *Macruronus magellanicus* (D'Amato and Carvalho, 2005). The detected significant genetic divergence indicates that restricted levels of gene flow do occur among stocks of *T. mediterraneus,* irrespective of geographical distance.

Levels of nucleotide diversity observed in Mediterranean horse mackerel are low (0.00716) compared with other pelagic species such as bigeye tuna *Thunnus obesus* (0.638; Chow et al., 2000), and yellowfin tuna *Thunnus albacares* (0.724; Ward et al., 1994b), but are consistent with other marine species such as Atlantic herring *Clupea harengus* (0.00809; Turan et al., 1998), and shortfin mako *Isurus oxyrinchus* (0.00347; Heist et al., 1996). Average haplotype diversity (0.38) within Mediterranean horse mackerel populations appeared as being not highly polymorphic when compared with haplotype diversity reported for other marine fish species such as Atlantic herring *Clupea harengus* (0.9205; Turan et al., 1998), and long-tailed hake *Macruronus magellanicus* (0.62; D'Amato and Carvalho, 2005). In rapidly expanding conditions, haplotype diversity increases more rapidly than nucleotide diversity, thus resulting in a large number of closely related haplotypes. *T. mediterraneus* appears to have moderate levels of haplotype diversity along with low nucleotide diversity. Such patterns are probably due to the population expansion and colonization of Aegean Sea and Black Sea regions after the Pleistocene glaciations. The Black Sea basin was formed during the Miocene epoch that formed mountain ranges and divided the ancient Tethys Ocean into several brackish basins. The Black Sea basin is connected to the Mediterranean Sea via the Turkish straits system in the southwest, which includes the Dardanelles, Bosporus and the Sea of Marmara. Post-glacial inflow of Mediterranean salt water into the Black Sea caused the invasion of marine organisms (Deuser, 1972; Rogl, 1999). The haplotype distribution of populations supports the expansion and colonization of the Mediterranean population towards the Aegean, Marmara and Black Sea regions. The common haplotype (BABBBB) of the Trabzon population (BS3, easternmost population in the Black Sea) is found only within the Iskenderun Bay population (NMS2, easternmost Mediterranean Sea population), which may indicate that the ancestral Mediterranean population divided into two and expanded towards the Black Sea and eastern Mediterranean regions. In mismatch analysis, the low values of the Harpening's raggedness index for each population support the sudden population expansions after genetic isolation (Harpening, 1994; Fu, 1997). Estimated effective female population size after expansion (θ_1) was about 3× and 10× higher than before expansion (θ_0) for BS3 and NMS2, respectively. Mismatch analyses suggest that effective population growth was approx. 10× higher for NMS2 than for BS1, BS2, MS, AS, NMS1 populations, and approx. 4× higher for NMS2 than for BS3. The variable estimates of the age expansion parameter should be interpreted as indicating that the most important demographic expansion of each lineage occurred at different evolutionary times. The most recent demographic expansion was detected within the BS1, BS2, MS, AS and MMS1 populations; this was also supported by the more reduced mtDNA diversity detected in these populations. In contrast, the demographic expansion was much older in the NMS1 and BS3 populations. It is presumed that marine environment conditions and over-fishing have an important impact on Mediterranean horse mackerel recruitment and distribution (Daskalov, 1999). Mediterranean horse mackerel fisheries are

rapidly increasing on the Black Sea coasts, resulting in fish stocks becoming over exploited. Recruitment in this species has been highly variable, with the stock biomass supported by sporadic strong year-classes followed by weak ones. Thus, the influence of a strong year-class can be traced through the subsequent few years of biomass increase (Daskalov, 1999). Moreover, pollution from the industrialized areas in the west and middle Black Sea has also been a major reason for the decline of marine stocks (Prodanov et al., 1997; Daskalov, 1998). Daskalov (1999) reported that environmental fluctuations are an important factor explaining recruitment variations; consequently, these must be considered in stock assessment and fisheries management in the Black Sea. Thus, the fisheries collapse in the early 1990s can be explained by the quasi-parallel decreasing trends in recruitment of the most important stocks, driven both by environment and over-exploitation.

These spatial differences indicate that groups of individuals of this species had spent a significant part of their lives in different environments and therefore suggested potential population separation. The detected pattern of differences at least shows that there is restriction to intermingling among populations. Therefore, from the management point of view, any depletion of one of these populations is unlikely to be compensated by emigration from other populations, at least at a sufficiently rapid rate. Precautions should be taken for the eastern Mediterranean (NMS2) and eastern Black Sea (BS2 & BS3) populations for the conservation of genetic diversity within this species. For conservation and management action, restrictions in catch quotas, fishing time and areas for the eastern Mediterranean and eastern Black Sea populations should be considered and applied.

In conclusion, the mtDNA 16S rDNA region provided evidence for genetic differentiation of *T. mediterraneus* in Turkish territorial waters. The results thus provide the first genetic evidence for the existence of three distinct *T. mediterraneus* populations (BS2, BS3 and NMS2) in the Black Sea and north-eastern Mediterranean Sea. Management implications in populations depend on whether marked variation persists over time. Consistent differentiation of these populations over at least one more year may indicate its temporal and spatial integrity and thus would also require its consideration as a separate population for management purposes. However, the potential power of mtDNA-RFLP analysis for the interpretation of population differences is relatively weak, and can only be of very limited information if not used in conjunction with nuclear markers. Therefore the utilization of nuclear genes with different genetic markers such as microsatellites (Shaw et al., 1999; Jiang et al., 2008; Ludwig et al., 2008) would extend the reliability of these findings.

Acknowledgements

This research was funded by the Scientific and Technological Research of Turkey (TUBITAK-104Y256).

References

Alheit, J.; Pitcher, T. J., 1995: Hake: biology, fisheries and markets Fish and Fisheries Series 15. Chapman and Hall, London, 478 p.

Avise, J. C., 1994: Molecular markers, natural history and evolution. Chapman and Hall, New York, USA, 511 p.

Brown, W. M.; Prager, E. M.; Wang, A.; Wilson, A. C., 1982: Mitochondrial DNA sequences of primates: tempo and mode of evolution. J. Mol. Evol. **18,** 225–239.

Carvalho, G. R.; Hauser, L., 1994: Molecular genetics and the stock concept in fisheries. Rev. Fish. Biol. Fish. **4,** 326–350.

Chow, S.; Okamoto, H.; Miyabe, N.; Hiramatsu, K.; Barut, N., 2000: Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. Mol. Ecol. **9,** 221–227.

D'Amato, M. E.; Carvalho, G. R., 2005: Population genetic structure and history of the long-tailed hake, *Macruronus magellanicus*, in the SW Atlantic as revealed by mtDNA RFLP analysis. ICES J. Mar. Sci. **62,** 247–255.

Daskalov, G., 1998: Pecheries et changement environmental a long-term en mer Noire. Ph.D. Thesis. Centre d'oceanologie de Marseille, Univ. Aix-Marseille II, 262 p.

Daskalov, G., 1999: Relating fish recruitment to stock biomass and physical environment in the Black Sea using generalized additive models. Fish. Res. **41,** 1–23.

Deuser, W. G., 1972: Late-Pleistocene and Holocene history of the Black Sea as indicated by stable isotope studies. J. Geophys. Res. **77,** 1071–1077.

DIE, 2001: Fishery Statistics. Prime Ministry Republic of Turkey. Turkish Statistics Institute, Ankara, Turkey.

DIE, 2004: Fishery Statistics. Prime Ministry Republic of Turkey. Turkish Statistics Institute, Ankara, Turkey.

Excoffier, L. G. L.; Schneider, S., 2005: An integrated software package for population genetics data analysis. Arlequin ver. 3.0. Evol. Bioinfor. Onl. **1,** 47–50.

Felsenstein, J., 1985: Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39,** 783–791.

Fischer, W.; Bauchot, M. L.; Schneider, M., 1987: Fiches FAO d'identification des espèces pour les besoins de la pêche Méditerranée et mer Noire. Zone de Pêche **37,** 761–1530.

Fu, Y. X., 1997: Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. **147,** 915–925.

Harpening, H., 1994: Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. Hum. Biol. **66,** 591–600.

Hauser, L.; Ward, R. D., 1998: Population identification in pelagic Fish: the limits of molecular markers. In: Advances in Molecular EcologyG. R. Carvalho (Ed.). . NATO Science Series IOS Press, Amsterdam. pp. 191–224.

Hauser, L.; Turan, C.; Carvalho, G. R., 2001: Haplotype frequency distribution and discriminatory power of two mtDNA fragments in a marine pelagic teleost (Atlantic herring, *Clupea harengus*). Heredity **87,** 1–10.

Heist, E. J.; Musick, J. A.; Graves, J. E., 1996: Genetic population structure of the shortfin mako (*Isurus oxyrinchus*) inferred from restriction fragment length polymorphism analysis of mitochondrial DNA. Can. J. Fish. Aquat. Sci. **53,** 583–588.

Jiang, X.; Yang, G.; Liao, M.; Liu, Y.; Gao, T.; Wang, D.; Wei, Q.; Zou, G., 2008: Microsatellite DNA polymorphism of Japanese sea bass (*Laterolabrax japonicus*) inhabiting Chinese and Japanese coasts. J. Appl. Ichthyol. **24,** 180–186.

Karaiskou, N.; Apostolidis, A.; Triantafyllidis, A.; Kouvatsi, A.; Triantafyllidis, C., 2003: Genetic identification and phylogeny of three species of the genus *Trachurus* based on mitochondrial DNA analysis. Mar. Biotechnol. **5,** 493–504.

Ludwig, A., 2008: Identification of Acipenseriformes species in trade. J. Appl. Ichthyol. **24**(S1)**,** 2–19.

Ludwig, A.; Arndt, U.; Lippold, S.; Benecke, N.; Debus, L.; King, T. L.; Matsumura, S., 2008: Tracing the first steps of American sturgeon pioneers in Europe. BMC Evol. Biol. **8,** 221.

Mamuris, Z.; Sfougaris, A. I.; Stamatis, C., 2001: Genetic structure of Greek brown hare (*Lepus europaeus*) populations as revealed by mtDNA RFLP-PCR analysis: implications for conserving genetic diversity. Biol. Conserv. **101,** 187–196.

McElroy, D.; Moran, P.; Bermingham, E.; Kornfield, J., 1991: Reap: The restriction enzyme analysis package, version 4.0. Department of Zoology, University of Maine, Orono, ME.

Meyer, A., 1994: Shortcomings of the cytochrome b gene as a molecular marker. Tree **9,** 278–280.

Miya, M.; Kawaguchi, A.; Nishida, M., 2001: Mitogenomic exploration of higher teleostean phylogenies: a case study for moderate-scale evolutionary genomics with 38 newly determined complete mitochondrial DNA sequences. Mol. Biol. Evol. **18,** 1993–2009.

Mohindra, V.; Singh, R. K.; Palanichamy, M.; Ponniah, A. G.; Lal, K. K., 2007: Genetic identification of three species of the genus

Clarias using allozyme and mitochondrial DNA markers. J. Appl. Ichthyol. **23,** 104–109.
Nei, M., 1987: Molecular Evolutionary Genetics. Columbia University Press, New York.
Nei, M.; Tajima, F., 1981: DNA polymorphism detectable by restriction endonucleases. Genetics **97,** 145–163.
Prodanov, K.; Mikhaylov, K.; Daskalov, G.; Maxim, K.; Ozdamar, E.; Shlyakhov, V.; Chashchin, A.; Arkhipov, A., 1997: Environmental Management of Fish Resources in the Black Sea and their Rational Exploitation. Studies and Reviews. GFCM, vol. 68. FAO, Rome, 178 p.
Rice, W. R., 1989: Analyzing tables of statistical tests. Evolution **43,** 223–225.
Roff, D. A.; Bentzen, P., 1989: The statistical analysis of mitochondrial DNA polymorphisms: chi-squared and the problem of small samples. Mol. Biol. Evol. **6,** 539–545.
Rogers, A. R.; Harpending, H., 1992: Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. **9,** 552–569.
Rogl, F., 1999: Mediterranean and Paratethys facts and hypothesis of Oligocene to Miocene Paleogeography. Geol. Carpat. **50,** 339–349.
Ryman, N.; Utter, F., 1987: Population genetics and fishery management. Univ. Washington Press, Seattle, WA.
Sambrook, J.; Fritsch, E. F.; Maniatis, T., 1989: Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Shaw, P.; Turan, C.; Wrigth, J.; O'connell, M.; Carvalho, G. R., 1999: Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*), with direct comparison to allozyme and mtDNA RFLP analyses. Heredity **83,** 490–499.
Slatkin, M.; Hudson, R.R., 1991: Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics **129,** 555–562.
Tabata, K.; Taniguchi, N., 2000: Differences between *Pagrus major* and *Pagrus auratus* through mainly mtDNA control region analysis. Fish. Sci. **66,** 9–18.
Tegelstrom, H., 1987: Genetic variability in mitochondrial DNA in a regional population of the great tit (*Pagrus major*). Biochem. Genet. **25,** 95–110.
Turan, C., 2004: Stock identification of Mediterranean horse mackerel (*Trachurus mediterraneus)* using morphometric and meristic characters. ICES J. Mar. Sci. **61,** 774–781.
Turan, C., 2006: The use of otolith shape and chemistry to determine stock structure of Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner). J. Fish Biol. **69,** 165–180.
Turan, C., 2008: Molecular systematics of Capoeta (Cyprinidae) species complex inferred from mitochondrial 16S rDNA sequence data. Acta Zool. Cracov. **51A** (1–2)**,** 1–14.
Turan, C.; Carvalho, G. R.; Mork, J., 1998: Molecular genetic analysis of atlanto-scandian herring (*Clupea harengus)* populations using allozymes and mitochondrial DNA markers. J. Mar. Biol. Assoc. UK **78,** 269–283.
Turan, C.; Öztürk, B.; Ergüden, D.; Gürlek, M.; Yağlıoğlu, D.; Uygur, N., 2007: Atlas of Marine Bony Fishes of Turkey. In: Atlas and Systematic of Marine Bony Fishes of Turkey, C. Turan (Ed.). (ISBN:978-9944-73-018-1). Nobel Publishing House, Adana, Turkey.
Waldman, J. R.; Doukakis, P.; Wirgin, I., 2008: Molecular analysis as a conservation tool for monitoring the trade of North American sturgeons and paddlefish. J. Appl. Ichthyol. **24**(Suppl. 1)**,** 20–28.
Waldron, E. M.; Kerstan, M., 2001: Age validation in horse mackerel (*Trachurus trachurus*) otoliths. ICES J. Mar. Sci. **58,** 806–813.
Ward, R. D.; Elliot, N. G.; Grewe, P. M.; Smolenski, A. J., 1994a: Allozyme and mitochondrial DNA variation in yellow fin tuna (*Thunnus albacares*) from the Pacific Ocean. Mar. Biol. **118,** 531–539.
Ward, R. D.; Woodwark, M.; Skibinski, D. O. F., 1994b: A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. J. Fish Biol. **44,** 213–232.

Author's address: Cemal Turan, Faculty of Fisheries, Fisheries Genetics Laboratory, Mustafa Kemal University, Iskenderun, Hatay, Turkey.
E-mail: cturan@mku.edu.tr