Reduced genetic variation of the Red Sea fish, Randall’s threadfin bream
*Nemipterus randalli*, invasive in the Mediterranean Sea

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**Abstract**

Randall’s threadfin bream (*Nemipterus randalli*) is a Red Sea species that has successfully invaded the Mediterranean via the Suez Canal (Lessepsian migrant). It has established a large population in its new region, shortly after arriving to the Mediterranean. In the present study, the mitochondrial DNA D-loop (control region) of Red Sea specimens of *N. randalli* was compared to that of specimens from the Mediterranean. A pronounced decrease in genetic variability was observed in the Mediterranean fish, indicating a bottleneck effect. Ten different haplotypes were found among 14 Red Sea specimens, while only six haplotypes were found among 42 Mediterranean specimens. The Simpson’s Index of Diversity was significantly larger for the Red Sea than for the Mediterranean fish. In addition, the average genetic distance between any pair of individuals was significantly lower in the Mediterranean samples (4.526 × 10⁻³) than that of the Red Sea samples (6.239 × 10⁻³). It can be concluded that the decrease in genetic variability did not hinder the success of the Lessepsian migrant *Nemipterus randalli* settlement in the eastern Mediterranean.

**Key words:** Lessepsian migration, bottleneck effect, population, mtDNA D-loop (control region)

**Introduction**

The opening of the Suez Canal in 1869 allowed a subsequent massive migration of Red Sea organisms into the Mediterranean Sea (Por 1978). Among these migrants are over 100 species of fishes (Golani et al. 2016). This phenomenon, known as “Lessepsian migration”, has provided a unique opportunity to test the theoretical hypothesis that the establishment of a new population will lead to a reduction in the genetic variability in the settled area. This hypothesis is based on the supposition that the new invasive population arose from a small number of individuals, constituting a sub-sample of the original population and carrying only a small portion of its genetic repertoire (Golani 2010).

One of the Red Sea Lessepsian species is the fish Randall’s Threadfin Bream *Nemipterus randalli* Russell, 1986. Its original distribution included...
Table 1. Number and of specimens and their accession number in the present study.

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<th>Location of catch</th>
<th>Number of specimens</th>
<th>HUJ voucher numbers</th>
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<td>MH142102–113</td>
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<tr>
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</tr>
</tbody>
</table>

the Red Sea, western India, the Gulf of Arabia and coast of east Africa to Durban (South Africa), including Madagascar (Russell 1990; Bakhsh 1994; Hanafi et al. 2010). *Nemipterus randalli* was first recorded in the Mediterranean by Golani and Sonin (2006) in Israel, under the name of *N. japonicus*. Shortly afterwards, it spread westward and was recorded in Lebanon (Lelli et al. 2008) and in Turkey in Iskanderun Bay (Bilecenoglu 2008; Erguden et al. 2010), Antalya Bay (Gokoglu et al. 2009) and Gokova Bay in the south-eastern Aegean Sea (Gülşahin and Kara 2013) as well as in the Mediterranean coast of Egypt (ElHaweet 2013). Soon after entering the Mediterranean it established a large population in the Levant, contributing significantly to the commercial trawl catch of the Israeli fleet (Edelist et al. 2013; Stern et al. 2014; Golani et al. 2017).

*Nemipterus randalli* inhabits open sandy or muddy substrate at depths of 30–100 m in the Mediterranean. It feeds mainly on crustaceans (chiefly *Processa* spp.) and, to a lesser extent, on small fishes, polychaetes and occasionally on mollusks and echinoderms (Gürlek et al. 2010; Gilaad et al. 2017). The spawning season in the Mediterranean begins in April and lasts until August (Gilaad 2011).

Molecular sequencing of *Nemipterus randalli* in the Mediterranean was conducted, among other species, without analyzing the results (Shirak et al. 2016; Karahan et al. 2017).

The purpose of this study was to explore the genetic structure of the origin population in the Red Sea and the targeted population in the Mediterranean of the Lessepsian fish *N. randalli*, in order to assess whether the invasive population experienced reduction of genetic variability.

**Materials and methods**

**Sample collection and DNA extraction**

Fifty-six specimens of *Nemipterus randalli* were collected by Dr. D. Golani (Table 1). All Mediterranean specimens were collected in the coastal waters of Israel. Voucher of all specimens, except the specimen from Eilat from 12/03/2009, were saved in Fish Collection of the Hebrew University of Jerusalem (HUJ).

Adult fish muscles (about 50 mg) were used for DNA sample preparation using the Accu-Prep® genomic DNA extraction kit (Bioneer, Daejeon, Korea).
PCR and sequencing

A segment of 401 bp was amplified from the 3′ region of the mitochondrial DNA D-loop (control region) using the following newly designed primers:

NR-MT15705F: 5′ CTGCCACTAACTCCCAAAGCTAGTATTCT 3′
NR-MT16242R: 5′ TGGGGAAATAAATAAGTTTATGCGCTGAA 3′

PCR reactions were carried out in 25 μl reaction volumes containing 1× PCR buffer (including 1.5 mM MgCl₂), 0.2 mM of each dNTP, 1 μM of each primer, 1 unit of Super-Term Taq polymerase (Hoffmann-La Roche), and about 100 ng of template DNA. PCR reactions were processed in an MJ Research thermal cycler with the following thermal regime: an initial step of 3 min at 95 °C followed by 35 cycles of 0.5 min at 95 °C, 0.5 min at 57 °C and 1 min at 72 °C, followed by 3 min at 72 °C and then held at 15 °C. PCR products were visualized on 1.5% agarose gels and sequenced bidirectionally using the PCR primers on an ABI 377 DNA Sequencer (Applied Biosystems, Foster City, CA) following the manufacturer’s instructions.

Data analysis

BioEdit Sequence Alignment Editor ver. 7.0.9.0 (Hall 1999) was used to align the different haplotypes. Neighbour-joining analysis was carried out using PHYLIP version 3.69 (Felsenstein 2009). Tree was constructed using the neighbour-joining approach. Bootstrap values were obtained using MEGA7 software (Kumar et al. 2016). In addition, we used the Index of Diversity by Simpson (1949) as following-

Simpson’s Index of diversity = 1 – the probability of inter-haplotype encounter

\[
D = 1 - \frac{\sum_{i=1}^{S} n_i (n_i - 1)}{N(N-1)}
\]

where N is the total sample size, S is the number of different haplotypes, and nᵢ is the number of individuals in haplotype i (i = 1,…,S).

The estimate for the variance of D is:

\[
\frac{\sum_{i=1}^{S} \left( \frac{n_i}{N} \right)^3 - \left[ \sum_{i=1}^{S} \left( \frac{n_i}{N} \right)^2 \right]^2}{N/4}
\]

Since our samples are not very large, we used the small sample formula for the estimate of the variance (Simpson 1949):

\[
\frac{4N(N-1)(N-2)\sum_{i=1}^{S} \left( \frac{n_i}{N} \right)^3 + 2N(N-1)\sum_{i=1}^{S} \left( \frac{n_i}{N} \right)^2 - 2N(N-1)(2N-3)\left[ \sum_{i=1}^{S} \left( \frac{n_i}{N} \right)^2 \right]^2}{[N(N-1)]^2}
\]
The Shannon's Diversity Index (Shannon 1948) was used to compare the diversity between the Mediterranean and the Red Sea populations.

Shannon’s Diversity Index

\[ H' = -\sum_{i=1}^{S} \frac{n_i}{N} \ln \left( \frac{n_i}{N} \right), \]

and the estimate for the variance of \( H' \) is

\[ \frac{\sum_{i=1}^{S} \frac{n_i}{N} [\ln(\frac{n_i}{N})]^2 - (H')^2}{N} + \frac{N-1}{2N^2}. \]

All sequences were sent to GenBank (accession numbers MH142087–2142).

**Results**

Out of the 56 samples, 42 were samples from the Mediterranean population and 14 were samples from the Red Sea population. Comparison of all 56 sequences showed 12 transition mutations. The number of the mutations provided a convenient comparison platform to perform a phylogenetic analysis based on genetic resemblance using Mega software (Figure 1). In an overall view of the phylogenetic tree created, we can notice that according to the first expectations, there is a wider genetic dispersal in the population of the Red Sea, compared to the Mediterranean population of the species. Further analysis of the phylogenetic tree suggests that Ea/Ma is the most ancient mitochondrial DNA type and therefore likely to have belonged to a founder population (Figure 1). In order to strengthen the hypothesis and the actual results we received, we decided to compare the mtDNA D-loop of 3 species from *Nemipterus* (*N. japonicus*, *N. bathybius*, *N. virgatus*) with our own sequences. The comparison showed that indeed the Ea/Ma haplotype is the most similar to these other species. To confirm that the Red Sea population is more dispersed genetically than the Mediterranean Sea population we used 3 statistical tests. First, the number of haplotypes: 6 in the Mediterranean and 10 in the Red Sea. Despite the fact that the Red Sea samples (n = 14) is much smaller than the Mediterranean samples (n = 42). Second, Simpson’s Index of Diversity is significantly larger for the Red Sea population than for the Mediterranean population; for the Mediterranean samples, \( D = 0.7491 \pm 0.0407 \) (estimate \( \pm \) SE) and for the Red Sea samples \( D = 0.9451 \pm 0.0451 \). Comparing both indexes, we get \( t_{35} = 3.2272 \) and \( P = 0.0027 \). Furthermore, the results for Shannon’s Diversity Index shows for the Mediterranean sample, \( H' = 1.4914 \pm 0.1121 \) (estimate \( \pm \) SE) and for the Red Sea sample \( H' = 2.2056 \pm 0.1945 \). Comparing both indexes, we get \( t_{22} = 3.1815 \) and \( P = 0.0043 \), and we conclude that Shannon’s Index of Diversity is significantly larger for the Red Sea population than for the Mediterranean population.
Last, when we used bootstrapping method to compare the average genetic distance between any pair of individuals, we could estimate the average distance in the Mediterranean and the Red Sea population:

Estimate of the average distance in the Mediterranean: $4.526 \times 10^{-3}$
Confidence interval for the average at 95% confidence level: $(4.366 \times 10^{-3}, 4.686 \times 10^{-3})$

Estimate of the average distance in the Red Sea: $6.239 \times 10^{-3}$
Confidence interval for the average at 95% confidence level: $(5.685 \times 10^{-3}, 6.793 \times 10^{-3})$.

**Discussion**

The existence of a “bottleneck effect” occurring in the migration of *Nemipterus ramdalli* from the Red Sea to the Mediterranean Sea is confirmed by the results. It seems that the Red Sea *N. ramdalli* has a diverse population of...
individuals with various genotypes, but only some of them actually succeeded in migrating and establishing a population in the Mediterranean Sea.

The main finding of the present study is that the colonizing population suffered a drastic reduction of genetic variation in comparison to its source population in the Red Sea due to a bottleneck or founder effect. Therefore, the authors expected to see wider genetic diversity in the population from the Red Sea. Indeed, a simple look at the phylogenetic tree reveals that Red Sea population spreads throughout the tree while the Mediterranean Sea population is much more grouped and concentrated. In addition, Ra and Ma groups were identified as the groups with the most ancient mtDNA, consequently, it may be assumed that Ma represents the founder population. Comparison between Ma sequences and the other species in family Nemipteridae showed that the groups identified as the founder population indeed possessed the genotype most similar to other species, identifying the earliest genotype of all the sequences produced. The statistical analysis performed namely, bootstrapping allowed analyzing the results even though the number of samples from the Red Sea was not ideal for statistical of, which generally require n > 30 samples. Similar to the observation from the phylogenetic tree, the statistical test showed that the Red Sea population is more diverse (see results).

The phenomenon of bottleneck effect has been studied in several Lessepsian migrant fish species. In *Atherinomorus forskali* (as *A. lacunosus*) (Family Atherinidae), *Upeneus moluccensis* and *U. pori* (family Mullidae), *Siganus luridus* and *S. rivulatus* (family Siganidae), no bottleneck effect was observed (Bucciarelli et al. 2002; Golani and Ritte 1999; Bonhomme et al. 2003; Hassan et al. 2003; Hassan and Bonhomme 2005). However, a genetic study of the Blue spotted cornetfish (*Fistularia commersonii*) revealed drastic reduction in the genetic variability of the colonizing population in the Mediterranean, compared to its Red Sea and Indo-Pacific source populations. This species is a very successful Lessepsian migrant which experienced a vast population increase immediately after entering the Mediterranean (Stern et al. 2014).

The difference between *F. commersonii* and the other Lessepsian fish species may be due to the mode of invasion, the rate of expansion and the sampling time. *Fistularia commersonii* was sampled a short time after the invasion and a drastic population growth. The other species were sampled many decades after their invasion into the Mediterranean, allowing multiple migration events which included a large number of individuals carrying a larger portion of the genetic repertoire from the source population.

Similarly, in the present study, the successful migrant, *Nemipterus randalli* was first sampled in the Mediterranean in 2007 only one year after its first record there (Golani and Sonin 2006).
This study provides an example for the need to further explore the drastic changes in the flora and fauna composition of the Mediterranean Sea, and how they are influenced by Lessepsian migration.

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References


Genetic reduction in invasive species


