

## Research Article

**Reduced genetic variation of the Red Sea fish, Randall's threadfin bream *Nemipterus randalli*, invasive in the Mediterranean Sea**Yaron Tikochinski<sup>1,\*</sup>, Noga Barak<sup>1</sup>, Rony Beiralas<sup>1</sup>, Vladimir Nepevny<sup>1</sup>, Ayala Raanan<sup>1</sup> and Daniel Golani<sup>2</sup><sup>1</sup>School of Marine Sciences, Ruppin Academic Center, 40297 Michmoret, Israel<sup>2</sup>National Natural History Collections and Department of Ecology, Evolution and Behavior, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

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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 ( $4.526 \times 10^{-3}$ ) than that of the Red Sea samples ( $6.239 \times 10^{-3}$ ). 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.

Date of catch	Location of catch	Number of specimens	HUJ voucher numbers	Accession Numbers
31/03/2007	Mediterranean	6	20076	MH142087–092
22/06/2007	Mediterranean	9	20789	MH142093–101
22/10/2008	Red Sea (Gulf of Suez)	12	20788	MH142102–113
05/05/2011	Red Sea (Eilat)	1	20070	MH142114
01/07/2011	Mediterranean	9	20167	MH142115–123
18/11/2014	Mediterranean	9	20412	MH142124–132
07/10/2016	Mediterranean	9	20649	MH142133–141
12/03/2009	Red Sea (Eilat)	1	not saved	MH142142

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' TGGGGAATAAATAAGTTTATGTGCCTGAA 3'

PCR reactions were carried out in 25 µl reaction volumes containing 1× PCR buffer (including 1.5 mM MgCl<sub>2</sub>), 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-haplotypic 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 n<sub>i</sub> 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

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

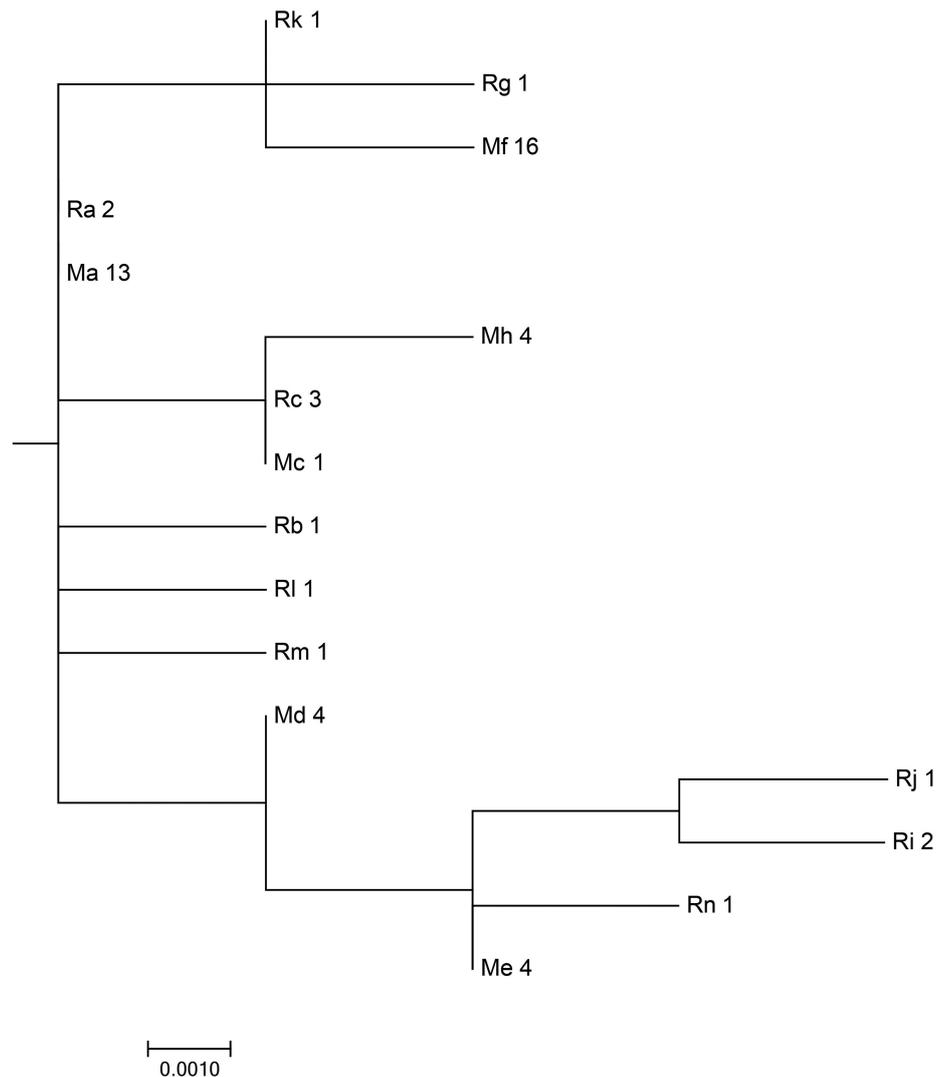
and the estimate for the variance of  $H'$  is

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

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.



**Figure 1.** A midpoint-rooted neighbor-joining tree based on sequences of mtDNA D-loop from 42 samples from the Mediterranean Sea and 14 from the Red Sea. Mediterranean samples are marked with M and Red Sea samples are marked with R. The different haplotypes are marked with a small letter (a–n). The numbers signify the number of individuals with the same phenotype.

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. commersoni* and the other Lessepsian fish species may be due to the mode of invasion, the rate of expansion and the sampling time. *Fistularia commersoni* 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

- Bakhsh AA (1994) The Biology of Thread Bream *Nemipterus japonicus* (Bloch) from Jizan Region of the Red Sea. *Journal of King Abdulaziz University - Marine Sciences* 7: 179–189, <https://doi.org/10.4197/mar.7-1.16>
- Bilecenoglu M (2008) Record of *Nemipterus randalli* Russell, 1986 (Nemipteridae) from Iskenderun Bay, Turkey. *Cybium* 32: 279–280
- Bonhomme FA, Baranes A, Golani D, Harmelin-Vivien M (2003) Lack of mitochondrial differentiations in Red Sea and Mediterranean populations of the Lessepsian rabbitfish, *Siganus rivulatus*. *Scientia Marina* 67: 215–217, <https://doi.org/10.3989/scimar.2003.67n2215>
- Bucciarelli G, Golani D, Bernardi G (2002) Genetic cryptic species as biological invaders: The case of a Lessepsian fish migrant, the hardyhead silverside *Atherinomorus lacunosus*. *Journal of Experimental Marine Biology and Ecology* 273: 143–149, [https://doi.org/10.1016/S0022-0981\(02\)00138-7](https://doi.org/10.1016/S0022-0981(02)00138-7)
- Edelist D, Rilov G, Golani D, Carlton JT, Spanier E (2013) Restructuring the Sea: profound shifts in the world's most invaded marine ecosystem. *Diversity and Distributions* 19: 69–77, <https://doi.org/10.1111/ddi.12002>
- ElHawet AE (2013) Biological studies of the invasive species *Nemipterus japonicus* (Bloch, 1791) as a Red Sea immigrant into the Mediterranean. *Egyptian Journal of Aquatic Research* 39: 267–274, <https://doi.org/10.1016/j.ejar.2013.12.008>
- Erguden D, Turan C, Gurlek M, Yaglioglu D, Gungor M (2010) Age and growth of the Randall's threadfin bream *Nemipterus randalli* (Russell, 1986), a recent Lessepsian migrant in Iskenderun Bay, northeastern Mediterranean. *Journal of Applied Ichthyology* 26: 441–444, <https://doi.org/10.1111/j.1439-0426.2009.01387.x>
- Felsenstein J (2009) PHYLIP<sup>®</sup> - Phylogeny Inference Package (version 3.69). Department of Genome Sciences, University of Washington, Seattle. Computer program
- Gilaad R-L (2011) Resource utilization by Indigenous and Migrant fish species in the Eastern Mediterranean. M.Sc. Thesis. Tel-Aviv University, 111 pp [Hebrew with English Abstract]
- Gilaad R-L, Galil BS, Diamant A, Goren M (2017) The diet of native and invasive fish species along the the eastern Mediterranean coast (Osteichthyes). *Zoology in the Middle East* 63: 235–235, <https://doi.org/10.1080/09397140.2017.1375196>
- Gokoglu M, Guven O, Balci BA, Colak H, Golani D (2009) First records of *Nemichthys scolopaceus* and *Nemipterus randalli* and second record of *Apterichthys caecus* from Antalya Bay, Southern Turkey. *Marine Biodiversity Records* 2: 1–3, <https://doi.org/10.1017/S175526720800033X>
- Golani D (2010) Colonization of the Mediterranean by Red Sea fishes via the Suez Canal-Lessepsian migration. In: Golani D, Appelbaum-Golani B (eds), Fish invasions in the Mediterranean Sea - change and renewal, Pensoft, Sofia, pp 145–188
- Golani D, Ritte U (1999) Genetic relationships in goatfishes (Mullidae: Perciformes) of the Red Sea and the Mediterranean, with remarks on Suez Canal migrants. *Scientia Marina* 63: 129–135, <https://doi.org/10.3989/scimar.1999.63n2129>
- Golani D, Sonin O (2006) The Japanese threadfin bream *Nemipterus japonicus*, a new Indo-Pacific fish in the Mediterranean Sea. *Journal of Fish Biology* 68: 940–943, <https://doi.org/10.1111/j.0022-1112.2006.00961.x>
- Golani D, Orsi-Relini L, Massuti E, Quignard JP, Dulcic J, Azzurro E (2016) CIESM atlas of exotic species in the Mediterranean. [www.ciesm.org/atlas/appendix1.html](http://www.ciesm.org/atlas/appendix1.html) (accessed 12 November 2018)
- Golani D, Edelist D, Lerner A, Sonin O, Motro U (2017) A long term (1949-2010) study of catch and effort in Israeli trawl fishery, Eastern Mediterranean Sea. *Acta Adriatica* 58: 57–163, <https://doi.org/10.32582/aa.58.1.13>
- Gülşahin A, Kara A (2013) Record of *Nemipterus randalli* Russell, 1986 from the southern Aegean Sea (Gökova Bay, Turkey). *Journal of Applied Ichthyology* 29: 933–934, <https://doi.org/10.1111/jai.12187>
- Gürlek M, Erguden S, Yaglioglu D, Turan F, Demirhan S, Gungor M, Ozbalcian B, Ozcan T (2010) Feeding habit of Indo-Pacific species *Nemipterus randalli* Russel [sic], 1986 (Nemipteridae) in Iskenderun Bay, eastern Mediterranean Sea. *Rapport Commission internationale Mer Méditerranée* 39: 539

- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98
- Hanafi NA, Mohamed AR, Al-Dubakel AY (2010) Fishery, growth and mortality of the threadfin bream, *Nemipterus japonicus* (Bloch, 1791) (Pisces: Nemipteridae) from the southern Sudanese waters, Red Sea. *Basrah Journal of Agricultural Science* 2: 187–199, <https://doi.org/10.33762/bagsr.2010.118386>
- Hassan M, Bonhomme F (2005) No reduction in neutral variability of mitochondrial and nuclear genes for Lessepsian migrant, *Upeneus moluccensis*. *Journal of Fish Biology* 66: 867–870, <https://doi.org/10.1111/j.0022-1112.2005.00643.x>
- Hassan M, Harmelin-Vivien M, Bonhomme F (2003) Lessepsian migration without bottleneck: example of two rabbitfish species (*Siganus rivulatus* and *Siganus luridus*). *Journal of Experimental Marine Biology and Ecology* 291: 219–232, [https://doi.org/10.1016/S0022-0981\(03\)00139-4](https://doi.org/10.1016/S0022-0981(03)00139-4)
- Karahan A, Douek J, Paz G, Stern N, Kideys AE, Shaish L, Goren M, Rinkevich B (2017) Employing DNA barcoding as taxonomy and conservation tools for fish species censuses at the southeastern Mediterranean, a hot-spot area for biological invasion. *Journal for Nature Conservation* 36: 1–9, <https://doi.org/10.1016/j.jnc.2017.01.004>
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874, <https://doi.org/10.1093/molbev/msw054>
- Lelli S, Colloca F, Carpentieri P, Russell BC (2008) The threadfin bream *Nemipterus randalli* (Perciformes: Nemipteridae) in the eastern Mediterranean Sea. *Journal of Fish Biology* 73: 740–745, <https://doi.org/10.1111/j.1095-8649.2008.01962.x>
- Por FD (1978) Lessepsian migration: the influx of the Red Sea biota into the Mediterranean by way of the Suez Canal. Springer-Verlag, Berlin, 228 pp, <https://doi.org/10.1007/978-3-642-66728-2>
- Russell BC (1990) Nemipterid fishes of the world (threadfin breams, whiptail breams, monocle breams, dwarf monocle breams, and coral breams). Family Nemipteridae. An annotated and illustrated catalogue of nemipterid species known to date. FAO Species Catalogue 125: (Vol. 12). Rome, FAO, 149 pp + VIII plates
- Shannon CE (1948) A mathematical theory of communications. *The Bell System Technical Journal* 27: 379–423, <https://doi.org/10.1002/j.1538-7305.1948.tb01338.x>
- Shirak A, Dor L, Serussi E, Ron M, Hulata G, Golani D (2016) DNA Barcoding of fish species from the Mediterranean coast of Israel. *Mediterranean Marine Science* 17: 459–466, <https://doi.org/10.12681/mms.1384>
- Simpson EH (1949) Measurement of diversity. *Nature* 163: 688, <https://doi.org/10.1038/163688a0>
- Stern N, Levitt Y, Galil BS, Diamant A, Yokes MB, Goren M (2014) Distribution and population structure of the alien Indo-Pacific Randall's threadfin bream *Nemipterus randalli* in the eastern Mediterranean Sea. *Journal of Fish Biology* 85: 394–406, <https://doi.org/10.1111/jfb.12421>