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Article

The First Spermatological Examination of New Commercial Fish Species of the Mediterranean: Randall's Threadfin Bream, *Nemipterus randalli* Russell, 1986

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Abstract: Determining spermatological characteristics in fish is critical for understanding species' reproductive biology, protecting fish stocks, increasing reproductive success, and ensuring fish farming efficiency. While reproductive biology research on native fish species of Mediterranean is restricted, there is no information on the spermatological characteristics of invasive Mediterranean species. Samples were collected from trawl surveys conducted in the Gulf of Antalya (Mediterranean-Turkey) between March and April 2024. The fish samples were promptly transported to the laboratory at the Department of Biology, Burdur Mehmet Akif Ersoy University. The individuals' sex determination, lengths, and weights were recorded. 17 (65.38%) males, 9 (34.62%) females were determined. Male specimens of N. randalli, ranging in size from 19 to 27.2 cm in total length and weighing between 80 and 270.5 g, were analyzed. Nemipterus randalli sperm viability and concentration were evaluated. Male fish gonads were handled, and testes were dissected in a tube containing 1 ml PBS. Sperm concentration was done with haemocytometric method. The viability of spermatozoa was analysed using a Cytoflex Flow Cytometry device and a Sybr-14/PI double staining method. Spermatozoa concentration of male individuals obtained from the Gulf of Antalya was 160.0±45.63 × 106 spz/ml and the viability rate were 51.71±17.01%. As a result, some spermatological characteristics of Nemipterus randalli, which is recorded as an invasive species and has a serious economic value, were revealed for the first time.

Keywords: Commercial species; Nemipteridae; Lessepsian fish; sperm parameters

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1. Introduction

The recruitment of wild fish populations and the controlled production in aquaculture systems are biological processes intrinsically linked to reproductive success, specifically through the fertilization of mature oocytes. Following this, further stages of the life cycle, such as successful larval development, particularly during critical periods like the initial intake of food, play a key role in recruitment dynamics. Typically, fish undergo seasonal gametogenesis and exhibit synchronized reproductive behaviors, resulting in the release of gametes into the aquatic environment for external fertilization. The success of fertilization is influenced by both the intrinsic characteristics of the gametes, such as quality and quantity and the environmental conditions at the site of gamete fusion. Consequently, fertilization serves as a complex integrative outcome of multiple interacting factors, potentially masking variations in the inherent quality of sperm. Recent comprehensive reviews Cabrita, Engrola [1], Bobe and Labbé [2] have demonstrated that while various sperm characteristics collectively influence overall sperm quality, none of these attributes

alone is sufficient to fully describe the fertilization potential of spermatozoa. Additionally, the evaluation of sperm quality can significantly benefit from the standardization of analytical methodologies and techniques.

In mammals, alterations in sperm structure, including changes in flagellum length or head size, have been observed under various conditions. During the 1990s, image analysis techniques were adapted for the study of mammalian sperm morphology, leading to the development of Automated Sperm Morphology Analysis (ASMA). This approach was first utilized to evaluate fish sperm by Van Look and Kime [3], who examined the impact of increasing mercury concentrations on sperm quality. Subsequently, ASMA has been applied in diverse contexts, such as evaluating the influence of HCG on spermiation in European eel [4], and exploring potential correlations between sperm morphology and swimming performance [5]. In thıs study we also observed N. randalli spermatozoa under the light microscobe (Figure 2).

Spermatozoa concentration in semen can be measured using various techniques, including microscopic counting, spectrophotometry, flow cytometry, and spermatocrit evaluation (as reviewed by Alavi, Psenicka [6]). However, each of these methods has certain limitations. Microscopic counting, considered the fundamental approach, provides sperm counts with reasonable accuracy, though subject to variability stemming from dilution and counting errors. For instance, an error margin of approximately 6% has been reported when counting 300 spermatozoa per observation using a 1/500 diluted sample over three repetitions [7]. While this method is the most cost-effective for measuring sperm concentration, its primary drawback is its labor-intensive nature, which makes it less practical for downstream applications such as sperm preservation or partitioning for genetic cross-breeding studies.

At the subcellular level, sperm quality is often evaluated based on the integrity of the spermatozoa's plasma membrane, which plays a crucial role in regulating ion and water exchange between the intracellular and extracellular environment, thereby influencing axonemal motion as described by Cosson, Groison [8] and Inaba [9]. Practically, membrane integrity has been examined using dye-penetration assays such as eosin/nigrosin or eosin staining alone, followed by microscopic observation, particularly in studies on mammals [10] and fish [11]. More recent approaches involve the use of fluorescent DNA-binding dyes like Hoechst 33258 and Propidium Iodide (PI), or more sophisticated kits with markers such as Sybr-14/PI, which enable concurrent visualization of both live and dead sperm cells. Viability percentages have been determined through direct counting or image analysis under the microscope [4, 12], flow cytometric analysis [13, 14].

The colonization of Red Sea species in the Mediterranean began sometime after the opening of the Suez Canal in 1869, a process that has had bioecological and economic impacts that continue today [15-18].

Nemipterus randalli Russell, 1986 is a Red Sea Lessepsian species [19]. It has a wide geographical distribution from the coasts of eastern and western India to Pakistan, the Persian Gulf, the Red Sea, the Gulf of Aden, East Africa, Seychelles and Madagascar in the western Indian Ocean [17, 20-22]. Golani and Sonin [23] recorded first time in the Mediterranean under the name N. japonicus in Israel. After a short time, it spread westward and was discovered in Lebanon by Lelli, Colloca [24], in Iskenderun Bay [20, 21], in Antalya Bay [25], in Gökova Bay [26], in the Mediterranean coast of Egypt [27], and in Greece [28].

Nemipterus randalli is a bottom fish that lives on sandy or muddy bottoms at depths ranging from 22 to 225 meters. It has an ellipsoid body shape and a silvery pink tint with three or four faint yellow stripes. The upper rays are recognized by a forked caudal fin with a long filament [29]. It feeds mostly on crustaceans, with minor amounts of small fish, polychaetes, molluscs, and spiny skins [21, 30, 31].

The number of studies on the distribution and ecology of N. randalli in the Mediterranean has increased in recent years [17, 20, 21, 23-27, 30-40].

There are limited studies on the reproductive biology of N. randalli [22, 31, 41]. In these studies, information on important reproductive parameters such as sex ratios, gonadasomatic index (GSI) values, fecundity values, etc. were presented. Ozen [42] provided histological information on male and female gonads. There are no studies on sperm parameters. Determination of sperm parameters is an important biological phenomenon in terms of understanding invasion biology. At the same time, this economically important species will also contribute to the evaluation of aquaculture potentials in the future. With this study, spermatological parameters will be determined for the first time in N. randalli species.

2. Materials and Methods

2.1. Ethics statement

This study follows all relevant international, national, and institutional guidelines for the collection and experimental use of fish samples. The fish species examined are not listed in the IUCN Red List of Threatened Species and are not classified as endangered, vulnerable, rare, or protected in Türkiye. Additionally, the sampling sites are situated outside of any designated protected areas, making an ethics statement unnecessary.

2.2 Study design

Samples were collected from trawl surveys conducted in the Gulf of Antalya (Mediterranean-Turkey) between March and April 2024. The fish samples were promptly transported to the laboratory at the Department of Biology, Burdur Mehmet Akif Ersoy University. The individuals' lengths (total length, TL; measured to a precision of 1 mm) and weights (total weight, W; measured to the nearest 0.1 g) were recorded (Figure 1). Gonads were examined macroscopically and microscopically, and sex was determined. After sex determination, sperm concentration and flow cytometric dead-live analysis were performed in terms of spermatological parameters with samples taken from males at Mehmet Akif Ersoy University Reproduction and Artificial Insemination Clinic. Gonads were separated from the fish by dissection and the weights of the gonads were recorded. A total of 9 male fish were analyzed. Testes were dissected in a tube containing 1 ml PBS and allowed to stand for two minutes to allow semen to come out of the tissue into the liquid.

Figure 1. A specimen of *Nemipterus randalli*

2.3 Sperm Concentration

The concentration of semen was determined using the haemocytometric method [43]. The testis was trimmed in 1 ml PBS and incubated in an incubator at 37°C for 2 hours. Subsequently, 10 µl of sperm sample was added to 490 µl of Hayem's solution, and the number of spermatozoa was counted using a Thoma counting chamber. The sperm concentration was expressed as the number of spermatozoa per ml. (spz. / ml) (Figure 3).

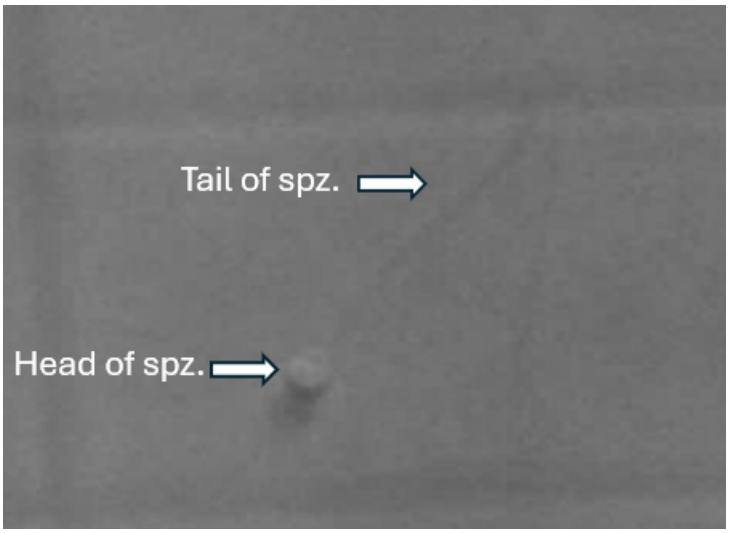

Figure 2. Sections of *N. randalli* spermatozoon

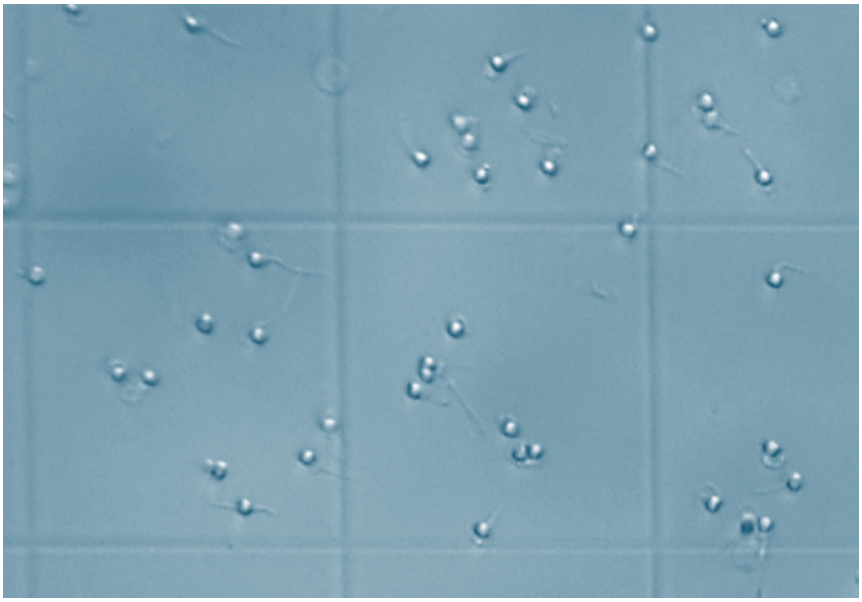

Figure 3. *N. randalli* sperm concentration analysis

2.4. Sperm Viability

The viability of spermatozoa was analysed using a Cytoflex Flow Cytometry device (Beckman Coulter, CA, USA) and a Sybr-14/PI (L7011, Invitrogen, CA, USA) double staining method. 50 µL of sperm sample, and 5 µL of Sybr-14 and 3 µL of PI stain were added to 442 µL of PBS solution. The mixture was then incubated in a dark room at 37 °C in a water bath for 5 minutes. The ratio of live to dead sperm was determined using CytExpert 2.3 software (Figure 4).

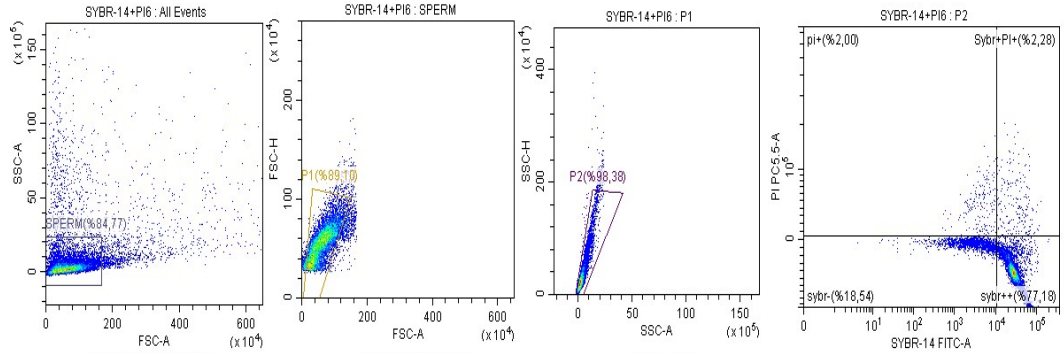

Figure 4. *N. randalli* flow cytometry viability analysis

2.5 Statistical analysis

All parameters, statistical analyses were performed using IBM Corp.'s SPSS (v.22, New York, NY, USA). Datas were given as MEAN±SEM.

3. Result

Based on the sex determination of *N. randalli*, we recorded 17 (65.38%) males, 9 (34.62%) females. The male–female ratio for all fish combined was 1.89:1 and differed statistically from the expected 1:1 ($P < 0.05$). During the study period, 17 male specimens of *N. randalli*, ranging in size from 19 to 27.2 cm in total length and weighing between 80 and 270.5 g, were analyzed.

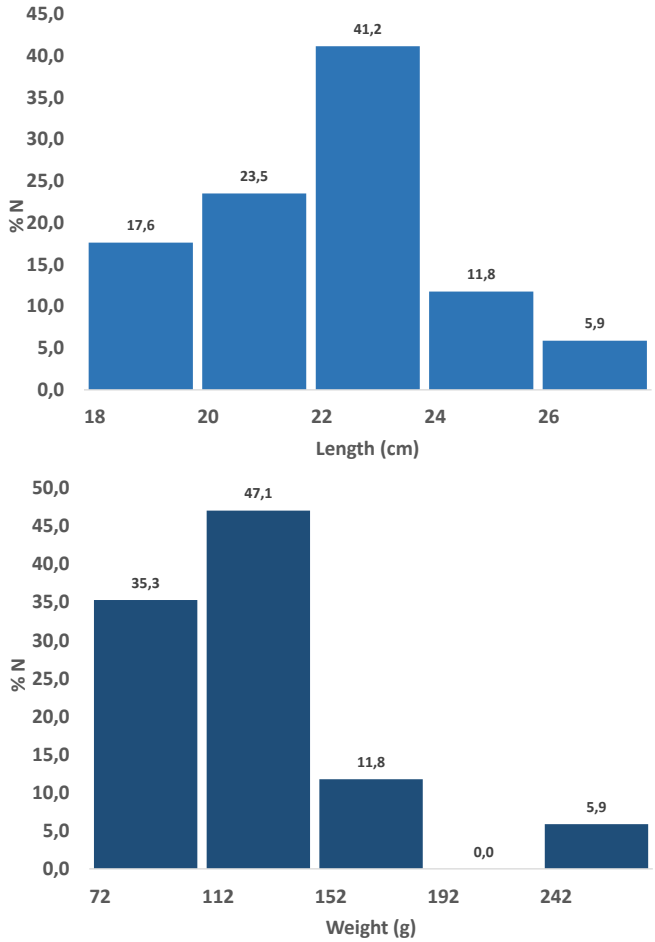

Figure 5. Length and weight distribution of *N. randalli* male specimens

The spermatozoa concentration was determined to be 160.0±45.63×10⁶ spz/ml. The flow cytometric determination of the sperm viability was 51.71±17.01% (Table 1).

Table 1. Total length (TL), total weight (W), sperm concentration, sperm viability values of male *N. randalli* species (Mean±SEM).

Mean Total length (mm)	21.75±0.50
Mean Weight (g)	131.31±10.75
Sperm Concentration (x10⁶/ml)	160.0±45.63
Sperm Viability (%)	51.71±17.01

4. Discussion

N. randalli, an Indo-Pacific fish migrating to the Mediterranean Sea, has established a sustainable population in the Gulf of Antalya, Turkey. No data on spermatological parameters of *N. randalli* were found in the literature. Therefore, in this study, the mean viability and concentration values of spermatozoa of *N. randalli* were investigated for the first time.

Reproductive biology studies on *N. randalli*, which has recently entered the Mediterranean Sea, revealed that reproductive periods differ according to habitats. Ozen [42] showed that the breeding period of *N. randalli* in the Gulf of Antalya is between June and October and the peak breeding period is between July and August. Taylan and Yapıcı [22] indicated that the reproductive activity of both female and male *N. randalli* occurs during the spring and summer months, with females being active

from April to August and males from April to July. Uyan [44], determine the spawning period of the species between May and June. Demirci, Demirci and Şimşek [41], in their study to determine the reproductive period, showed that the gonadosomatic index value of the species increased in February and reached the highest value in April and May. The result of this study that male individuals of *N. randalli* are dominant over female individuals is similar to other studies [17, 21, 22, 31, 35, 44, 45]. To better understand the importance of sperm quality in *N. randalli* reproductive performance, it should be compared with many parameters. In this study, viability and sperm concentrations were revealed. *N. randalli* sperm concentration was found to be 160.0±45.63×10^6 spz/ml. Flow cytometric analysis of sperm viability resulted in a value of 51.71±17.01%. In the literature, spermatozoa concentration values are given for some species. The spermatozoa concentration of Barbus barbus (Teleostei: Cyprinidae) decreased from 18.81× 10^9 spz/ml to 12.45 × 10^9 spz/ml in March to in May and decreased towards the end of the breeding season [6]. Hatipoğlu [46] determined that the average concentration of Abant trout was 17.85x10^9 spz/ml. While the concentration of spermatozoa in rainbow trout was found to be 11.80x10^9 spz/ml, reported it as 6.90 x 10^9 spz/ml by hemacytometric method in their study on the same species[47].

In *N. randalli* viability analysis by flow cytometric method was found 51.71±17.01%. There are some studies on spermatozoa viability rates in natural fish stocks. Trigo, Merino [48] was found the spermatozoa viability in rainbow trout (Oncorhynchus mykiss) 95.1 ± 0.68%. Also Nynca, Dietrich [49] reported that rainbow trout sperm viability was 97.00 ± 0.99% by flow cytometry and 86.22 ± 1.16% by fluorescence microscopy analyse. In a study conducted on Galaxias argenteus, a giant cockle grown in farms, sperm viability was determined to have 4.621 live cells and 1.168 dead cells by flow cytometry [50]. In these studies, it was determined that spermatozoa viability rates differed between species.

Differences in sperm concentration and sperm viability between species may be due to changing habitat conditions and abiotic parameters such as temperature, pH, salinity and dissolved oxygen. For some populations, sperm analyses cover a single sampling period. This may alter sperm values. In addition to seasonality and habitat conditions, the observed variation in sperm parameters may be due to biological characteristics of fish species, sex ratio, invasiveness, reproductive strategies, sampling and analysis methods.

Ozen [42] showed that the breeding period of *N. randalli* in the Gulf of Antalya is between June and October and the peak breeding period is between July and August. Taylan and Yapıcı [22] stated that both female and male *N. randalli* are reproductively active during the spring and summer, with the spawning season for females lasting from April to August and for males from April to July. Likewise, Demirci, Demirci and Şimşek [41] observed in their study on the species' reproductive period that the gonadosomatic index started rising in February and reached its highest levels in April and May.

During the present study males were more abundant in the catch of *N. randalli*. In the *N. randalli* populations under study, the sex ratio which varies from population to population within the same species was 1.89 males to 1 females. The small sample size prevented a clear determination of the gender ratio. According to Bohlen, Freyhof and Nolte [51], the sex ratio in a population can change from year to year, suggesting that genetics or environmental variables play a role in its determination. There are a variety of potential causes for this variation, such as seasonal variations, feeding and

maturation schedules, disparities in male and female growth rates, mortality differences between the sexes, and potentially size-selective impacts of fishing gear.

5. Conclusions

Determining fish sperm parameters is a crucial scientific phenomenon that helps comprehend the biology of invasion. The first information on spermatological characteristics of *N. randalli*, such as spermatozoa concentration and viability, was reported in this study. These data will help determine the invasion ecology, assess the possibility for aquaculture in the future, and comprehend the reproductive biology of this commercially significant species. The normospermic traits of this species will be determined in part by analyzing spermatological traits over time and with a larger number of individuals.

Supplementary Materials: Figure S1: A specimen of Nemipterus randalli, Figure S2: Sections of N. randalli spermatozoon, Figure S3: N. randalli sperm concentration analysis, Figure S4: N. randalli flow cytometry viability analysis, Figure S: Length and weight distribution of N. randalli male specimens, Table S1: Total length (TL), total weight (W), sperm concentration, sperm viability values of male N. randalli species (Mean±SEM)

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References

1. Cabrita, E., et al., Successful Cryopreservation of sperm from sex-reversed dusky grouper, Epinephelus marginatus. *Aquaculture*, **2009**. 287: p. 152-157. 10.1016/j.aquaculture.2008.10.019
2. Bobe, J. and C. Labbé, Egg and sperm quality in fish. *General and Comparative Endocrinology*, **2010**. 165(3): p. 535-548. https://doi.org/10.1016/j.ygcen.2009.02.011
3. Van Look, K.J.W. and D.E. Kime, Automated sperm morphology analysis in fishes: the effect of mercury on goldfish sperm. *Journal of Fish Biology*, **2003**. 63(4): p. 1020-1033. https://doi.org/10.1046/j.1095-8649.2003.00226.x
4. Asturiano, J.F., et al., Effects of hCG as spermiation inducer on European eel semen quality. *Theriogenology*, **2006**. 66(4): p. 1012-20. 10.1016/j.theriogenology.2006.02.041
5. Tuset, V.M., E.A. Trippel, and J. De Monserrat, Sperm morphology and its influence on swimming speed in Atlantic cod. *Journal of Applied Ichthyology*, **2008**. 24(4): p. 398-405. https://doi.org/10.1111/j.1439-0426.2008.01125.x
6. Alavi, S.M.H., et al., Changes of sperm morphology, volume, density and motility and seminal plasma composition in Barbus barbus (Teleostei: Cyprinidae) during the reproductive season. *Aquat Living Resour*, **2008**. 21(1): p. 75-80. https://doi.org/10.1051/alr:2008011

7. Suquet, M., M.H. Omnes, Y. Normant, and C. Fauvel, Assessment of sperm concentration and motility in turbot (Scophthalmus maximus). *Aquaculture*, **1992**. 101(1): p. 177-185. https://doi.org/10.1016/0044-8486(92)90241-C
8. Cosson, J., et al., Marine fish spermatozoa: racing ephemeral swimmers. *Reproduction*, **2008**. 136(3): p. 277-94. 10.1530/rep-07-0522
9. Inaba, K., Molecular mechanisms of the activation of flagellar motility in sperm. 2008; 267-279.
10. Björndahl, L., I. Söderlund, and U. Kvist, Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. *Hum Reprod*, **2003**. 18(4): p. 813-6. 10.1093/humrep/deg199
11. Zilli, L., et al., Adenosine Triphosphate Concentration and β-d-Glucuronidase Activity as Indicators of Sea Bass Semen Quality. *Biology of Reproduction*, **2004**. 70(6): p. 1679-1684. 10.1095/biolreprod.103.027177
12. Flajšhans, M., J. Cosson, M. Rodina, and O. Linhart, The application of image cytometry to viability assessment in dual fluorescence-stained fish spermatozoa. *Cell Biology International*, **2004**. 28(12): p. 955-959. https://doi.org/10.1016/j.cellbi.2004.07.014
13. De Baulny, B.O., Y. Le Vern, D. Kerboeuf, and G. Maisse, Flow cytometric evaluation of mitochondrial activity and membrane integrity in fresh and cryopreserved rainbow trout (Oncorhynchus mykiss) spermatozoa. *Cryobiology*, **1997**. 34(2): p. 141-149.
14. Cabrita, E., et al., Evaluation of DNA damage in rainbow trout (Oncorhynchus mykiss) and gilthead sea bream (Sparus aurata) cryopreserved sperm. *Cryobiology*, **2005**. 50(2): p. 144-153. https://doi.org/10.1016/j.cryobiol.2004.12.003
15. Galil, B.S., et al., 'Double trouble': the expansion of the Suez Canal and marine bioinvasions in the Mediterranean Sea. *Biol Invasions*, **2015**. 17: p. 973-976. http://dx.doi.org/10.3391/mbi.2016.7.2.01
16. Golani, D., The marine ichthyofauna of the Eastern Levant—history, inventory, and characterization. *Isr J Ecol Evol*, **1996**. 42(1): p. 15-55. https://doi.org/10.1080/00212210.1996.10688830
17. Innal, D., et al., Age and growth of Nemipterus randalli from Antalya Gulf-Turkey. *Int J Fish Aquat Stud*, **2015**. 2(4): p. 299-303.
18. Spanier, E. and B.S. Galil, Lessepsian migration: a continuous biogeographical process. *Endeavour*, **1991**. 15: p. 102-106. https://doi.org/10.1016/0160-9327(91)90152-2
19. Tikochinski, Y., et al., Reduced genetic variation of the Red Sea fish, Randall's threadfin bream Nemipterus randalli, invasive in the Mediterranean Sea. *Aquat Invasions*, **2019**. 14(4): p. 716-723. https://doi.org/10.3391/ai.2019.14.4.10
20. Bilecenoglu, M. and B.C. Russell, Record of Nemipterus randalli Russell, 1986 (Nemipteridae) from Iskenderun Bay, Turkey. *Cybium*, **2008**. 32(3): p. 279-280. https://doi.org/10.26028/cybium/2008-323-014
21. Erguden, D., et al., Age and growth of the Randall's threadfin bream Nemipterus randalli (Russell, 1986), a recent Lessepsian migrant in Iskenderun Bay, northeastern Mediterranean. *J Appl Ichthyol*, **2010**. 26(3): p. 441-444. https://doi.org/10.1111/j.1439-0426.2009.01387.x
22. Taylan, B. and S. Yapıcı, Reproductive biology of non-native Nemipterus randalli Russell, 1986 and native Pagellus erythrinus (Linnaeus, 1758) from the Aegean Sea. **2021**.
23. Golani, D. and O. Sonin, The Japanese threadfin bream Nemipterus japonicus, a new Indo - Pacific fish in the Mediterranean
24. Lelli, S., F. Colloca, P. Carpentieri, and B. Russell, The threadfin bream Nemipterus randalli (Perciformes: Nemipteridae) in the eastern Mediterranean Sea. *J Fish Biol*, **2008**. 73(3): p. 740-745. http://dx.doi.org/10.1111/j.1095-8649.2008.01962.x
25. Gökoglu, M., et al., First records of Nemichthys scolopaceus and Nemipterus randalli and second record of Apterichthus caecus from Antalya Bay, Southern Turkey. *Mar Biodivers Rec*, **2009**. 2: p. e29. http://dx.doi.org/10.1017/S175526720800033X
26. Gülşahin, A. and A. Kara, Record of Nemipterus randalli Russell, 1986 from the southern Aegean Sea (Gokova Bay, Turkey). *J. Appl. Ichthyol*, **2013**. 29( ): p. 933–934. http://dx.doi.org/10.1111/jai.12187
27. ElHaweet, A.E.A., Biological studies of the invasive species Nemipterus japonicus (Bloch, 1791) as a Red Sea immigrant into the Mediterranean. *Egypt J Aquat Res*, **2013**. 39(4): p. 267-274. https://doi.org/10.1016/j.ejar.2013.12.008
28. Kampouris, T.E., N. Doumpas, I. Giovos, and I.E. Batjakas, First record of the Lessepsian Nemipterus randalli Russell, 1986 (Perciformes, Nemipteridae) in Greece. *Cah Biol Mar*, **2019**. 60(6): p. 559-561. http://dx.doi.org/10.21411/CBM.A.53CEC126
29. Russell, B.C., *FAO Species Catalogue: Nemipterid Fishes of the World (Threadfin Breams, Whip Tail Breams, Monocle Breams, Dwarf Monocle Breams and Coral Breams*; FAO, 1990.
30. Gurlek, M., et al., Feeding habits of indo-pacific species Nemipterus randalli Russel, 1986 (Nemipteridae) in Iskenderun bay, Eastern Mediterranean Sea. *Rapp p.-v. réun - Cons. int. explor mer*, **2010**. 39: p. 539.
31. Yapıcı, S. and H. Filiz, Biological aspects of two coexisting native and nonnative fish species in the Aegean Sea: Pagellus erythrinus vs. Nemipterus randalli. *Mediterr Mar Sci*, **2019**. 20(3): p. 594-602. https://doi.org/10.12681/mms.19658

32. Akgun, Y. and E. Akoglu, Randall's Threadfin Bream (Nemipterus randalli, Russell 1986) Poses a Potential Threat to the Northeastern Mediterranean Sea Food Web. *Fishes*, **2023**. 8(8): p. 402.
33. Ali, M., A. Saad, C. Reynaud, and C. Capapé. Fırst Records Of Randall's Threadfın Bream Nemıpterus Randallı (Osteıchthyes: Nemıpterıdae) Off The Syrıan Coast (Eastern Medıterranean)/Prıme Segnalazıonı Dı Nemıpterus Randallı (Osteıchthyes: Nemıpterıdae) Al Largo Della Costa Della Sırıa (Medıterraneo Orıentale). in *Ann Ser Hist Nat*. 2013. Scientific and Research Center of the Republic of Slovenia.
34. Aydın, İ. and O. Akyol, Occurrence of Nemipterus randalli Russell, 1986 (Nemipteridae) off Izmir Bay, Turkey. *Egypt J Aquat Res*, **2016**. 18(39): p. 267-74. https://doi.org/10.1111/jai.13331
35. Özen, M.R. and O. Çetinkaya, Population Composition, Growth and Fisheries of Nemipterus randalli Russell, 1986 in Antalya Gulf, Mediterranean Sea, Turkey. *Acta Aquat Turc*, **2020**. 16(3): p. 330-337. https://doi.org/10.22392/actaquatr.681309
36. Tartar, Ü. and H. Yeldan, Some population dynamic parameters of Northeastern Mediterranean (Iskenderun Bay) threadfin bream, Nemipterus randalli Russell, 1986. *Acta Aquat Turc*, **2022**. 35(3): p. 3-1-7.
37. Uyan, U., et al., Fish length and otolith size of in Nemipterus randalli Russel, 1986 (Actinopterygii: Perciformes: Nemipteridae) collected from Gökova Bay, Turkey. *Thalass Sal*, **2019**. 41: p. 137-146. http://dx.doi.org/10.1285/i15910725v41p137
38. Yazıcı, R., et al., The length-weight (LWR) and length-length (LLR) relationships of Nemipterus randalli (Russel, 1986), an invasive species in Iskenderun Bay. *Acta Bio Turc*, **2024**. 37(1): p. 7-1-7.
39. Yazici, R., Sex ‐ linked variations in the sagittal otolith biometry of Nemipterus randalli (Russell, 1986) from the eastern Mediterranean Sea. *J Fish Biol*, **2023**. 102(1): p. 241-247. https://doi.org/10.1111/jfb.15256
40. Stern, N., et al., Distribution and population structure of the alien Indo ‐ Pacific Randall's threadfin bream Nemipterus randalli in the eastern Mediterranean Sea. *J Fish Biol*, **2014**. 85(2): p. 394-406. https://doi.org/10.1111/jfb.12421
41. Demirci, S., A. Demirci, and E. Şimşek, Spawning season and size at maturity of a migrated fish, Randall's Threadfin Bream (Nemipterus randalli) in Iskenderun Bay, Northeastern Mediterranean, Turkey. *Fresenius Environ Bull*, **2018**. 27(1): p. 503-507. https://dx.doi.org/10.17582/journal.pjz/20180327130349
42. Ozen, M.R., Some histological reveals on reproduction of one of the lessepsian species, Nemipterus randalli in Antalya (Turkey). **2021**. https://doi.org/10.3906/vet-2007-96
43. Caille, N., et al., Quantity, motility and fertility of tench Tinca tinca (L.) sperm in relation to LHRH analogue and carp pituitary treatments. *Aquac Int*, **2006**. 14(1): p. 75-87. http://dx.doi.org/10.1007/s10499-005-9015-0
44. Uyan, U., Nemipterus Randalli Russell, 1986nin Gökova Körfezinde bazı biyolojik özelliklerinin belirlenmesi. PhD Thesis, PhD Thesis, University of Muğla Sıtkı Koçman, Muğla, 2017.
45. Al-Kiyumi, F., S. Mehanna, and N. Al-Bulush, Growth, mortality and yield per recruit of the Randall's threadfin bream Nemipterus randalli (Russell, 1986) from the Arabian Sea off Oman. *Thalassas*, **2014**. 30(1): p. 67-73.
46. Hatipoğlu, T., Abant Alabalığında (salmo trutta abanticus) bazı reprodüktif özelliklerin saptanması, spermanın kısa süreli saklanması ve dölverimi. **2007**.
47. Seçer, S., E. Akçay, Y. Bozkurt, and S. Kayam, Gökkuşağı Alabalıklarında (Oncorhynchus mykiss W., 1792) Yaşın Spermatolojik Özellikler Üzerine Etkisi. *Turk J Vet Anim Sci*, **2003**. 27: p. 37-44.
48. Trigo, P., et al., Effect of short ‐ term semen storage in salmon (O ncorhynchus mykiss) on sperm functional parameters evaluated by flow cytometry. *Andrologia*, **2015**. 47(4): p. 407-411. https://doi.org/10.1111/and.12276
49. Nynca, J., et al., Usefulness of a portable flow cytometer for sperm concentration and viability measurements of rainbow trout spermatozoa. *Aquaculture*, **2016**. 451: p. 353-356. http://dx.doi.org/10.1016/j.aquaculture.2015.09.027
50. Pearce, J., et al., Assessing sperm membrane viability using flow cytometry in farmed New Zealand giant kokopu Galaxias argenteus. *N Z J Mar Freshw Res*, **2018**. 52(3): p. 362-371. https://doi.org/10.1080/00288330.2017.1394883
51. Bohlen, J., J. Freyhof, and A. Nolte, Sex ratio and body size in Cobitis elongatoides and Sabanejewia balcanica (Cypriniformes, Cobitidae) from a thermal spring. *Folia Zool*, **2008**. 57(1/2): p. 191.