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Some histological reveals on reproduction of one of the lessepsian species, *Nemipterus randalli* in Antalya (Turkey)

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Abstract: In this study, the reproduction of *Nemipterus randalli*, one of the lessepsian fish catch from the Gulf of Antalya, Turkey was interpreted histologically. Regarding the fish used in this study (N = 1715), it is noteworthy to mention that male individuals are larger than females. The first sexual maturity in males was determined 17 cm TL and 60 g in BW, whereas 13 cm TL and 25 g BW in females. Females reached sexual maturity at age I+ and males at the age of II+. Seven different stages (O1–O7) were identified in oocyte maturation in the ovaries. The spawning time of *N. randalli* in the Gulf of Antalya extended from June to October (Peak on July–August), given the histological interpretation of the development in gonads and the GSI % data. According to this data, *N. randalli* may be described to have a Batch spawner characteristic.

Key words: *Nemipterus randalli*, histology, reproduction, batch spawner, Gulf of Antalya

1. Introduction

The Lessepsian migration, a one-way movement of various marine species from the Red Sea to the Eastern Mediterranean via the Suez Canal, was first described by Por [1,2]. These invasive species having more extensive adaptability against biotic and abiotic barriers established their colonies and have imposed significant ecological and economic impact in the Eastern Mediterranean [2–4]. Among several others, one of the species is Randall's Threadfin Bream *N. randalli* RUSSELL, 1986 (Osteichthyes: Nemipteridae) are widely available in different sizes in tropical and subtropical Indo-Pacific region and significantly contributes to fishery [3,4]. Golani and Sonin study identified Lessepsian immigrants for the first time accidentally on the Mediterranean coast of Israel [4]. In Turkey, Bilecenoğlu and Russel reported similar migration in İskenderun Bay on the east coast [5] and Gökoğlu et al. in Antalya Bay [6].

Shape, colour, texture, and size are the most important parameters in the macroscopic determination of gonadal development and maturity stages in fish [7]. Gametogenesis and the ripening phases in the gonads among teleost fish are similar.

Spermatogenesis in male fish: Spermatogonia consists of universal stages called spermatocytes, Spermatid, and spermatozoa. The reproductive cells of male gonads can be produced year-round without interruption. The stages of regression and regeneration observed in the female fish are never seen [7]. Oogenesis in female fish occurs in stages as follows: oogonia, primary growth oocyte, pre-vitellogenic oocytes, cortical alveolar stage (egg yolk vesicles), vitellogenic stage, oocyte maturation, and ovulation (spent) stages, respectively [8]. In this study, it is aimed to better understand the reproductive activities of *N. randalli* and to interpret the data on reproductive studies in the most accurate way with histology.

2. Materials and methods

In this study, R/V AKDENIZ SU, which is equipped with the bottom trawl belonging to Akdeniz University Faculty of Fisheries, was used. Throughout the research period (2014–2015), sampling was carried out every month from the depth of 25–55 m in the Gulf of Antalya, Turkey (Figure 1 A) between Lara and Side Lighthouse (36° 41'N, 31° 22' E; 36° 41' N, 30° 56' E) with cod end mesh size of 22 mm. The fish of *N. randalli,* which, at first glance, resembles *Sparus pagrus*, selected from among the caught fish, is understood from the continuing extension of the caudal fin at the tip of the upper lobe (Figure 1 B). Length (cm), weight (g) and age determination of the caught fish (Immature + Female + Male = 1715) were done; average total weight (TW) was 0.73–171.78 gr, average total length

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(TL) was 3.9–23.8 cm; were found to be. Sex composition was also calculated.

They were dissected and their gonadal differentiation and status identified and recorded. Gonad samples were fixed in 10% neutral buffered formaldehyde solution [9–12]. Gonado Somatic Index (GSI) was calculated by using the formula GSI % = Weight of Gonad (g)/Weight of Fish (g) ×100 [13].

The fixed gonad samples were processed by routine paraffin embedding techniques and were cut in 5-micron thickness by a rotary microtome. The sectioned tissues were stained by haematoxylin-eosin staining routine technique and examined under photo-microscope and photographed [9–12]. Brown-Peterson et al. [14]. proposed a universal terminology for the phases in the reproductive cycle applicable to all male and female elasmobranches and teleost fish. Prominent stages of development include immature, developing, ovulating, regressing, and regenerating phases. The development stages of ovarian and eggs of this species were identified as I. Rest, II. Development, III Mature, IV. Ovulation and V. Spent [15].

3. Results and discussion

3.1. Macroscopic observations

In this study, the immature gonad (stage Ist) and spent gonads (stage VIIth) of specimens could not be distinguished due to their very slender and transparent cords structure macroscopically.

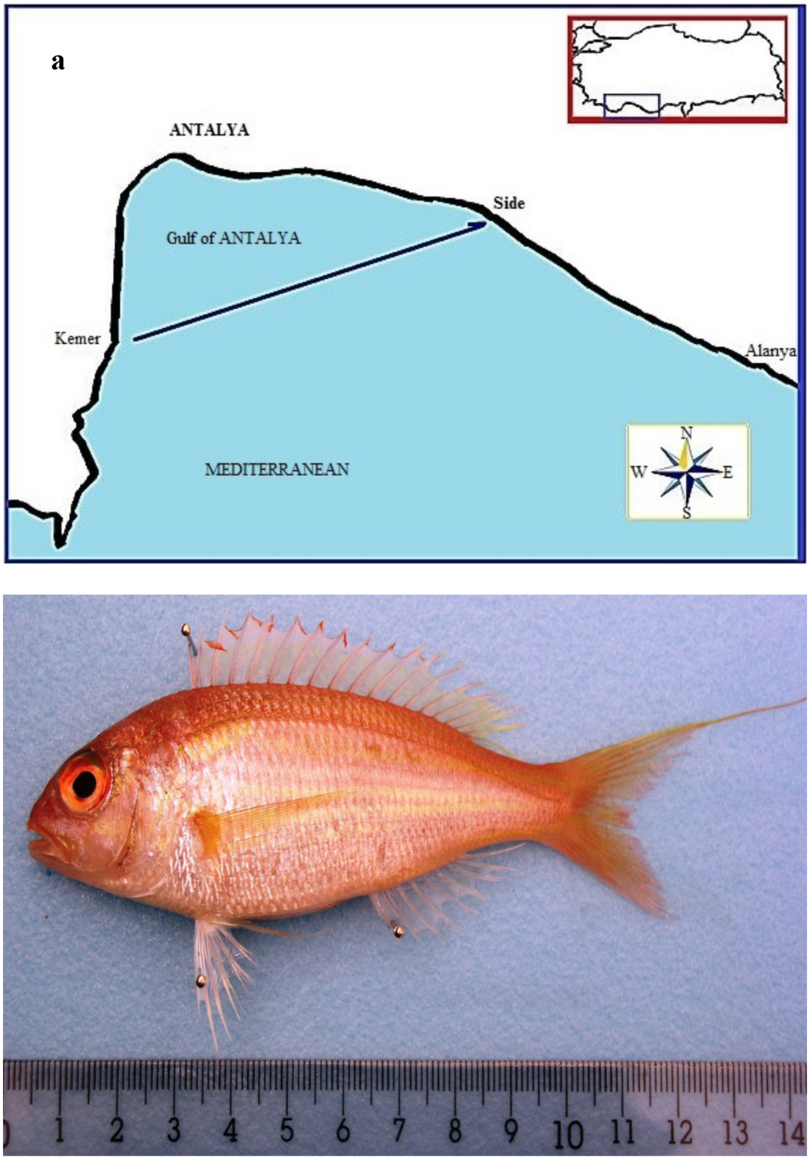

Figure 1 a. Sampling area. Gulf of Antalya, Mediterranean, Turkey, **b**. *N. randalli.*

Macroscopically, in mature male fishes, testes are paired and situated ventrally (Figure 2 A). Testes are relatively smaller than the ovaries and whitish in colour, smoothly shaped; they lie just below the thick salmon pink coloured peritoneum (Figure 2 A). An accessory kidney was found that as embedded in the viscera near the urinary tract, covered with peritoneum, brown in colour (Figure 2 A). In male individuals, the size of this structure increased by the progress of sexual maturation (Figure 2 A). Macroscopically, the mature ovaries are larger, pink-orange in colour, granulated, and veined structure (Figure 2 B). It is also seen that the ovaries are under the salmon-pink coloured peritoneum (Figure 2 B). In females, accessory kidney structure remained small in all stages of maturation (Figure 2 B).

The maturing ovaries (II-IV stages), which are at various stages of maturation; that have not spawning maturity are smooth, little in size, and pink-white in colour .

The sex determination of *N. randalli* that has immature gonads (stage I) could not be done by naked eye due to their gonads' very slender and transparent appearance. The gonads of female fish are usually in Ist, IInd, and

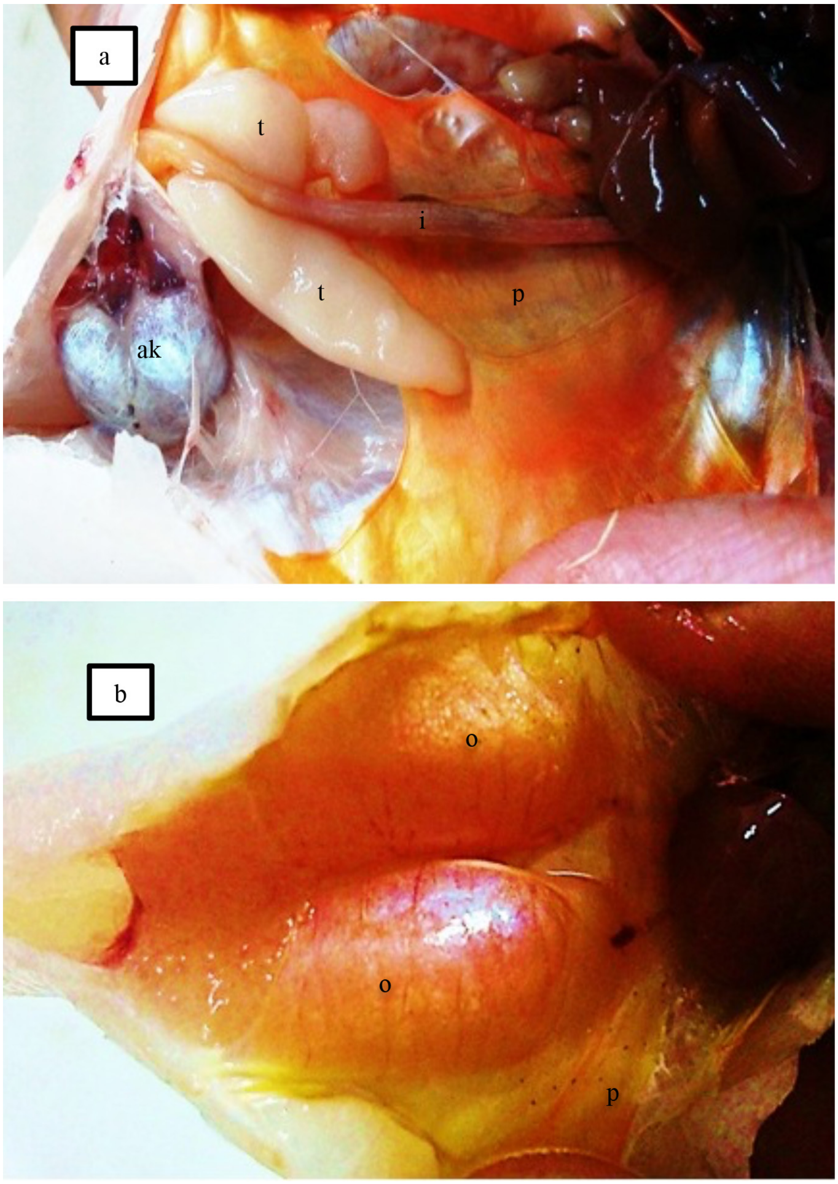

Figure 2. Gonadal morphology of male and female of *N. randalli.* **a.** Male gonads (Testes). t: testis, p: peritoneum, ak: accessory kidney, i: intestine. **b.** Female gonads (Ovaries). **o**: ovary, **p**: peritoneum.

IIIrd stages in winter and mid-spring. In late spring, in summer, and early autumn, the presence of IVth, Vth, and VIth stages of ovaries clearly indicate reproductive activity. IVth and Vth stages are the stages of egg maturity and spawning. Stage VI is an empty ovary stage. But, due to its transparent appearance, it can be confused with the immature (I. stage) stage.

3.2. Histologic observations

3.2.1. Male fish

The testicular structure of *N. randalli* was observed to be in lobular testicular form. It was determined that sperm production continued all months. Testis tissue was found to be divided into lobes by connective tissue septas (testicular lobulation) (Figure 3). In the lobes, reproductive cells

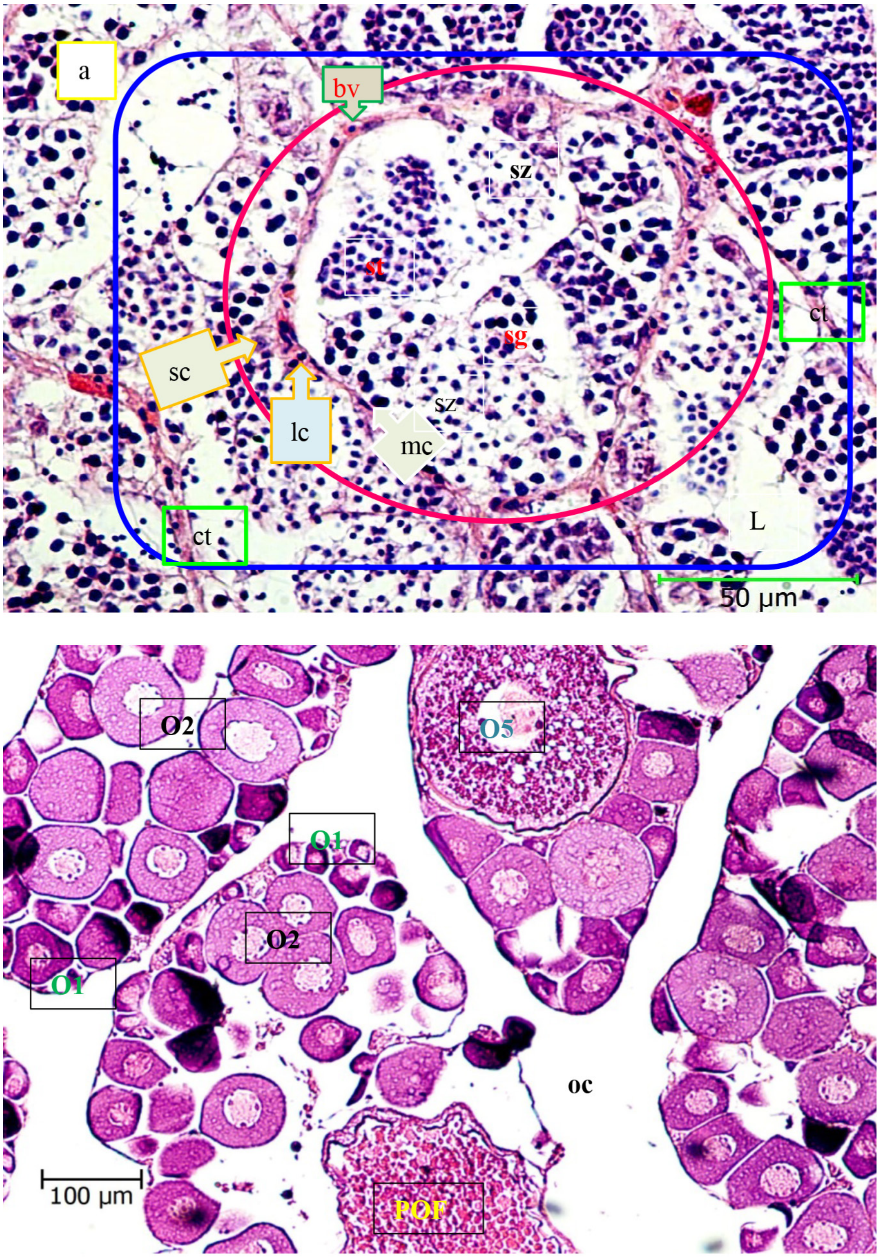

Figure 3. Testis tissue and ovary tissue of *N. randalli* (January) H&E. **a.** Testis tissue. Blue frame: germinal compartment, Pink circle: interstitial compartment. sz : spermatozoa, lc: leydig cell, sc: sertoli cell, sd: spermatid, sg: spermatogonia, L: lumen, bv : blood vessel, mc: myoid cell, ct: connective tissue. **b**. Ovary tissue. POF : post ovulatory follicle (O7), O5: matured oocyte, O1; primary growth, O2:perinucleolar oocyte, oc: ovarian cavity.

(Figure 3) were seen in different stages of development of sperm cells to function in reproductive activities. In the testicular tissue, parts of the germinal compartment (Figure 3 A) supported by sertoli cells (Figure 3A) and interstitial compartments (Figure 3 A) containing connective tissue (ct), blood vessels (bv), myoid cells (mc) and leydig cells (Lc) were also seen (Figure 3 A).

3.2.2. Female fish

Histological examinations of gonads in the young females showed many developing sex cells covered superficially by tunica albuginea. The immature ovary of the *N. randalli* was also found as cist ovarian type, the oocytes composed of different stages of chromatin nuclear and peri-nucleolar oocytes in the ovarian cavity (Figure 3B). The oocytes (primary egg cells) mature in a series of stages of development known as oocyte development or oocyte growth before they spawned. Seven different phases that generally reported in oocyte development have also been found in *N. randalli*. These oocyte development stages were determined as O1, O2, O3, O4, O5, O6, and O7. In O1 stage, which is the first growth stage of oocyte development, a large nucleolus is seen in the nucleus surrounded by a small amount of cytoplasm at the centre of the oocytes. Primary growth cells (O1) densely staining basophilic with H&E (Figure 3B) was found in all samples in January, February, and March. This phase, also known as the chromatin nucleus phase, consists of cells (O1) that have not yet acquired reproductive properties and grow for the first time (Figure 3B). However, some January samples, the presence of several O5 (matured oocytes) and their remaining from autumn spawning post-ovulatory follicle (POF) cell among the O1 cells (Figure 3 B) suggest that these fish may have had a regression phase. Sperm cells of various developmental stages were observed in the testes tissue of the male fish that were captured in April (Figure 4 A). In the ovaries of female fish, after the cytoplasm of the oocytes reaching the second stage of the growth phases (O3: Cortical alveolar stage), it was noticed that their previous dark blue colour turned to lighter shades (Figure 4 B).

It was determined that the egg yolk vesicles that emerged during this phase grew in proportion to the growth of the oocytes. It was also seen that a sheath known as Zona radiata or chorion became more prominent around the oocyte (Figure 4 B). Later, the amount of vitellus increased further in the cell, leading to oocyte growth. It is the vitellogenesis stage in which oocytes emerge, the most important stage of secondary growth, in the ovaries of specimens captured in May. This indicates that females now have a fully mature ovarium.

Some degenerated oocytes and translucent granules called cortical alveolar, which is one of the distinguishing features of the third stage, have been noticed in microscopic examination. It was revealed that the zona radiata (chorion) became thicker (Figures 4, 5) and the nucleoli were pushed towards the oocyte wall along with the cortical alveoli at this stage.

Additionally, these structures began to be seen around the nucleus and moved toward the cell periphery according to oocyte development. There has been an increase in the size of the nucleus and cytoplasm compared to the previous stage (Figure 5).

At the end of the resting and re-maturation process from May to July, *N. randalli* had shown a complete breeding activity in Antalya Bay during the June to October period. Following spawning that started in June (peak on July–August), the GSI% data has begun to decline after August (Figure 6).

In the histological examinations of the gonads of the female fish; hydrated oocytes (Figure 7 A), un-ovulated and degenerated oocytes at the end of spawning, the follicular atresia, and the apoptotic appearance were observed (Figures 7A, 7B). This indicates that a reproductive activity is realized and finished at this period of time (Figures 7A, 7B). This result was also confirmed both of sex with GSI% graphs (Figure 6). Until October, with the help of appropriate climatic conditions, however, some individuals still continue to show also a little reproductive activity.

In the histological examination of November's ovaries, a few mature oocytes (O5) and some remnant oocyte (O6) along with primary growth oocytes were seen (Figure 8). November to May, the resting stage is a period of preparation for a new reproductive activity in the gonads.

4. Discussion

Invasive species are reported to tend to reach sexual maturity in younger ages in order to become more dominant in their habitats [16]. In İskenderun Bay, the first sexual maturity length of *N. randalli* is 11:02 cm [15]; in Gökova Bay, the first sexual maturity lengths were reported as 12.86 cm in males and 15.35 cm in females [16].

The sizes of the sampled fish ranged between 3.9–23.8 cm. Male individuals were found to be generally larger than females ($p < 0.05$). Except for September 2014 specimens, females are more dominant (M:F 1:1.402). Sex ratio of *N. randalli* was found not significant ($p > 0.05$). The first sexual maturity size in males was determined 17 cm TL and 60 g in BW, whereas 13 cm TL and 25 g BW in females. The minimum length for both sexes was defined as 9–10 cm TL. While females reached sexual maturity at I + age, males reached sexual maturity after the age of II + years old. So, the females of the fish samples reached sexual maturity at an earlier age than males. The females of this species were also smaller than males and were lighter than males. These results reveal that *N.randalli* fish

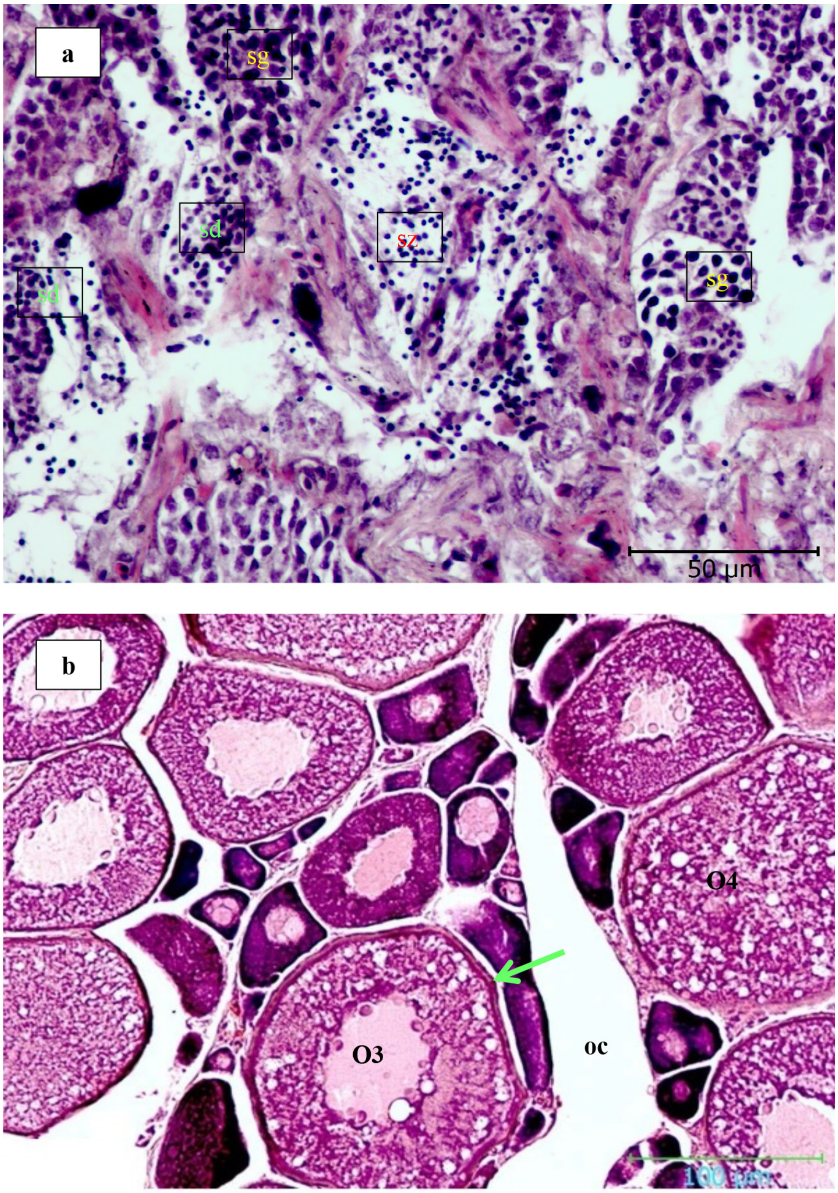

Figure 4. Ovary and testis tissues of *N. randalli* (April) H&E **a.** Testis tissue. sg: spermatogonia, sd: spermatid, sz: spermatocytes. **b.** Ovary tissue. oc: ovarian cavity; O1: chromatin nucleus; O3: cortical alveolar; O4 : mature oocytes, arrow**:** zona radiata.

reached sexual maturity early in their life, as Yapıcı and Filiz stated [16].

A previous study had reported delimited type rudimentary hermaphroditism in the bisexual gonads of the juvenile stage of *N. bathybius* and *N. virgatus* species belonging to the genus Nemipterus [17].

In the current study, as a result of histological examinations of ovarian and testicular tissues, *N. randalli* was determined as a fish that had bisexual reproduction but with no sign of hermaphroditism.

In Table 1, it is also given M:F ratios of *N. japonicus* and *N. randalli* species living in various seas of the world. According to these data, overall female fish are reported to be either equal to or higher than male ones [16, 18–23].

Although most exploited marine teleost are highly fecund and produce either pelagic or demersal eggs, their reproductive timing strategies vary widely. All fish undergo sexual maturation. They participate in one or more reproductive cycles, spawn once or more per cycle, age, and die.

However, reproductive timings range from spawning only once during a lifetime to spawning multiple times within an extended spawning season for many years [8].

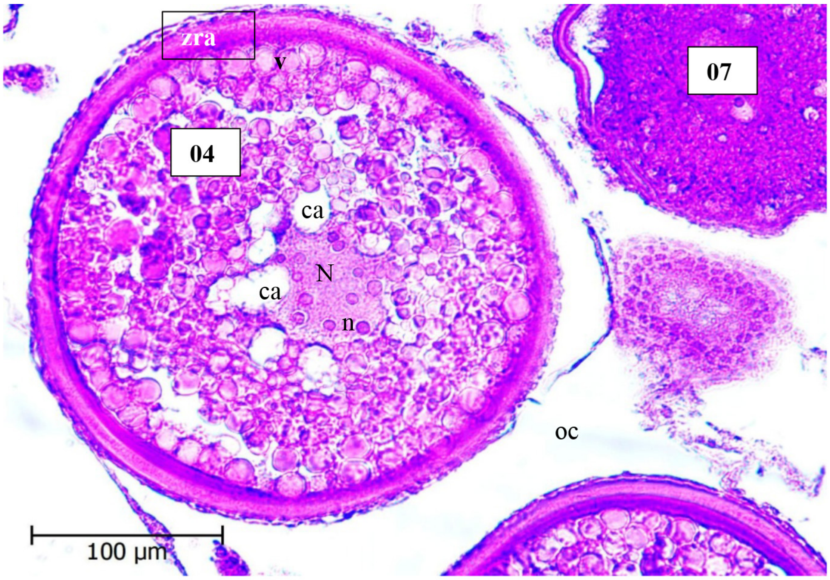

Figure 5. Ovary tissue of *N. randalli* (May) H&E. zr: zona radiata, oc: ovarian cavity; ca: cortical alveolar; N: nucleus, n: nucleolus; O4: mature oocytes; O7: degenerated oocyte.

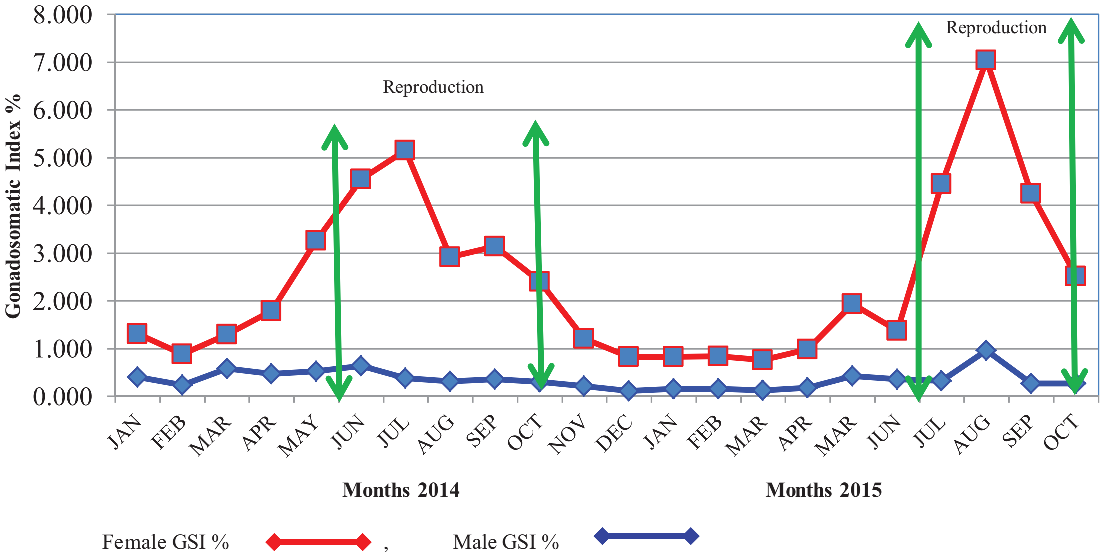

Figure 6. GSI % changes in male and female of *N. randalli* in Gulf of Antalya.

The reproductive cycle of female fish can be divided into the period of oocyte growth followed by oocyte maturation along which the oocyte goes through different stages of development before ovulation and spawning [24].

Brown–Peterson proposed terminology for the reproductive cycle in both males and females of cartilaginous and bony fishes [14]. Gonadal development stages, according to this proposal, are called as immature (1), developing (2), spawning capable (3), regressing (4), and regenerating (5).

The other study has revealed that maturation and spawning stages of *N. japonicus* fish on Mangalore coasts reported immature, immaturing, maturing I, maturing II, ripe, and spent stages by RAJESH et al. [25]. Along with similar lines in *N. japonicus*, the spawning season was extended from May to September in Suez Gulf, Red Sea, Egypt, and gonadal development stages were also reported in five classes: I. immature stage, II. maturing stage, III. mature stage, iv. ripe stage and v. spent stage [26]. Nettely et al. argued that ovarian maturity of *N. japonicus* occurred

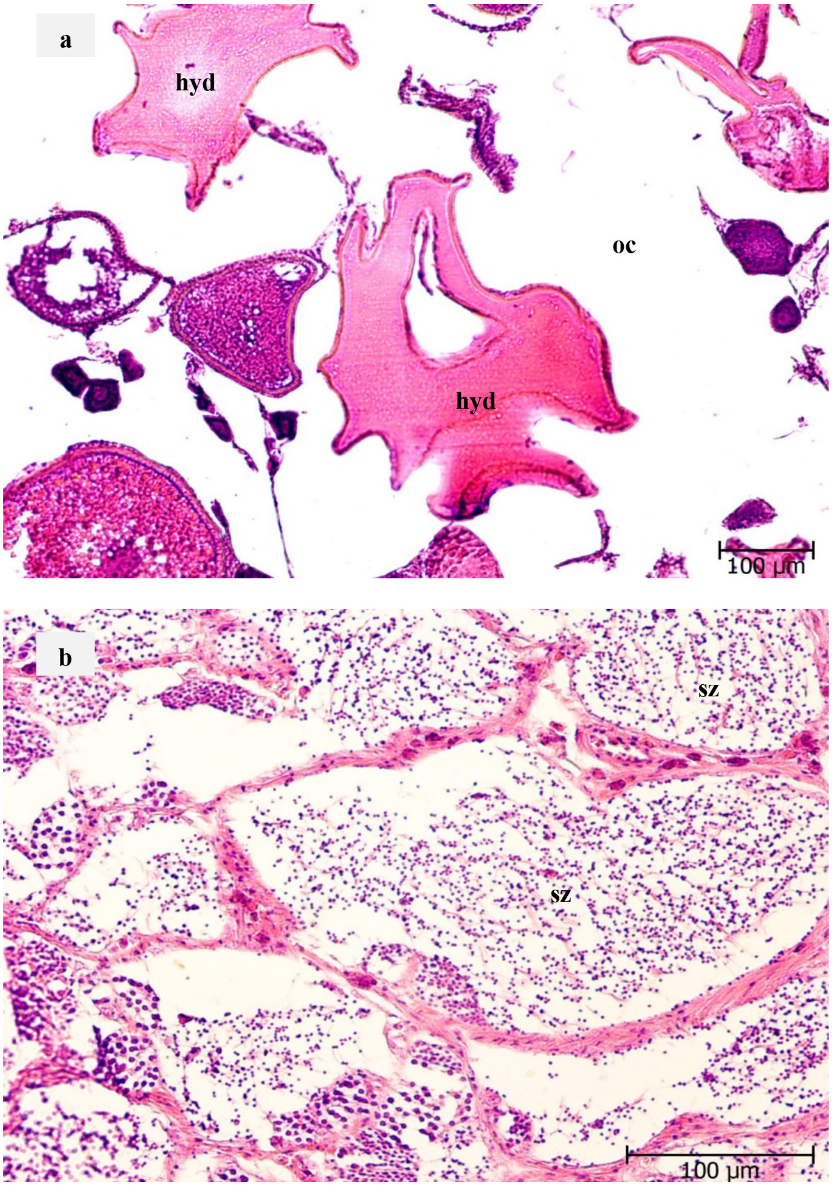

Figure 7. Ovary and Testis tissues of *N. randalli* (August) H&E.a: Ovary tissue; oc: Ovarian cavity; hyd: Hydrated oocyte, b. Testis tissue; sz: Spermatozoa.

in seven stages histologically, including immature I, immature II, maturing III, mature IV, mature V, ovulation VI, VII being spent [27].

In the present study, the gonadal development and ovarian maturity stages of the *N. randalli* species are as follows: immature, maturing, mature, spawning, and spent (totally in five stages). The immature phases can be only one time during the lifespan of all fishes (14). The matured ovaries contained egg cells in all stages of maturity [28].

The findings of the study revealed that, in the cached fish, females (I $^{+}$ years old) reached sexual maturity earlier than males (II $^{+}$ years old), but their height was smaller than males. In other studies, *N. peroni, N. mesoprion, N. japonicus* species were reported as the first sexual maturity length (M, F), spawning and peak time in the year. These stages of development, which continue until the seasonal release of some of the eggs, can be repeated several times a year in some fish genera such as Nemipterus. This type of fish leaves their eggs from time to time; batch spawner [14,29,30].

Research conducted in various seas of the world; it is reported that the spawning season of *N. japonicus and N. randalli* occurs in different months depending on the environmental factors of each region. In Table 2, some Nemipterid species (*N. japonicus* and *N. randalli*)

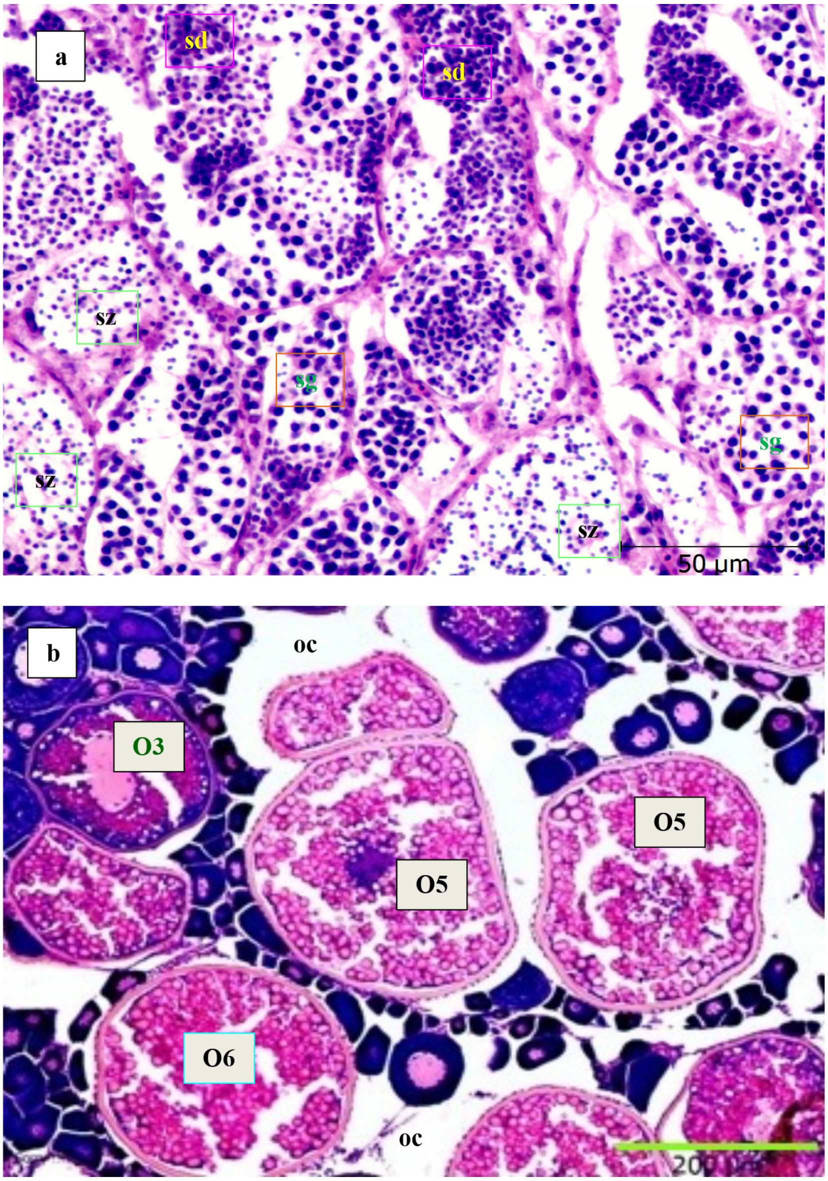

Figure 8. Ovary and testis tissues of *N. randalli* (November) H&E. a. Testis tissue. sg: Spermatogonia, sd: Spermatid, sz: Spermatocytes. b. Ovary Tissue. O1: Chromatin nucleus, O3: Cortical alveolar, O5: Mature oocyte; O6: Remnant oocyte.

have undergone spawning periods according to where they live [15,16,18,19,21,28,31]. July October may, July and October 2014-2015 breeding activities of *N. randalli* species in Antalya Bay (Turkey) were determined in this study.

5. Conclusion

The seasonal shift, which was a result of global warming, also caused deviations in the spawning periods of the fish. In this study, *N. randalli*, living in the Gulf of Antalya, spawned between June–October (peak on July-August). When the two sampling years (2014 and 2015) are evaluated together, reproductive activities have been continued from June to October. The months of most intense spawning has been observed in July–August. In addition, changing environmental conditions of both years (for example, temperature changes) caused differences in the timing of fish breeding activities.

Nemipterus randalli, one of the lessepsian species settled in Antalya Bay (Turkey), laid its matured eggs for the first time in June. Those that mature until June were observed to lay eggs in July and August. Their eggs,

Table 1. Male and female ratios (*M:F*) of *N. japonicus* and *N.randalli* of various seas.

M:F	Species	Location	Reference
1:0,53	*N. japonicus*	Eastern Mediterranean Sea	(3)
1.70:1	*N. randalli*	İskenderun Bay; Turkey	(15)
1:1.28	*N. randalli*	Gökova Bay, Turkey	(16)
F: a lot	*N. japonicus*	Abu Qir, Alexandria, Egypt	(19)
0.9:1	*N. japonicus*	Arab Sea Coast, Oman	(20)
0.9:1	*N. randalli*	Antalya Gulf, Turkey	(20)
1:2,6	*N. japonicus*	Northern Persian Gulf; Iran	(21)
1:1,01	*N. japonicus*	Veraval (Gujarat); India	(22)
1:1.08	*N. japonicus*	Saurashtra (Gujarat); India	(23)
1:0,90	*N. japonicus*	Mangalore Coast	(25)
1:1	*N. japonicus*	Bintulu, Sarawak, Malaysia.	(27)
1.29:1	*N. japonicus*	İskenderun Gulf, Turkey	(32)
1:1,402	*N. randalli*	Antalya Bay; Turkey	Present Study

Table 2. Spawning periods of *N. japonicus* and *N. randalli.*

Species	Location	Spawning	Reference
N. randalli	İskenderun Bay, Turkey	Apr–May	(15)
N. japonicus	Gulf of Suez	Sep–May	(18)
N. japonicus	Abu Qir, Egypt	Apr–Nov	(19)
N. randalli	Antalya Bay, Turkey	Apr–Oct	(20)
N. japonicus	Northern Persian Gulf	May–Sep	(21)
N. japonicus	Veraval, India	Nov–Dec	(22)
N. japonicus	At Waltair	Dec–Feb	(28)
N. japonicus	Gujarat, India	Sep–Apr	(31)
N. randalli	Antalya Bay, Turkey	June–Oct	Present Study

which mature until September, were found to spawn until the end of October. This is a recurring and prolonged spawning period. Based on the observations, histological examinations and GSI data evaluated together, we may say that *N.randalli* was able to spawn more than one egg during a spawning period. All of these indicate that *N.randalli* has also batch spawner characteristics.

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