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Preliminary results on the ovarian histology of rabbitfish *Siganus rivulatus* (Forsskal, 1775) in the southern Aegean Sea, Turkey

by

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Abstract

The study aimed to determine the annual reproductive cycle of *Siganus rivulatus* females based on monthly variation of the gonadosomatic index (GSI) and histological changes in gonads. A total of 240 fish samples were collected monthly from small-scale (trammel nets) and recreational (angling and pot fishery) fishing from Gökova Bay, the southern Aegean Sea, between July 2017 and June 2018. The length and weight of female fish varied from 10.8 to 26.1 cm with a mean length of 18.7 ± 0.2 cm and from 14.98 to 293.42 g with a mean weight of 91.1 ± 2.93 g. GSI values began to increase in May, peaked in June and gradually decreased in July, indicating that the spawning season was early summer. A total of 240 gonads were histologically examined. Granular, pinkish structure of eggs was observed primarily between April and June, which was consistent with the increase in the gonadosomatic index. Post-spawning follicles were determined in July. Values of the gonadosomatic index gradually decreased after August and they were minimal between September and November. During this period, ovaries were surrounded by immature oocytes at the chromatin and peri-nucleolus stages. These results revealed that *Siganus rivulatus* started vitellogenesis in April and the gonadosomatic index peaked in June.

Key words: fisheries management, *Siganus rivulatus*, ovary, histology

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Introduction

Rabbitfish or spinefoots (Siganidae) are distributed in the Western Indian Ocean, in several localities in East Africa and from the Red Sea to the eastern Mediterranean via the Suez Canal. They are represented by 29 species all over the world (Froese & Pauly 2019), which occur in shallow waters, usually in protected areas, generally in schools of 50 to several hundred individuals grazing on algae (Froese & Pauly 2019). The marbled spinefoot *Siganus rivulatus* (Forsskål 1775) belonging to Siganidae is of commercial importance for Turkish small-scale fisheries, although it has not yet been included in the national fishery statistics.

As the marbled spinefoot is a commercial fish species, it is one of the target species for small scale fishermen in the southern coast of Turkey. Due to its flavor, it is also an important species for recreational anglers. The capture-based Siganidae production was estimated at 86.229 tons in 2015 from the Eastern and Western Indian Ocean, the Mediterranean Sea and the Western Central Pacific (Fisheries and aquaculture software 2019). Although this is of global and regional importance to the fisheries economy, there are no official landing data for Turkey.

There have been many studies on the growth, mortality and yield per recruit of the spinefoot in the Red Sea (El-Gammal 1988; El-Ganainy 2002; Mehanna & Abdallah 2002). In addition, different biological aspects were investigated, including age, growth, mortality, and yield per recruit of *Siganus rivulatus* in the Mediterranean basin (Mohammed 1991; Bilecenoglu & Kaya 2002; Bariche 2005; El-Far 2008). However, reproduction of the species was the subject of only one comprehensive research from the Turkish Mediterranean coast (Yeldan & Avşar 2000). The literature review reveals that there is insufficient information on the spawning of this species, not only from the southern Aegean Sea, but also from Turkey. Therefore, it is very important to determine the reproductive season, sexual maturity and fecundity of the species in order to manage fisheries of the species, as no regulatory tool has been applied for the marbled spinefoot in Turkey.

The current study was carried out to determine the annual reproductive cycle of *Siganus rivulatus* based on monthly variation of the gonadosomatic index and histological changes in gonads. This is the first study on gonad histology of the species performed in Turkey. Results of this study are expected to contribute to the fisheries management of the species and will lead to further detailed studies.

Materials and methods

Fish samples were collected monthly, 20 fish each month, from small-scale (trammel nets) and recreational (angling and pot fishery) fisheries in Gökova Bay, the southern Aegean Sea, between July 2017 and June 2018 (Fig. 1). The total length (TL) was measured in the natural body position to the nearest millimeter. The total weight (W) and weight of gonads

Figure 1

Sampling locations (indicated by black dots) to determine the ovarian histology in the marbled spinefoot from Gökova Bay, the southern Aegean Sea

(W_g) were measured to the nearest 0.01 g, and sex was determined after the dissection of a specimen. The spawning season was established based on monthly variation in the gonadosomatic index (GSI, %), using the equation GSI = [W_g / (W – W_g)] × 100, where W_g is the weight of gonads (g) and W is the total weight (g) of the fish (Ricker 1975). Sex and maturity were determined based on macroscopic analysis of gonads. Maturity stages were assigned using Gunderson's (1993) five-stage classification system: stage I = immature, stage II = resting, stage III = developing, stage IV = mature, and stage V = spent. Histological examination was carried out to determine seasonal changes in the gonads. A total of 240 gonad samples were histologically examined. Gonadal tissue samples were fixed in 10% formalin buffered solution for 24 h. They were dehydrated through a graded series of ethanol, cleared in xylene, embedded in paraffin, and sectioned in a Leica 2125 rotary microtome at a thickness of 5 µm. Sections were stained with haematoxylin and eosin (Luna 1982; Bancroft & Stevens 1996) and then examined under an Olympus JX31 light microscope. Samples were photographed by an Olympus E330 digital camera.

Results

The length of female fish varied from 10.8 to 26.1 cm, with a mean length of 18.7 ± 0.2 cm. Furthermore, the weight of females was in the range of 14.98–293.42 g, with a mean weight of 91.1 ± 2.93 g. The length–weight relationship was $W = 0.0065L^{3.25}$ ($R^2 = 0.956$), indicating positive allometric growth (t-test, $p < 0.05$, Fig. 2).

GSI values began to increase in May, peaked in June and gradually decreased in July, indicating that the spawning season was in early summer. Furthermore, the minimum value was recorded in September, while from September to early May the trend was almost stable (Fig. 3).

Maturity stages of gonads

Stage I (immature): Samples identified as immature were observed in December and January with an average weight of 112.72 g. The color of ovaries was dirty yellowish-red and gonads were very small and thinly structured.

Stage II (resting): During the sampling process in February and March, ovaries of females were clearly visible with the naked eye. Female ovaries began to have a granular, symmetrical, light pink or transparent structure.

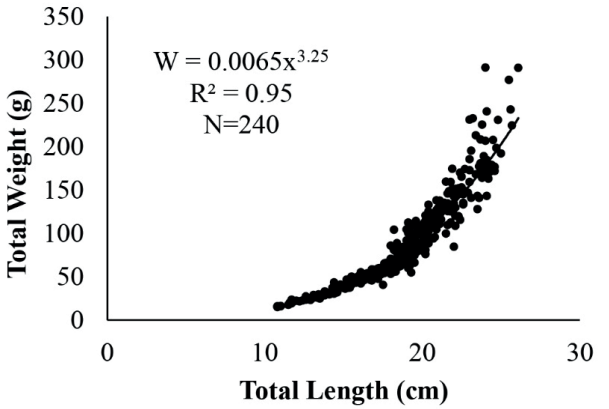

Figure 2

Length–weight relationship for female marbled spinefoot from the southern Aegean Sea

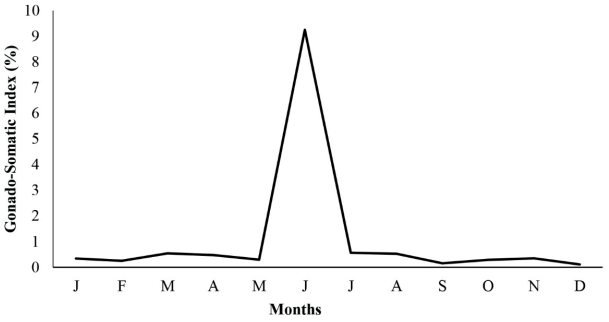

Figure 3

Monthly gonadosomatic index (GSI) for females of the marbled spinefoot *Siganus rivultaus* in the southern Aegean Sea

Stage III (developing): Sex can be easily determined with the naked eye. This stage was observed in April, May and June. Eggs had a pinkish and granular structure.

Stage IV (mature): Samples obtained in July included ovaries that filled the medio-dorsal part of the whole body (Fig. 4). Ovaries were orange or pink and surrounded with developed veins. Eggs were composed of big and mature cells.

Stage V (spent): This phase continued from August to November. Ovaries were flaccid, vascularized, salmon pink, oocytes were smaller with the presence of hyaline spaces. The fourth stage was defined as the peak time of matured eggs in July. Spawned females were observed between August and November. Gonads were in the resting phase until December–January and immature individuals were observed starting from January. February and March were considered to be the period when eggs become mature (Table 1).

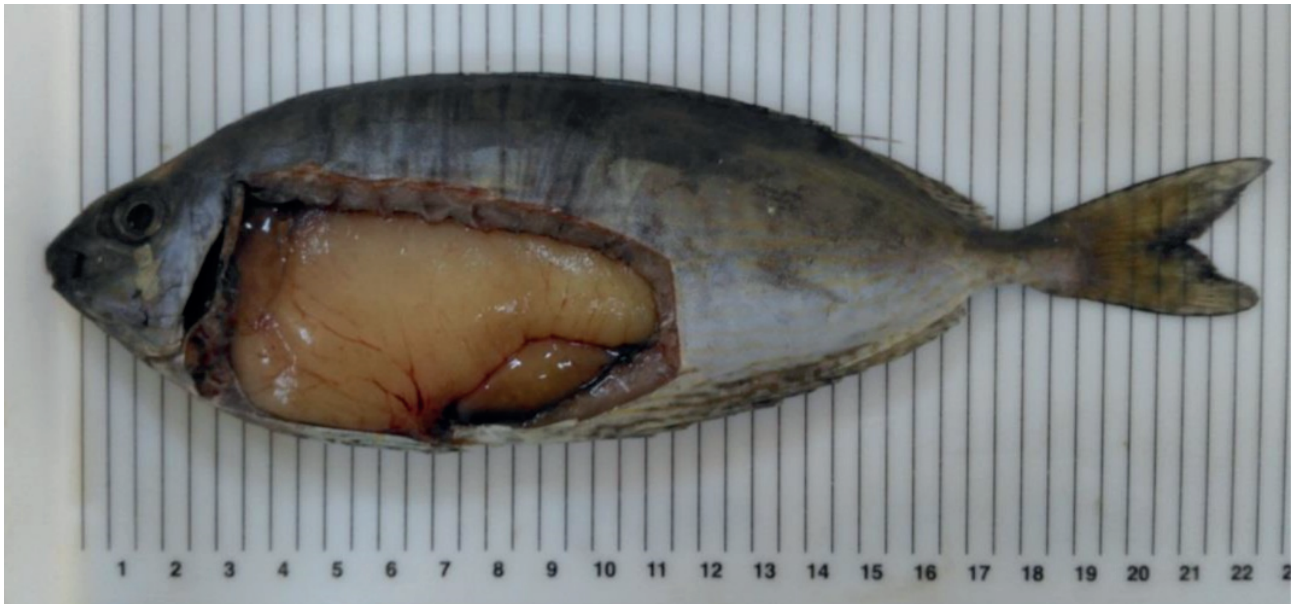

Figure 4

Mature (stage IV) female marbled spinefoot sampled from the southern Aegean Sea

Histological analysis of the ovarian cycle and ovarian development

The ovarian cycle of *S. rivulatus* was determined based on vitellogenic ovarian cells, which form the first and second development phase. A detailed presentation of the ovarian cycle based on sections is presented in Figures 5 and 6.

First development phase

Ovarian cells were observed on the edges of the ovarian lamellae. Cells were colorless, with spherical or ellipsoidal shapes. They contained basophilic and homogeneous cytoplasm (Fig. 7A). A membrane-bound yolk sphere was found between cortical alveoli in the peripheral ooplasm. Its location cells was more central compared to the perinuclear space. Egg yolk vesicles lined up around the cytoplasm (Fig. 7B).

Table 1

Monthly changes in the sexual maturity stages of *Siganus rivulatus* in the southern Aegean Sea

Years	Months	Sexual maturity stages
2017	February	II
	March	II
	April	III
	May	III
	June	III
	July	IV
	August	V
	September	V
	October	V
	November	V
	December	I
2018	January	I

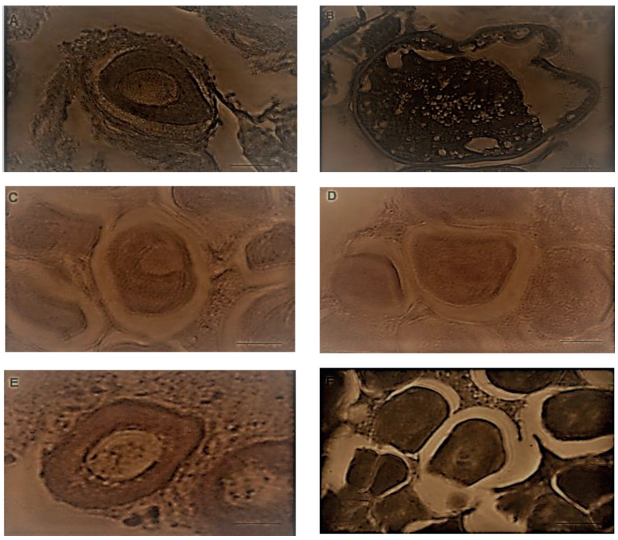

Figure 5

Six-month ovarian cycle of *S. rivulatus*. While ovarian cells in the first and second development phase were observed in June and July, only the first development stage and mitotic ovarian cells were found during the other months; A – June, B – July, C – August, D – September, E – October, F – November; **scale – 50 µm**

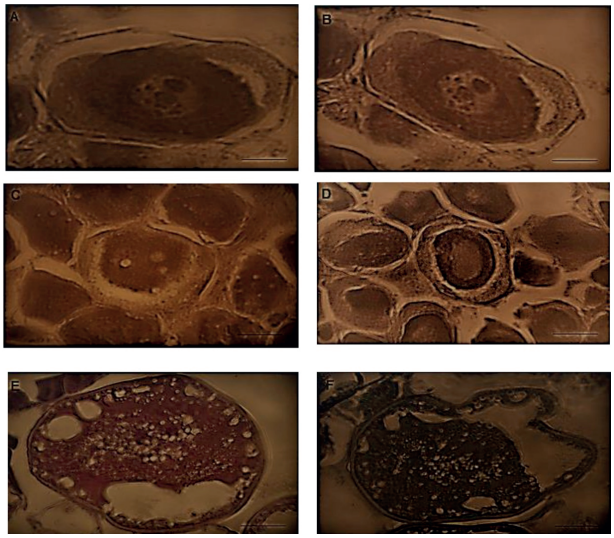

Figure 6

Six-month ovarian cycle of *S. rivulatus*. While ovarian cells of the first and second development phase were observed in April and May, only the first development stage and ovarian cells were found during the other months; A – December, B – January, C – February, D – March, E – April, F – May; **scale – 50 µm**

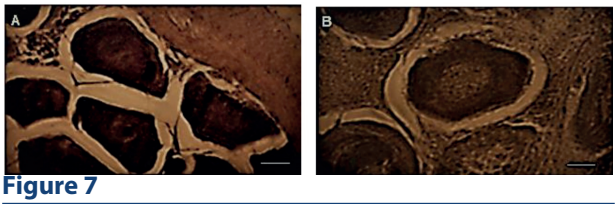

Figure 7

First development phase of ovarian cells; A – chromatin-nuclear period, B – chromatin-nuclear period, perinuclear period; **scale – 30 µm**

Ovarian cells were surrounded by a series of theca and granulosa cells, which form a follicle from the early stage of the perinuclear phase transition (Fig. 8A). The basal membrane was present within these two cell groups. The follicle was surrounded externally by a series of epithelial cells and internally by zona radiata, which formed the corion.

In the progressive/upcoming stages of the perinuclear phase, the chromatin material became dense, enclosed within the nucleus wall and the number of nucleoli increased (Fig. 8B). The number of nucleoli reached 16 units. The dimensions of these nucleoli did not vary as much as they did in the chromatin-nuclear phase and they were more or less the same (Fig. 8C).

Second development phase

At the beginning of this phase, the entrance of vitelline globes into the ovum was observed at the cytoplasm periphery. This phase was very rarely observed in ovarian cells, which is related to the short duration of this period. This is yolk phase I, which is the first stage of vitellogenesis (Fig. 9A).

It was observed at the beginning of this phase that vitelline globes entered into the cytoplasm periphery. This phase was rarely determined in ovarian cells, which was associated with its very short duration. This is yolk phase I of the first part of early vitellogenesis (Fig. 9A).

During this phase, vitelline globules grew quickly and accumulated around the nucleus (Fig. 9B). The size of vitelline globules increased between the nucleus and zona radiata, which resulted in the granular form of the cytoplasm. Zona radiata became clear and visible in this phase.

Maturing Phase

During this phase, which is known as a germinal vesicle or a nucleus migration stage, fat droplets (Fig. 10A) began to accumulate around the germinal vesicle. Fat globules located in this zone began to form

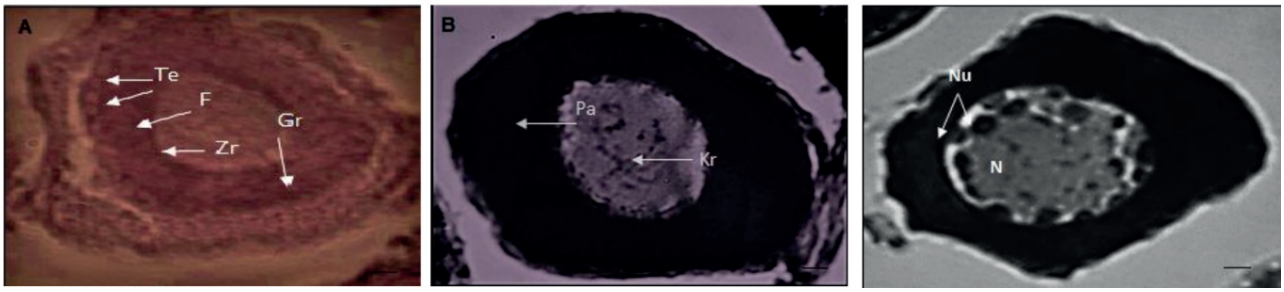

Figure 8

Components of ovarian cells; A – post-vitellogenesis period, B, C – perinuclear period, Pa – perinuclear area, Kr – chromatin, N – nucleus, Nu – nucleolus, Te – theca cells, Gr – granulosa cells, Zr – zona radiata, F – follicle

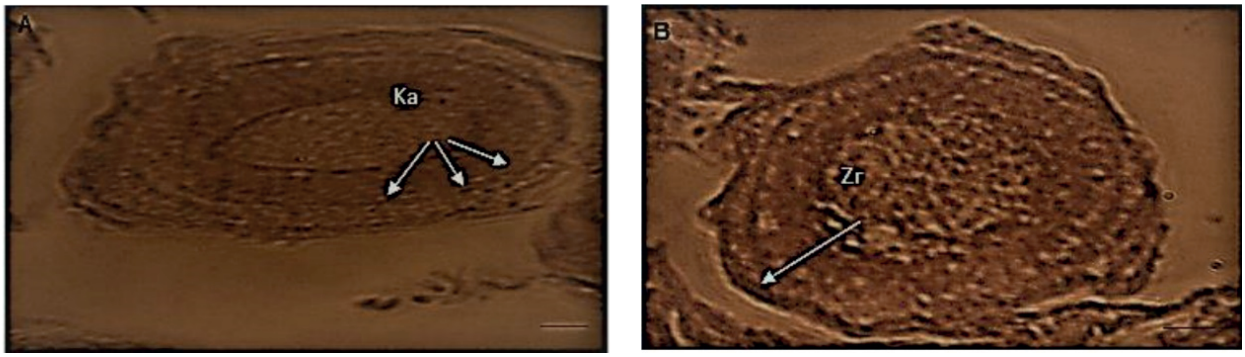

Figure 9

First stage of the second development phase, early vitellogenesis; yolk phase I; A: cortical alveoli phase, B: accumulated vitellin globules around the nucleus observed during the period of cortical alveoli; Ka: cortical alveoli, Zr: zona radiata; **scale: 30 µm**

fat droplets due to the deformation of the nucleus (Fig. 10B). After accumulation of fat droplets, it was observed that the germinal vesicle and those droplets moved to the animal pole of the ovarian cell (Fig. 10C).

Ovaries were hydrated and grew in size after this stage. The ovarian follicle covering the ovarian cell became thinner and the ovary became very large, occupying almost the entire abdominal cavity. Mature oocytes were observed in May–July.

Discussion

The marbled spinefoot *Siganus rivulatus* (Forsskål 1775) is one of the two Siganidae species (together with *Siganus luridus*) that inhabit Turkish waters. It is one of the Lessepsian migrants (Ben-Tuvia 1985) that invaded the Mediterranean and became one of the commercial exotic fishes, not only in terms of capture fishery but also aquaculture. Furthermore, coastal areas of the Aegean Sea are rich in terms of gulfs and bays, but the narrow continental shelf with hilly and jagged bottom structures limits the fishing activity in the area (Soykan et al. 2016), especially in the south, where there are very limited zones for demersal trawling. Therefore, the main sampling gear to collect *Siganus rivulatus* individuals are gillnets, trammel nets, pots and fishing lines, which have limited sampling capacity compared to large-scale fishing gear such as trawlers and purse seiners. That is why the marbled spinefoot can be considered a species difficult to investigate due to its habitat preferences and fishery regulations in Turkey.

This study describes the annual gonadal development and the gonadal cycle of *S. rivulatus* in the southern Aegean Sea. As shown by our study, the gonadal development in *S. rivulatus* starts in the southern Aegean Sea in April. This period continued until December and the gonadal immaturity was observed between December and February. Based on the GSI values, June was characterized by the maximum gonadal development. This result was supported by histological studies.

A six-month ovarian cycle was observed in June and July. While the ovarian cells of both the first and second development phase were determined in this

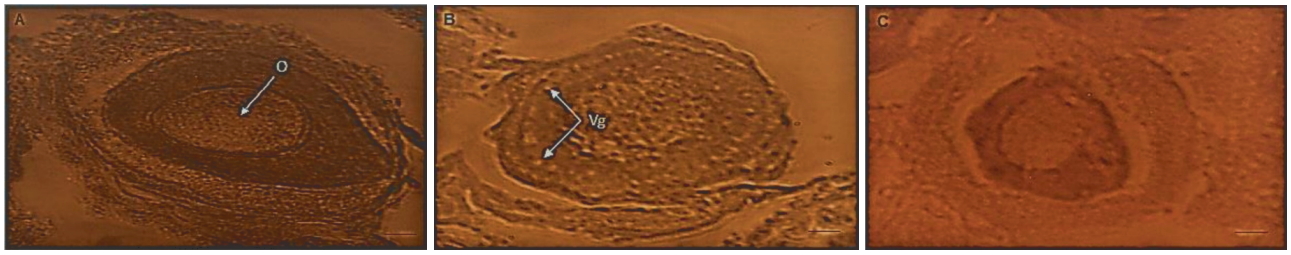

Figure 10

Maturing phase of ovarian cells; A – accumulated fat globules around the nucleus, B – fat globules come together and form fat droplets, C – movement of the germinal vesicle into the animal pole O – oil droplets, Vg – vitelline globules; **scale: 10 µm**

period, only the first development phase and mitotic ovarian cells were observed during the other months. It was found that the results of this study and of the previous studies on the reproduction biology of spinefoots show some discrepancies. Ben Tuvia et al. (1966) reported the occurrence of mature females in the southeastern Mediterranean waters between July and November. Amin et al. (1985) stated that the gonadal development of the species starts earlier in the Jeddah part of the Red Sea compared to the Eastern Mediterranean. In fact, the results of Amin (1985), obtained based on various samples collected in the Red Sea, revealed that gonadal maturity was achieved in three months (January–March) and spawning lasted approximately seven months (March–September). This result may be attributed to water temperature, which is higher in the Red Sea than in the Eastern Mediterranean. Ben Tuvia (1985) reported that the spawning season for the species lasts from July to August. According to Hussein (1986), the spawning season of this species in the Mediterranean coast is between July and September. On the other hand, Golani (1990) reported that the spawning season on the Israeli Mediterranean coast lasts from May to August.

In addition to studies from other parts of the Mediterranean Sea, some works included information on the reproductive biology of the species from the Turkish coast. Akşıray et al. (1954) observed that the marbled spinefoot spread to the southern Aegean Sea from the Mediterranean coast and its spawning season lasted from early to mid-summer. Torcu et al. (1994) reported the reproduction season of the species in the Mediterranean between April and August. Results of the histological analysis conducted in our study (Table 1) revealed that the marbled spinefoot in the southern Aegean Sea reproduces in June. Furthermore, the reproduction period of the species was determined by Yeldan and Avşar (2000) as July and August. The difference between our study and the study by Yeldan and Avşar (2000) is not only attributed to water temperature, but may also be due to feeding habit, stomach fullness of the examined individuals and the sampling method.

Research on the histology of *Siganus rivulatus* gonads are limited and scarce. However, some researches focused on the gonadal histology of other Siganid species. Hoque et al. (1998) conducted a study on *Siganus canaliculatus* and revealed that oocytes reaching the first tertiary yolk stage were primarily observed in March and vitellogenesis also started in the same month. Then, while oocytes filled with yolk grew almost synchronously, oocytes during the maturing phase were found in gonads between April and June. It was reported that the ovary development of *S. guttatus* was classified into three stages: immature (August–April), pre-spawning (May) and spawning (June–July). During the immature phase, even though the GSI was very low, vitellogenic oocytes from first to tertiary egg yolk phases became visible in the ovary. In some ovaries, many vitellogenic oocytes and empty follicles were observed in June and July (Rahman et al. 2000). Hoque et al. (1998) reported that *S. canaliculatus* showed synchronized development of the ovarium, same as *S. guttatus*. However, it was found that the ovarian development in *S. argenteus* was asynchronous. Various types of vitellogenic oocytes were determined during the spawning season of *S. argenteus* (Salaki 1993). This histological evidence proved that at least two types of oocyte development occur in the *Siganus* genus.

Histological studies of gonads in the Siganidae family were performed for only three species. Particularly evident is the lack of studies focusing on the spawning season and gonadal development of invasive *Siganus rivulatus* in the southern Aegean waters of Turkey. The results of the present work are expected to contribute to the fisheries management of the species and will lead to further detailed studies.

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