University of Cyprus
Department of Biological Sciences

Reproductive biology and growth of the alien Red Sea goatfish *Parupeneus forsskali* (Fourmanoir & Guézé, 1976) off the coast of Cyprus

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A Research-Based Master's Thesis
for the
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ABSTRACT

Goatfish (family Mullidae) are of great commercial and economical importance around the world, including in the Mediterranean Sea, where six goatfish species have been recorded. Four of those species are alien to the Mediterranean, migrating either from the Atlantic Ocean or from the Red Sea through the Suez Canal. The most recent mullid species to arrive in the Mediterranean is the Red Sea goatfish (*Parupeneus forsskali*), which was first spotted there in the year 2000. *P. forsskali* is endemic to the Red Sea and the Gulf of Aden, but has increased its range considerably in recent years, spreading throughout the eastern Mediterranean and all the way out to Tunisia in the west. It was first confirmed around Cyprus in 2014 and has since then established a thriving population around the island, already becoming commercially important. Its arrival in Cyprus seems to have negatively impacted the populations of native goatfish species through competition, indicating that it could become invasive at some point in the future. In the present study, the spawning season and gonad development of *P. forsskali* off the coast of Cyprus were studied, along with important biological parameters. 466 individuals were sampled from the southern coast of Cyprus, from November 2020 to October 2021. Macroscopic and histological analyses of the gonads indicated six clear maturity stages for both ovaries and testes. The gonadal maturity stages and the gonadosomatic index indicated that *P. forsskali* spawns in the summer months, with the peak of the spawning season in July. Males were bigger, heavier, and more abundant than females, but their length-weight relationship did not differ. The overall length-weight relationship was found to be $TW = 0.0065 \times TL^{3.17}$, indicating positive allometric growth. The size at first sexual maturity (L_{50}) did not differ between males and females, and was 14.3 cm for all fish combined. Five age groups were identified from the length-frequency distribution (0-4 years old). Age group 1 (one year old fish) was the most dominant one, containing almost 54% of the sampled individuals. The parameters of the von Bertalanffy growth function were L_∞ = 31.81 cm, K = 0.27 year^{-1}, and t_0 = -1.67. Overall, this study provides information on the spawning season of the Red Sea goatfish, detailed descriptions of its gonadal maturity stages which can be helpful to other researchers studying the species, and estimations of biological parameters that are important for fisheries management.

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I would like to thank Niki Chartosia and Anna Papadopoulou for allowing me to work on this research and for giving me valuable notes and feedback. Special thanks go to my local fisherman, Kostas Kontakkis from Latchi Fishmarket, for going out to catch my samples every month. I would also like to thank Panicos Eleutheriou for catching some juvenile fish for me, and Spyros Sfenthourakis for giving me the consumables I needed to do this research.

COMPOSITION OF THE EXAMINATION COMMITTEE

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SEMINAR ANNOUNCEMENT

University of Cyprus
Department of Biological Sciences

Master Research Dissertation in Biodiversity and Ecology (BIO 831/601)

Student Presentation

Wednesday, 15 December 2021 at 10:00

This seminar is open to the public via Zoom at the following link:

https://ucy.zoom.us/w/92319771749?tk=gJF4hWdlimMPtdBDt_-FjhsVbMswPkvMaif-UKVgLfI.DQMAAAAVfg_sZRYyTG1RdkFzVlJ1Ty10WHZOTnpkOUtBAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA&pwd=b3NPRUhlOFdWVDRwUElOWDFOK2FFUT09

Sigurdur Finnbogi Saemundsson

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Goatfish (family Mullidae) are of great commercial and economical importance around the world, including in the Mediterranean Sea, where six goatfish species have been recorded. Four of those species are alien to the Mediterranean, migrating either from the Atlantic Ocean or from the Red Sea through the Suez Canal. The most recent mullid species to arrive in the Mediterranean is the Red Sea goatfish (*Parupeneus forsskali*), which was first spotted there in the year 2000. *P. forsskali* is endemic to the Red Sea and the Gulf of Aden, but has increased its range considerably in recent years, spreading throughout the eastern Mediterranean and all the way out to Tunisia in the west. It was first confirmed around Cyprus in 2014 and has since then established a thriving population around the island, already becoming commercially important. Its arrival in Cyprus has negatively impacted the populations of native goatfish species through competition, indicating that it could become invasive at some point in the future. In the present study, the spawning season and gonad development of *P. forsskali* off the coast of Cyprus were studied, along with important biological parameters. 466 individuals were sampled from the southern coast of Cyprus, from November 2020 to October 2021. Macroscopic and histological analyses of the gonads indicated six clear maturity stages for both ovaries and testes. The gonadal maturity stages and the gonadosomatic index indicated that *P. forsskali* spawns in the summer months, with the peak of the spawning season in July. Males were bigger, heavier, and more abundant than females, but their length-

TABLE OF CONTENTS

INTRODUCTION

Goatfish are members of the family Mullidae and can be found worldwide in tropical, subtropical, and temperate waters (Uiblein 2007). The family contains six genera and around 90 species (Nelson et al. 2016; WoRMS 2021) that live mostly in shallow coastal marine waters (the littoral zone) on muddy or sandy bottoms, or on coral reefs (Fischer and Bianchi 1984). All goatfish are benthic carnivores, using a pair of sensory barbels on their snout to detect prey on or below the surface of the substrate (Gosline 1984). They are pelagic broadcast spawners, meaning that they release their eggs and sperm into the open sea for external fertilization, and some species show seasonal migrations where they aggregate during the spawning season (Claydon 2004).

Goatfish are of great commercial and economical importance on a global scale (Whitehead et al. 1984; Nelson et al. 2016), including in the Mediterranean Sea (Tsikliras et al. 2013). There are six confirmed mullid species present in the Mediterranean, two of which are native: the red mullet (*Mullus barbatus*, Linnaeus, 1758) and the surmullet (*Mullus surmuletus* Linnaeus, 1758) (Whitehead et al. 1984; Evagelopoulos et al. 2020). The West African goatfish, *Pseudupeneus prayensis* (Cuvier, 1829), is originally from the Atlantic Ocean but has migrated into the Mediterranean in recent years (Azzouz et al. 2011). The other three goatfish species have migrated from the Indian Ocean or the Red Sea through the Suez Canal, so-called Lessepsian migrants. The Suez Canal was opened in 1869, linking the Red Sea and the Mediterranean, which has allowed hundreds of new species to migrate between them and cause considerable problems (Galil et al. 2014). Many non-indigenous species introduced through the Suez Canal have been able to establish thriving populations in the Levantine Sea, and some have managed to spread to the western parts of the Mediterranean. Some species are more harmful than others and the impact of several of those species has adversely affected the conservation status of native species, as well as the function of whole ecosystems (Galil et al. 2015). Por's goatfish (*Upeneus pori* Ben-Tuvia & Golani, 1989) was first reported in the Mediterranean by Kosswig (1950) around Turkey and is now common in the eastern Mediterranean. The golden-banded goatfish (*Upeneus moluccensis* Bleeker, 1855) was first reported off the coast of Israel by Haas and Steinitz (1947) and is now also quite common in the Levantine Sea (EastMed 2010). The most recent alien mullid species to

arrive in the Mediterranean is the Red Sea goatfish (*Parupeneus forsskali* Fourmanoir & Gueze, 1976), which was first spotted off the coast of Turkey in 2000 and again in 2004 (Çinar et al. 2006). It was not until 2012 that the first specimen was collected and recorded in the Mediterranean, off the coast of Lebanon (Bariche et al. 2013).

P. forsskali is endemic to the Red Sea and the Gulf of Aden, and is the most common goatfish in the area (Randall 1983, 2004), as well as being one of the most exploited goatfish in the Red Sea (Farrag et al. 2018). Its coloration can vary a bit, but it is characterized by a broad black stripe running from the tip of the snout, through the eye, and along the upper side of the body, ending beneath the second dorsal fin. It also has a roundish black spot on the upper caudal peduncle. The body is grayish green or even red above the stripe, and white or reddish pink below it. The caudal fin is bright yellow, as well as the dorsal part of the caudal peduncle (Randall 2004). *P. forsskali* inhabits sandy bottoms and coral reefs (Golani and Diamant 1999), and probes the substrate with its sensory barbels for prey. Its diet mainly consists of benthic invertebrates, such as crustaceans and molluscs (Evagelopoulos et al. 2020). In its native range, it can live for up to five years (Mehanna et al. 2018), reach up to 28.5 cm in total length (Sabrah 2015), and a size at first sexual maturity (i.e. the length at which 50% of individuals are sexually mature) of 16 cm (Farrag et al. 2018).

The range of *P. forsskali* has increased considerably in recent years. Since it was first spotted around Turkey in 2000, it has spread throughout the eastern Mediterranean (Evagelopoulos et al. 2020), and has been found as far west as Tunisia (Capapé et al. 2018). It was first confirmed around Cyprus in 2014 when a single specimen was caught in the area of Cape Pyla (Chartosia and Michailidis 2016). Since then it has established itself around the island, possibly becoming the most abundant goatfish in shallow waters (< 50 m deep) around Cyprus, and has already become commercially important. Despite having clearly replaced native mullids, to an extent, in the Cypriot artisanal fisheries catches in the last few years, indicating negative effects on the native species through competition, it is not yet considered an invasive species in Cyprus (Evagelopoulos et al. 2020).

Only a few studies have been done on *P. forsskali* in the Mediterranean, and none of them has focused on its reproduction and spawning period. At a certain length or age, fish become sexually mature and the gonads start to go through a seasonal cycle of ripeness or maturity stages, which are reflected in the appearance of the gonads (Mous et al. 1995). Environmental changes,

such as in food supply, temperature, and photoperiod, are major drivers of the reproductive cycle of many fish species (Wootton and Smith 2015). Knowing when fish become mature and when they spawn is important for fisheries management and conservation since it can indicate when and at which size the species should be protected. It allows for better monitoring of the population and of recruitment, so changes in the population, such as in size, can be observed more closely (Hunter et al. 1992). Determining the maturity stages of fish is either done by examining the gonads macroscopically (or visually) or with histological analyses of the gonads. Visual examination of gonads is fast and inexpensive, so a large number of specimens can be examined, but examining them histologically is more accurate and is often needed to verify visual observations (Tomkiewicz et al. 2003). When examining gonads histologically, the maturity of oocytes in ovaries and spermatocytes in testes are used to determine the maturity stage of the gonads. Oocytes develop through a process called oogenesis, which starts with the development of primary oocytes (Tyler and Sumpter 1996). Primary oocytes go through different stages of development, beginning with the chromatin nucleolus stage, where the oocyte is small and roughly spherical with a single prominent nucleolus. As the primary growth phase continues, the oocyte becomes larger and can become irregular in shape. The nucleus also gets bigger and multiple nucleoli become arranged around its periphery. These are called perinucleolar oocytes. As the oocyte develops further, oil droplets and small yolk vesicles called cortical alveoli start to appear in the periphery of the cytoplasm, marking the end of the primary growth phase (Wootton and Smith 2015). The secondary growth phase begins when the oocyte reaches a certain size. During this phase is when the oocyte acquires most of its dry mass, mainly due to the accumulation of yolk (Tyler and Sumpter 1996). The major process of this stage is vitellogenesis, which is the synthesis and processing of vitellogenin by the oocyte, transforming it into vital proteins stored in the yolk globules. The number of oil droplets and yolk vesicles increases in the oocyte, ultimately almost filling up the entire cytoplasm as the oocyte develops. The oocyte then enters the maturation phase, and hydration of the oocyte occurs by the uptake of water. Once the oocyte is hydrated, the maturation phase is complete and ends with the ovulation of the oocyte (Tyler and Sumpter 1996; Wootton and Smith 2015). Spermatocytes also develop through different stages: primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa. Primary spermatocytes are the largest and have a round shape. After the first phase of meiosis, they produce two secondary spermatocytes. They rapidly go through the second phase of meiosis and produce haploid

spermatids. The spermatids then go through spermiogenesis, where their morphology changes drastically and eventually produce spermatozoa with flagella (Wootton and Smith 2015).

Estimations of biological parameters are important for fisheries management, and can also be used for conservation purposes. As mentioned before, size at first sexual maturity (L_{50}) is the length at which 50% of individuals in the population are sexually mature, and accurately knowing the timing of maturity is important for effective fisheries management (King and McFarlane 2003). The length-weight relationship (LWR) is used to predict the weight of fish from the length, which is useful for estimating the biomass of a sample from length-frequency data. It is also used to compare parameter estimates of the relationship of a population to other parameter estimates, for example, from previous years or from other populations to determine the relative condition of the population (Le Cren 1951; Froese 2006). The development of fish gonads can be described at a macroscopic level using the gonadosomatic index. The gonadosomatic index (GSI) is the relationship between body weight and gonad weight, and is used to determine the spawning season of a species. Monthly values of the GSI are compared and spawning activity is at its peak in the months where the GSI is at its highest (Wootton and Smith 2015). Growth rate is determined by the relationship between size and age, and sufficient knowledge of growth rate parameters is important in fisheries management since they can be used to predict the status of the population in the future, for example. The von Bertalanffy growth equation is likely the most commonly used growth model in fishery biology, and is used to report growth parameters. It has the form $L_t = L_\infty(1-e^{-K(t-t_0)})$, where L_t = length at time t, L_∞ = maximum asymptotic length, K = growth rate coefficient, and t_0 = time when length is theoretically zero (Lugert et al. 2016).

In the present study, the reproductive cycle and spawning season of the Red Sea goatfish were examined for the first time, along with estimations of some important biological parameters. The results of this study can be compared with those regarding native species in order to see differences and similarities among the species, and to make it easier to monitor the population around Cyprus and to assess whether actions are needed to prevent possible further negative impacts on native species and biodiversity around Cyprus caused by this fish.

MATERIALS AND METHODS

Sampling and laboratory processing

Fish samples were collected in the first or second week of each month along the southern coast of Cyprus, from November 2020 to October 2021 (12 months, n = 466). They were fished by a local fisherman at 15-20 m depth using a net with an 18-20 mm net opening, and examined shortly after. A portion of the samples fished in July 2021 were fished with a smaller net opening to ensure that fish of all sizes could be examined. The samples were mostly kept and examined fresh, but some samples had to be stored in a freezer or in a 10% buffered formalin solution prior to examining them due to unavoidable circumstances. Total length (TL) and standard length (SL) (Figure 1) were measured to the nearest 0.1 cm with a measuring tape, and total weight (TW) and gutted weight (GW) were measured to the nearest 0.01 g with a digital scale.

Figure 1. Total length and standard length.

Histological analysis

The gonads were extracted and weighed to the nearest 0.001 g. The sex of the fish was determined by examining the gonads, but the sex of juvenile fish could not be determined since the male and female gonads are too similar. Macroscopic maturity stages of the gonads were determined based on size, shape, color, stiffness, and presence of blood vessels, and by using Follesa and Carbonara

(2019) and Ungaro (2008) for reference. After weighing the gonads they were fixed and stored in 10% formalin until further examination. 10 gonad samples from each month were selected for histological analysis based on the difficulty of determining their macroscopic stage. For the histological analyses, about 0.5 cm thick tissue samples were taken from the middle part of either lobe of the ovaries and testes. The samples were then dehydrated in a graded series of ethanol, embedded in paraffin wax, sectioned at 7-10 µm, and then mounted on microscope slides and stained with haematoxylin and eosin. The sections were examined under a Zeiss Primo Star microscope, and pictures of the sections were taken with a Zeiss Axiocam ERc 5s microscope camera. The histological maturity stages were determined based on oocyte and spermatocyte development, using Follesa and Carbonara (2019) for reference.

Data analysis

Data analysis was done by using R version 3.3.2 (www.R-project.org/). The Shapiro-Wilk test was used to test normality of the data, and equal variance was tested with an F-test where needed. The sex ratio between males and females was tested using the exact binomial test at a significance level of 0.05 to see if it differed from 1:1. The two-sample t-test was used to test the difference in mean length between sexes. Total weight of males and females did not have the same variance, so Welch's t-test was used to test the difference there.

Spawning season

Monthly changes in the gonadosomatic index (GSI) of mature individuals and maturity stages were used to determine the spawning season of *P. forsskali*. GSI was calculated using the following formula:

$$GSI = 100 \times (\text{gonad weight}/\text{gutted weight}).$$

The non-parametric Kruskal-Wallis test was used to test for differences in the mean gonadosomatic index among months. The pairwise Wilcoxon rank-sum *post hoc* test was used to compare the means to determine which ones were significantly different.

Size at first sexual maturity

Total length was used to estimate the size at which 50% of individuals were sexually mature. The fish were split into length classes of 0.5 cm (10-10.4 cm fish are in length class 10, 10.5-10.9 cm fish are in class 10.5, etc.) and the proportion of mature individuals calculated in each length class. A logistic regression model was fitted to the data to estimate the relationship between length and maturity, and the size at first sexual maturity was determined for both sexes by analyzing the logistic curve. The male and female logistic curves were compared using the likelihood ratio test to test if there was a significant difference in their size at first sexual maturity.

Length-weight relationship

The relationship between total length and total weight was expressed by the following equation:

$$W = aL^b$$

where W is total weight, L is total length, and a and b are constants. The parameter b (called the allometric coefficient) indicates the rate at which weight increases for a given increase in length, so it is considered a shape parameter for the body form of the species. When $b = 3$, the growth is isometric, so the shape of the fish does not change as it grows and the condition of small and large individuals is the same. If $b > 3$, the growth is positive allometric, so the fish increases in height or width faster than in length (becomes rounder), which could indicate that larger individuals are in better nutritional condition than smaller ones. Conversely, $b < 3$ indicates negative allometry, so the body shape becomes more elongated as the fish grows in size, which could indicate that smaller individuals are in better nutritional condition than larger ones. The parameters of the LWR can be affected by numerous factors, such as the availability of prey, environmental conditions, and gonadal maturity stages (Froese 2006).

The data were logarithmically transformed to meet the assumptions of linear regression, and the LWR equation was obtained from the regression line. The significance of the regression was assessed by analysis of variance (ANOVA). The t-test was used to test whether the b-value was significantly different from the isometric value of 3 for both sexes and for the pooled data. Analysis of covariance (ANCOVA) was used to check if the LWR differed between males and females.

Age and growth

Since no age data were collected during this research, the age had to be estimated using the frequency distribution of the 0.5 cm length classes. Bhattacharya's method (Bhattacharya 1967) was used to create age groups from modes in the length-frequency distribution. This method splits a composite distribution into separate normal distributions, each representing a generation (or age group) of fish. The program FISAT II was used to do this analysis. Each fish was assigned an age depending on its estimated age group, and the age data were then used in a length-at-age analysis. The non-linear least squares method was used to estimate the parameters of the von Bertalanffy growth function, which was fitted to the mean lengths of the estimated ages.

RESULTS

Morphometric characteristics of the species

A total of 272 male and 140 female *P. forsskali* were sampled, along with 54 others that could not be sexed, 37 of which were juveniles. The other 17 fish were not sexed due to complications with extracting their gonads. The sex ratio of *P. forsskali* differed significantly from the expected ratio (1:1), since a disproportionately large proportion of the samples were male (0.66, SE = 0.02; binomial test, n = 412, $P < 0.05$), or 1.94:1. Data on total length and total weight can be seen in table 1. Total length ranged from 12.1 to 26.1 cm for males, with an average of 19.1 cm (SD = 2.5), and 10.3 to 24.1 cm for females, with an average of 17.4 cm (SD = 2.1). The smallest recorded individual was 8.2 cm and was a juvenile, but the smallest sexually mature fish was a 10.4 cm long female. Total weight ranged from 16.69 to 204.22 g for males, with an average of 79.36 g (SD = 33.45), and 11.07 to 148.19 g for females, with an average of 58.90 g (SD = 22.49). The lightest individual was 4.49 g and was a juvenile, but the lightest sexually mature fish was a 11.94 g female (not the same as the smallest sexually mature fish). On average, males were heavier and bigger than females, with a significant difference in total weight ($t = 7.36$, $df = 382$, $P < 0.05$) and total length ($t = 6.64$, $df = 410$, $P < 0.05$).

Table 1. Total length and total weight.

Data	Number of individuals	Total length (cm)	Mean ± SD	Total weight (g)	Mean ± SD
Male	272	12.1 - 26.1	19.1 ± 2.5	16.69 - 204.22	79.36 ± 33.45
Female	140	10.3 - 24.1	17.4 ± 2.1	11.07 - 148.19	58.90 ± 22.49
Juvenile	37	8.2 - 18.1	13.6 ± 3.1	4.49 - 66.52	29.77 ± 18.61
All	466	8.2 - 26.1	18.1 ± 2.8	4.49 - 204.22	68.48 ± 32.49

Note: SD = standard deviation.

Morphology of gonads

Ovaries and testes were divided into six clear maturity stages, both macroscopically (Table 2) and histologically (Table 3). As the ovaries matured, they became heavier, blood vessels became more apparent, and eventually eggs became visible to the naked eye. The testes became thicker and heavier, and blood vessels became visible as they matured. Juveniles with immature gonads could not be sexed macroscopically, but immature testes were observed under the microscope after histological processing. All macroscopic maturity stages are shown in figures 2-9, and all histological maturity stages are shown in figures 10-21.

Table 2. Definitions of the macroscopic maturity stages and descriptions of male and female gonads in each stage (modified from Follesa and Carbonara (2019) and Ungaro (2008)).

Maturity stage	Definition	Female	Male
Immature	Juveniles, i.e. sexually immature fish that have not spawned yet. Sex indistinguishable.	Small and thin ovaries with little color, can be grayish.	Small and thin testes with little color, can be grayish.
Developing virgin	Sexually immature fish, but mature enough to distinguish sex.	Small but plump and roundish, not a lot of color but can be reddish.	Small and transparent, but bigger than immature ones.
Recovering	Sexually mature fish in the period between spawning seasons.	Plump, thick lobes as long as 1/4 - 1/3 of the body cavity. Color can range from red, pink and red-orange. Blood vessels usually not visible.	Length ranging from 1/5 - 1/3 of the body cavity. White or reddish with no visible blood vessels.
Maturing	Fish getting ready for the spawning season with enlarging gonads.	Roundish pink/red lobes, blood vessels visible in some cases.	White/reddish and long lobes with visible blood vessels. Thicker than recovering ones.

Mature/spawning	Fish ready to spawn/actively spawning.	Pink/red, round and plump lobes with highly visible blood vessels. About 1/2 - 2/3 the length of the body cavity. Eggs clearly visible to the naked eye.	White/reddish/pink and thick with clearly visible blood vessels. About 1/2 the length of the body cavity.
Spent	Fish that have recently released their spawn.	Pink/red and round but quite flaccid. Still contains some eggs. Blood vessels usually visible.	White/pinkish or even grayish and flaccid. Blood vessels sometimes visible.

Figure 2. Immature gonads.

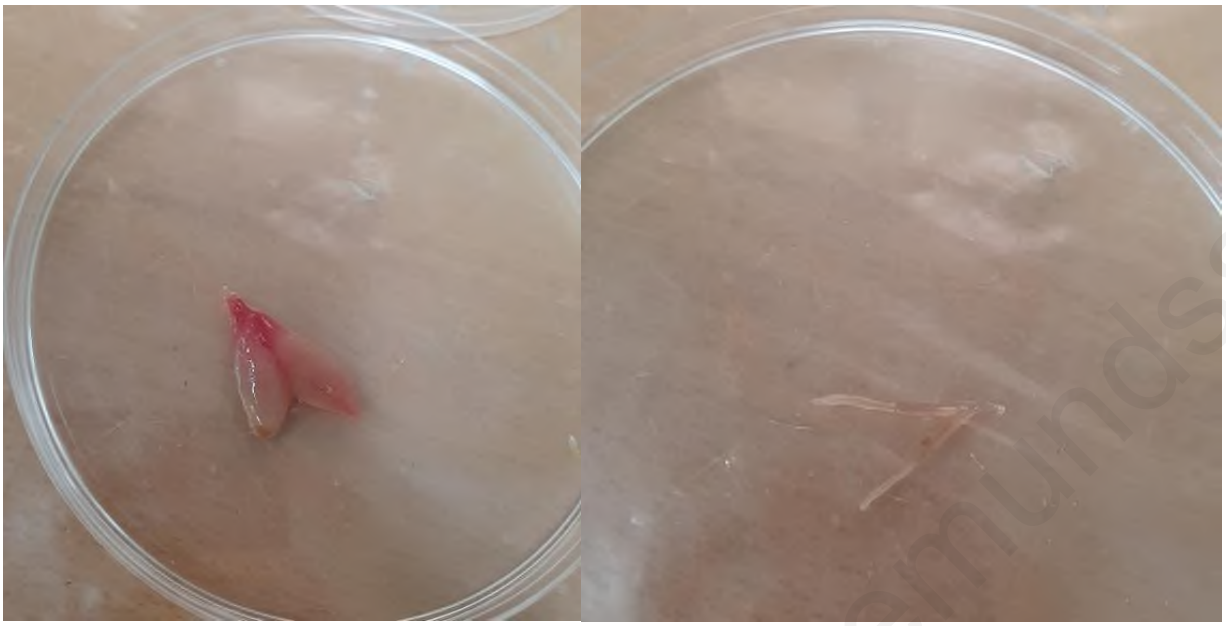


Figure 3. Gonads in the developing virgin stage. Left: female. Right: male.

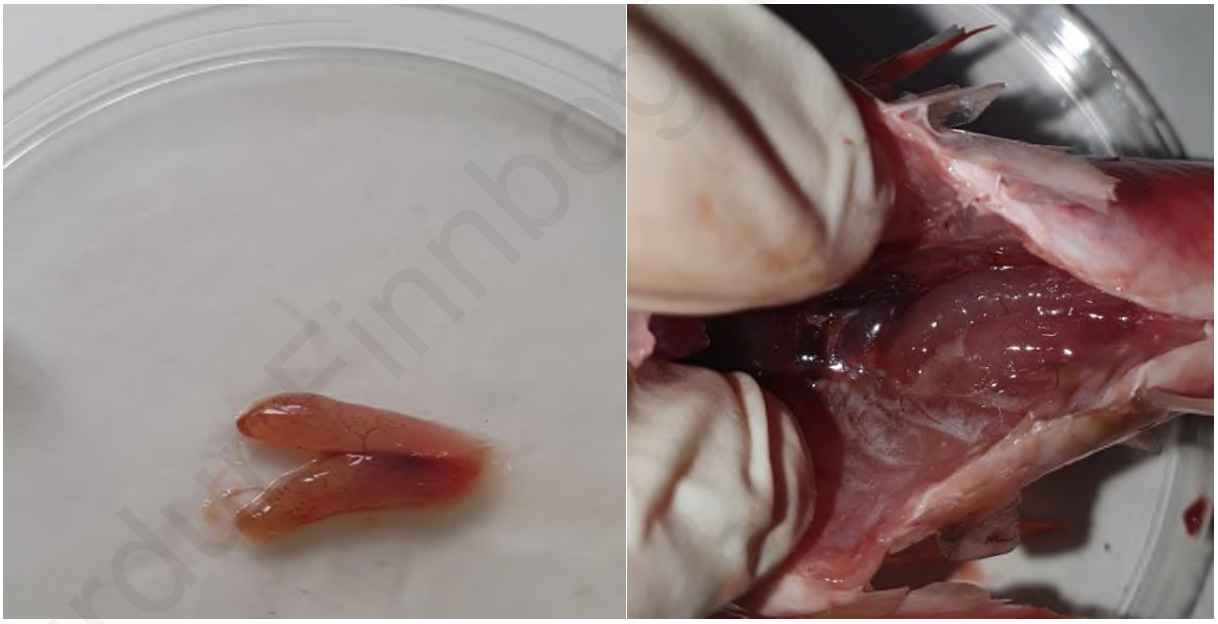


Figure 4. Ovaries in the recovering stage.

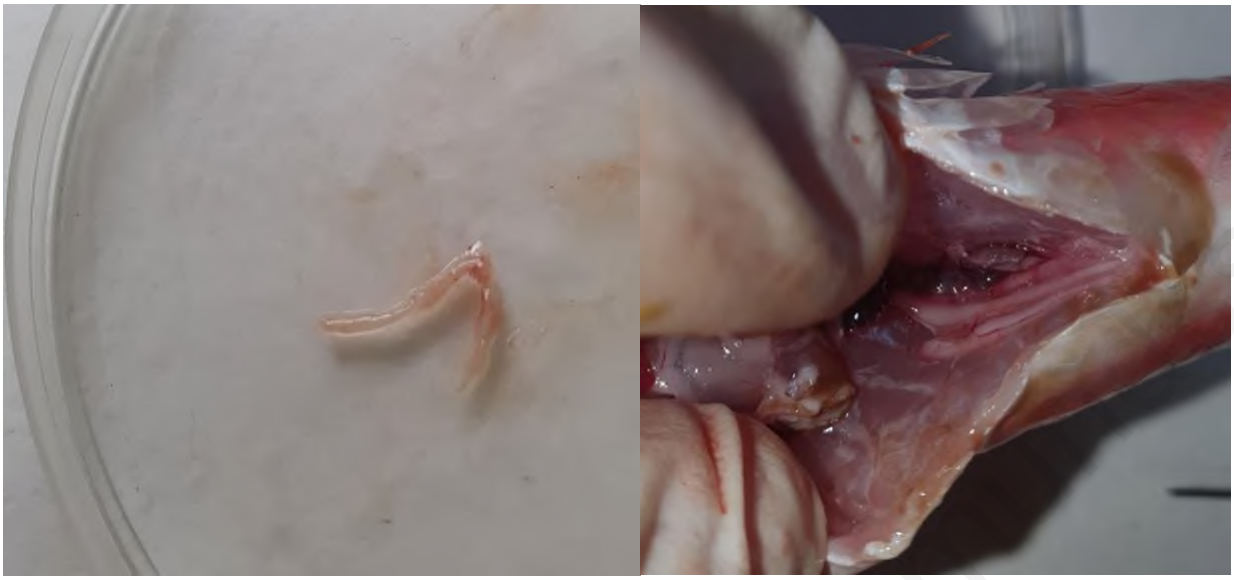


Figure 5. Testes in the recovering stage.

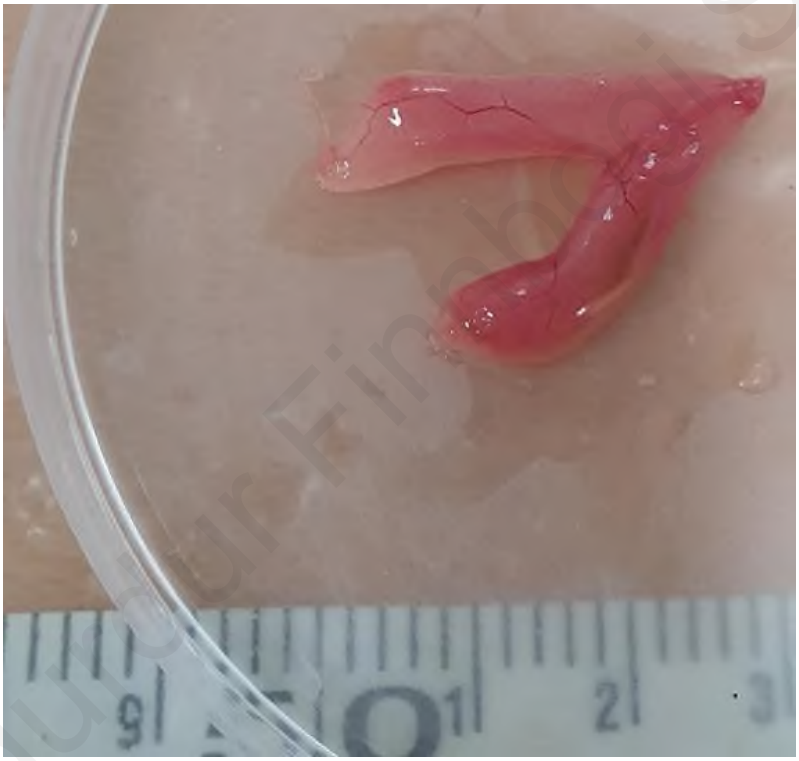


Figure 6. Ovaries in the maturing stage.

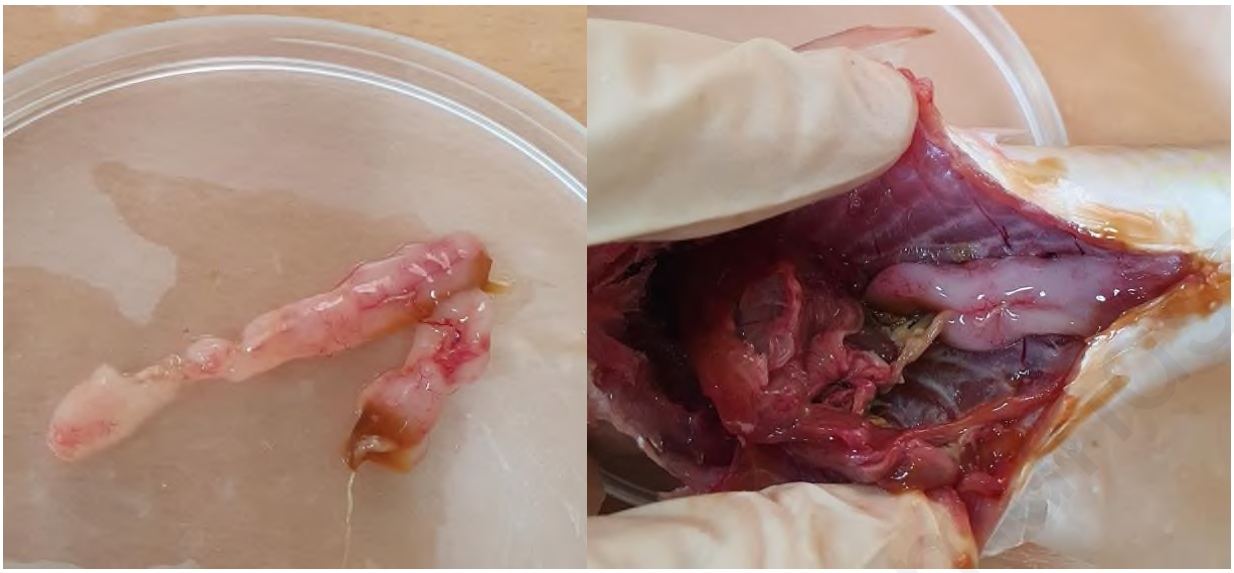


Figure 7. Testes in the maturing stage.

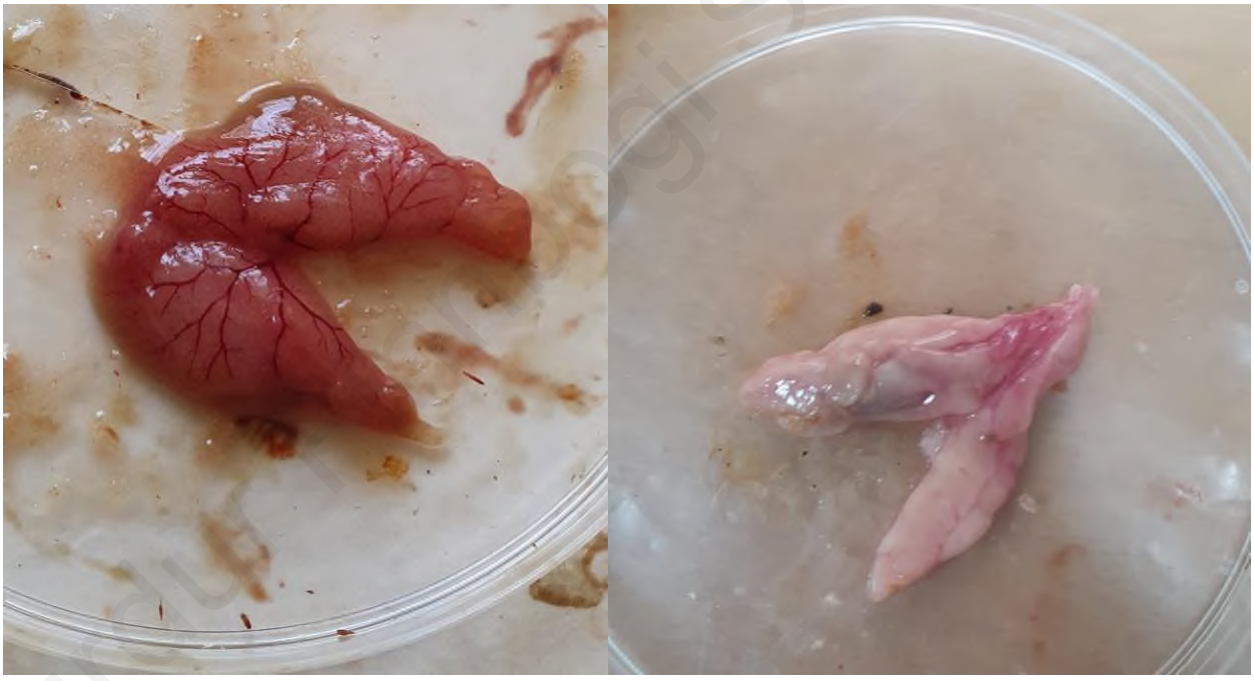


Figure 8. Gonads in the mature/spawning stage. Left: female. Right: male.

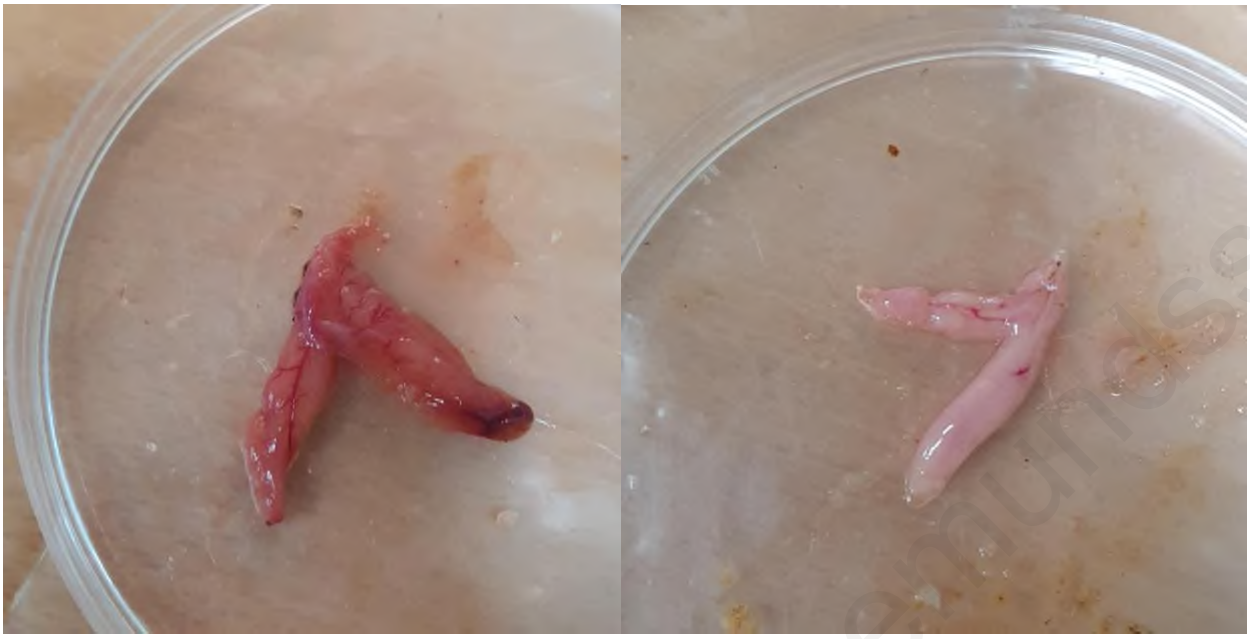


Figure 9. Gonads in the spent stage. Left: female. Right: male.

Table 3. Histological maturity stages of male and female gonads (modified from Follesa and Carbonara (2019)).

Maturity stage	Female	Male
Immature	X	Spermatocytes dominant, spermatids sometimes present. No spermatozoa.
Developing virgin	Only pre-vitellogenic oocytes present. Perinucleolar oocytes dominant.	Spermatids and spermatocytes present, no spermatozoa. Appearance very similar to the immature stage.
Recovering	Perinucleolar oocytes dominant. Cortical alveoli oocytes may be present. Appearance similar to the developing virgin stage.	Spermatocytes, spermatids, and spermatozoa present, but not in large quantities.
Maturing	Vitellogenic and pre-vitellogenic oocytes present. Perinucleolar and cortical alveoli oocytes mixed in with larger vitellogenic oocytes.	Spermatozoa abundant, spermatids and spermatocytes present as well.
Mature/spawning	Vitellogenic and mature oocytes are dominant. Some less developed oocytes present.	Spermatozoa abundant and dominant. Spermatids and spermatocytes present.
Spent	All stages present, but irregularly shaped vitellogenic oocytes are dominant. Post-ovulatory follicles present.	Spermatozoa present but not abundant. Spermatids and spermatocytes also present.

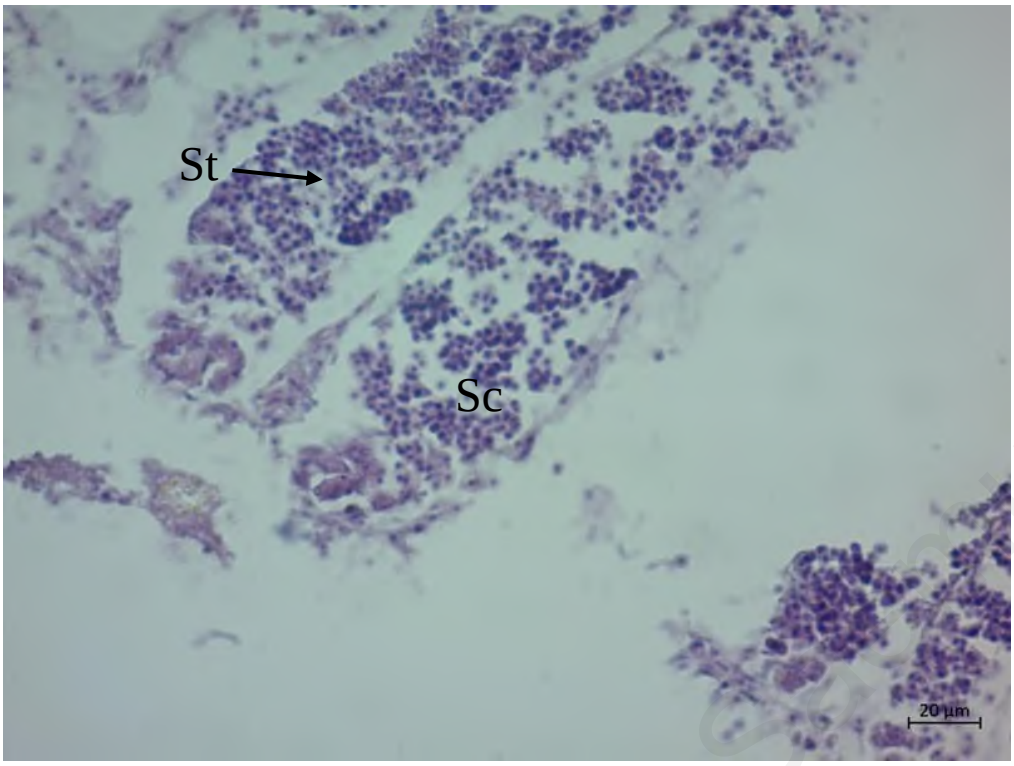


Figure 10. Histological appearance of immature testes.
Sc = spermatocytes; St = spermatids; bar = 20 µm.

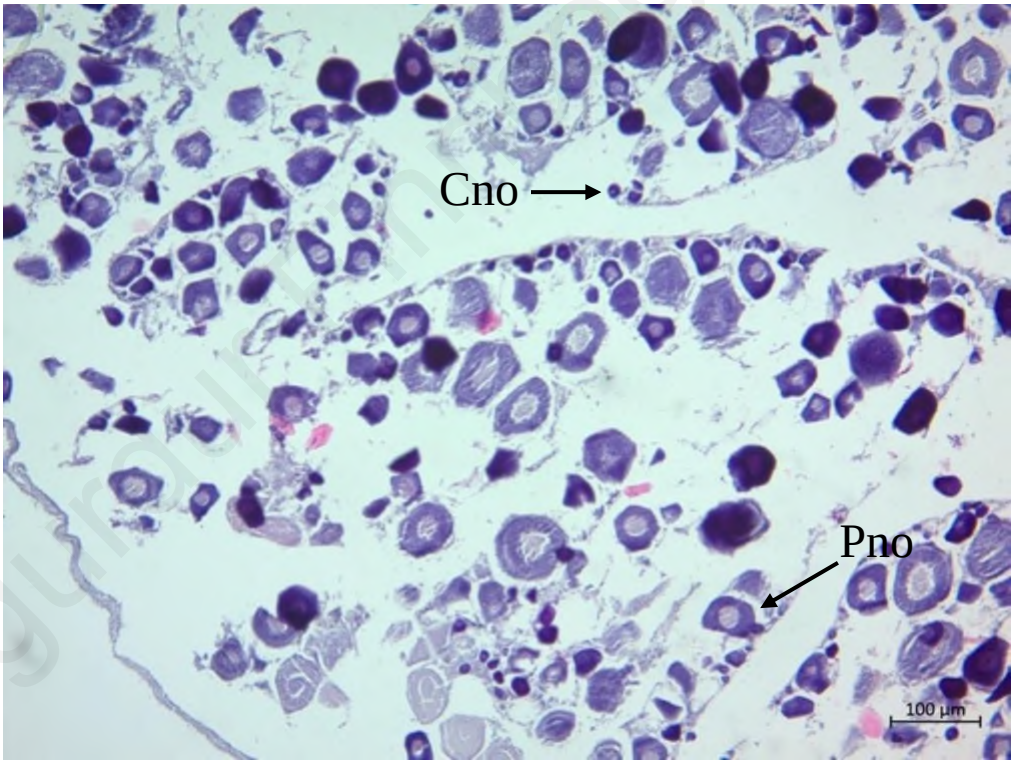


Figure 11. Histological appearance of developing virgin ovaries.
Cno = chromatin nucleolus oocyte; Pno = perinucleolar oocyte; bar = 100 µm.

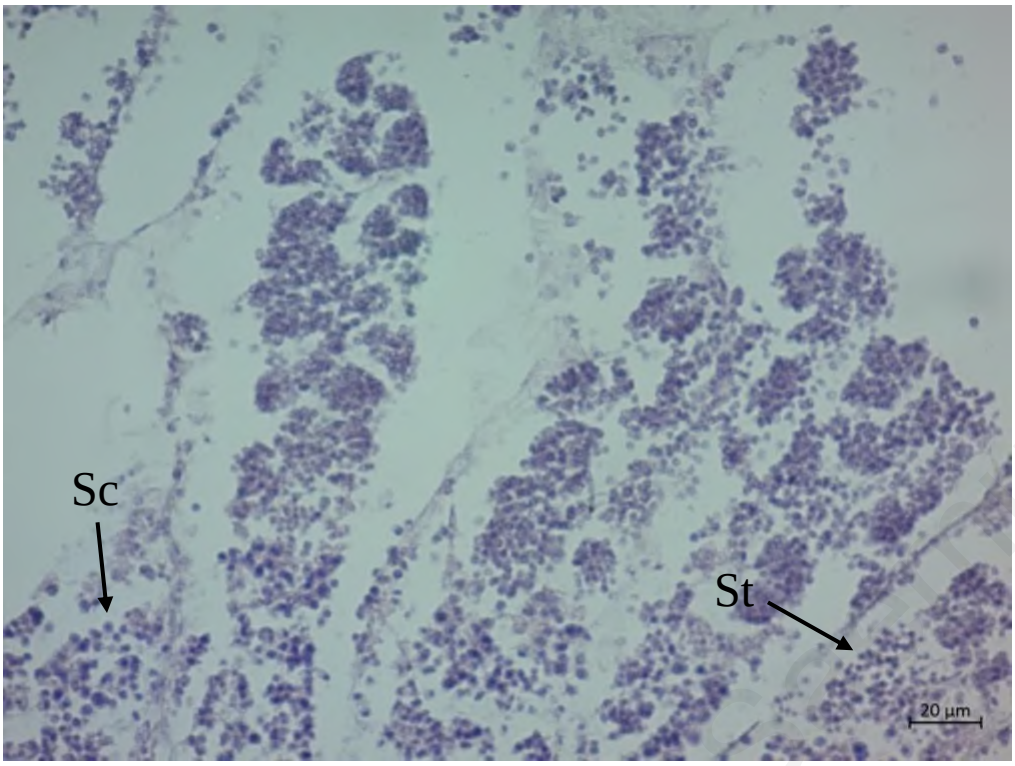


Figure 12. Histological appearance of developing virgin testes.
Sc = spermatocytes; St = spermatids; bar = 20 µm.

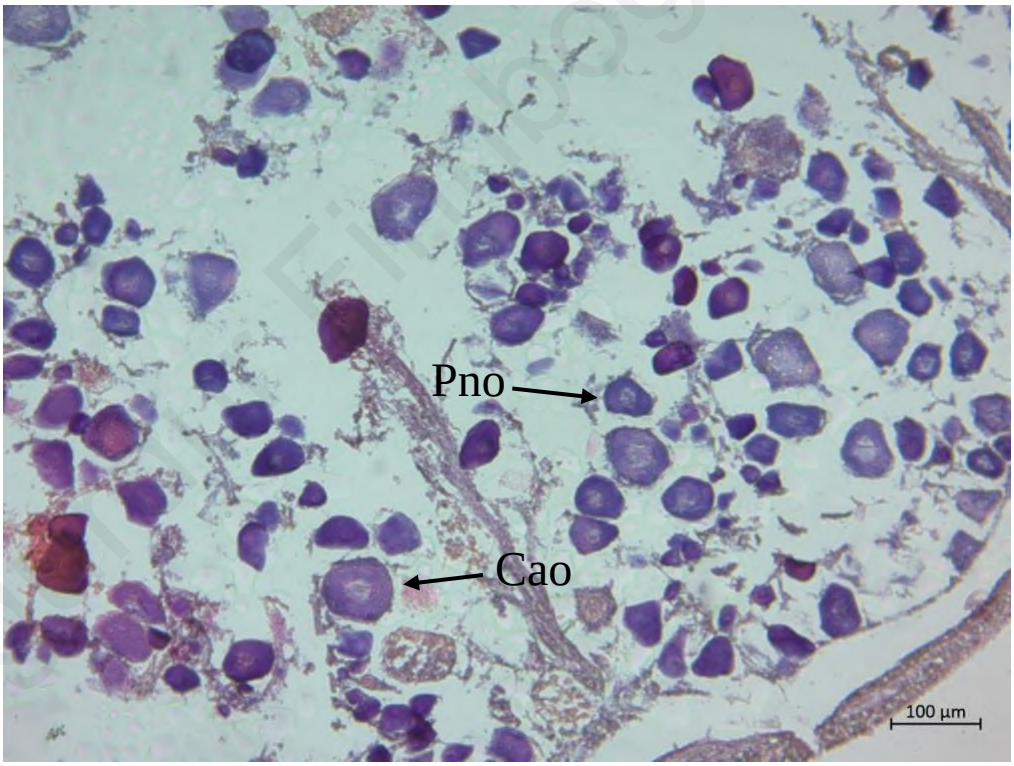


Figure 13. Histological appearance of recovering ovaries.
Pno = perinucleolar oocyte; Cao = cortical alveoli oocyte; bar = 100 µm.

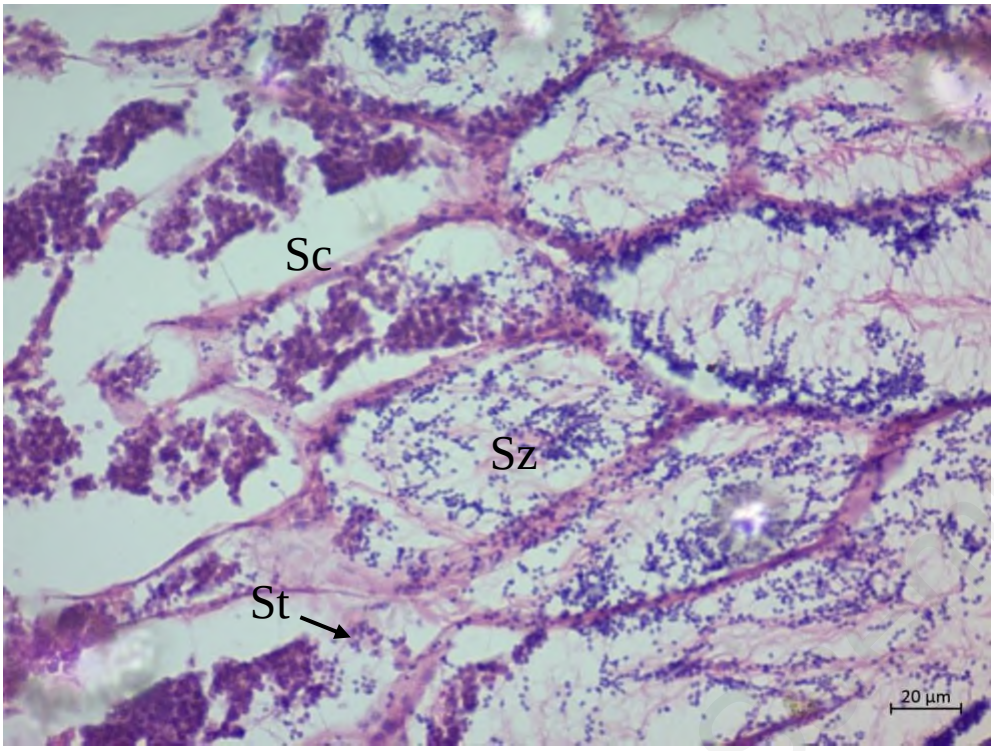


Figure 14. Histological appearance of recovering testes.
Sc = spermatocytes; St = spermatids; Sz = spermatozoa; bar = 20 µm.

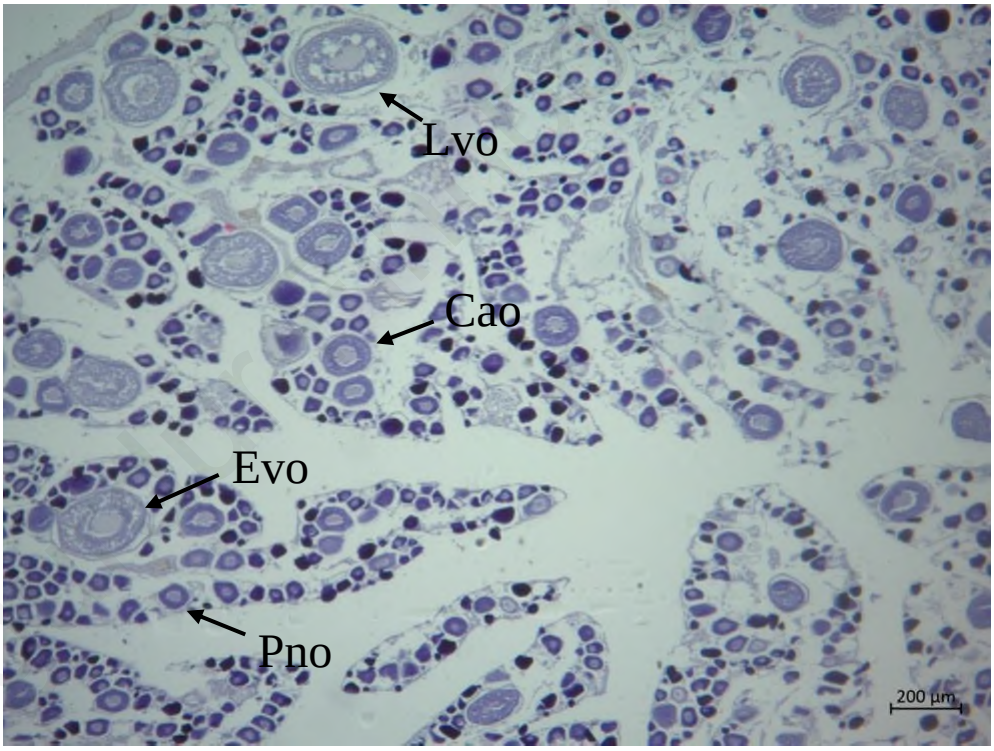


Figure 15. Histological appearance of maturing ovaries.
Pno = perinucleolar oocyte; Cao = cortical alveoli oocyte;
Evo = early vitellogenic oocyte; Lvo = late vitellogenic oocyte; bar = 200 µm.

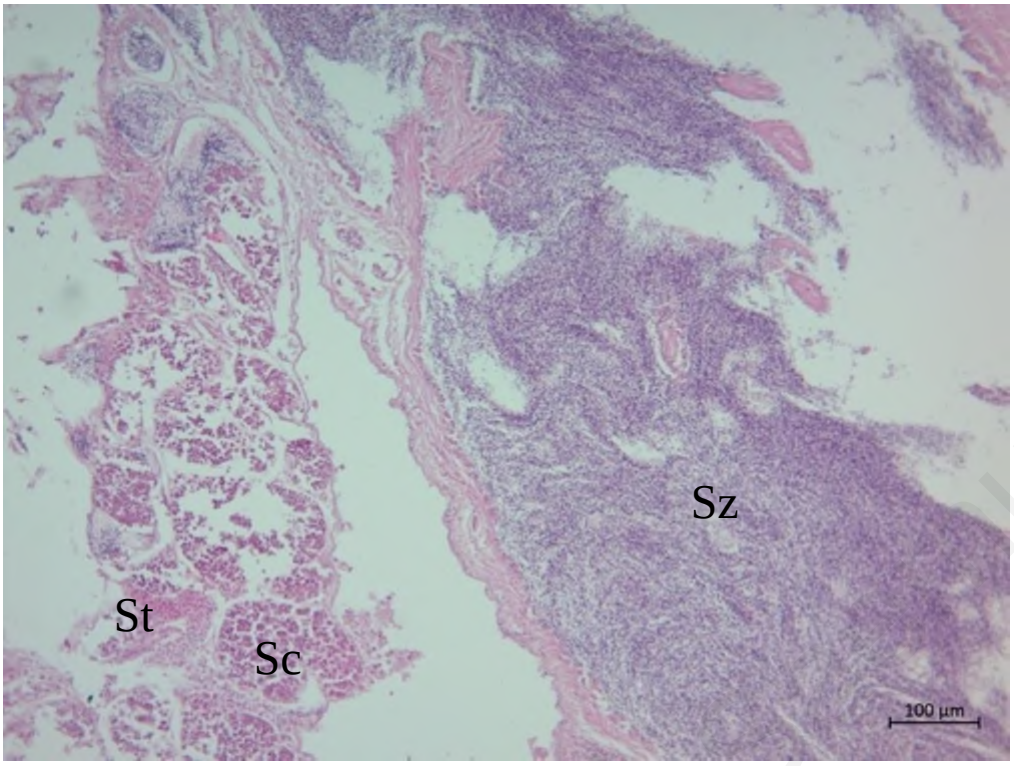


Figure 16. Histological appearance of maturing testes.
Sc = spermatocytes; St = spermatids; Sz = spermatozoa; bar = 100 µm.

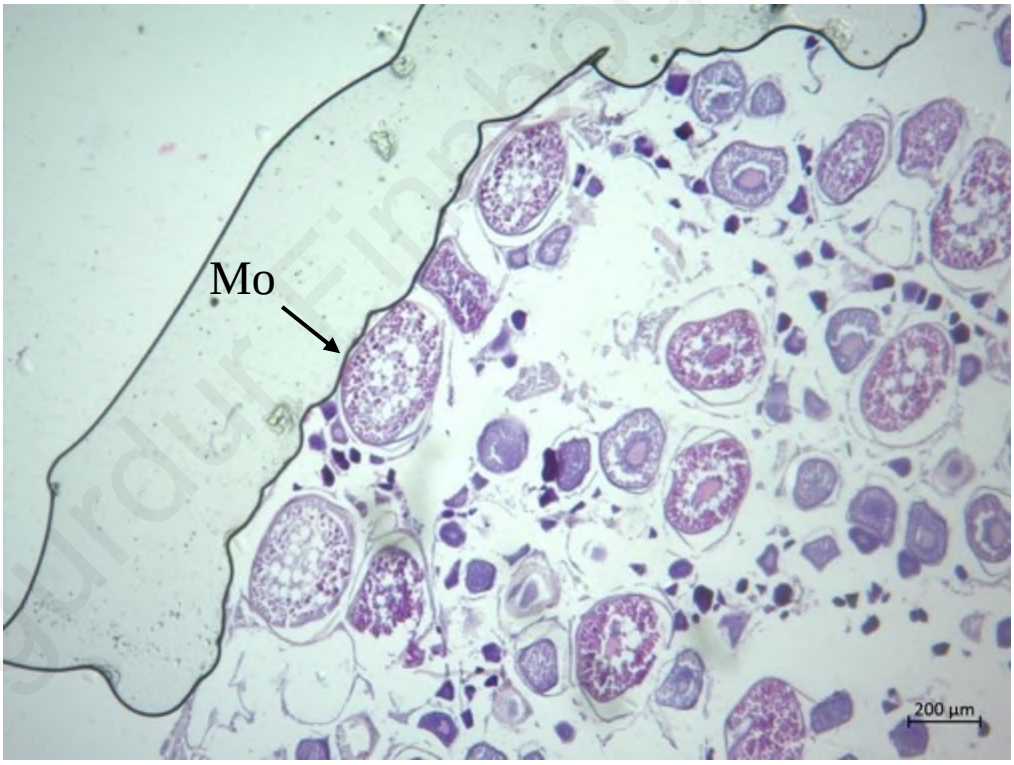


Figure 17. Histological appearance of mature ovaries.
Mo = mature oocyte; bar = 200 µm.

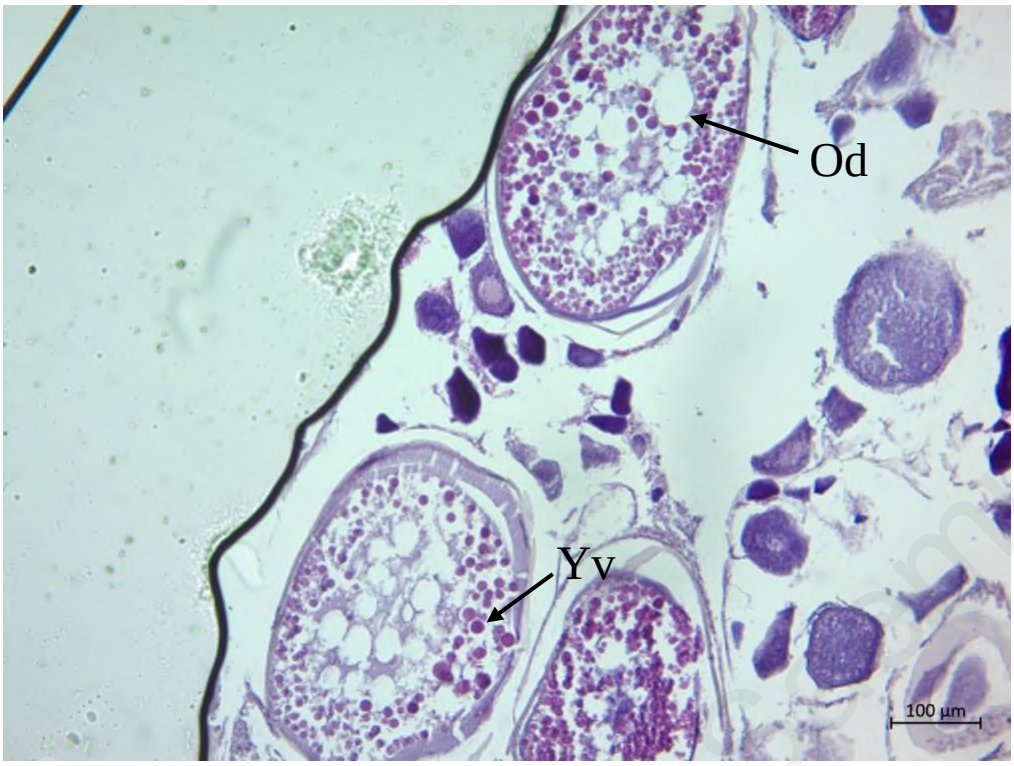


Figure 18. Mature oocytes.
Od = oil droplet; Yv = yolk vesicle; bar = 100 µm.

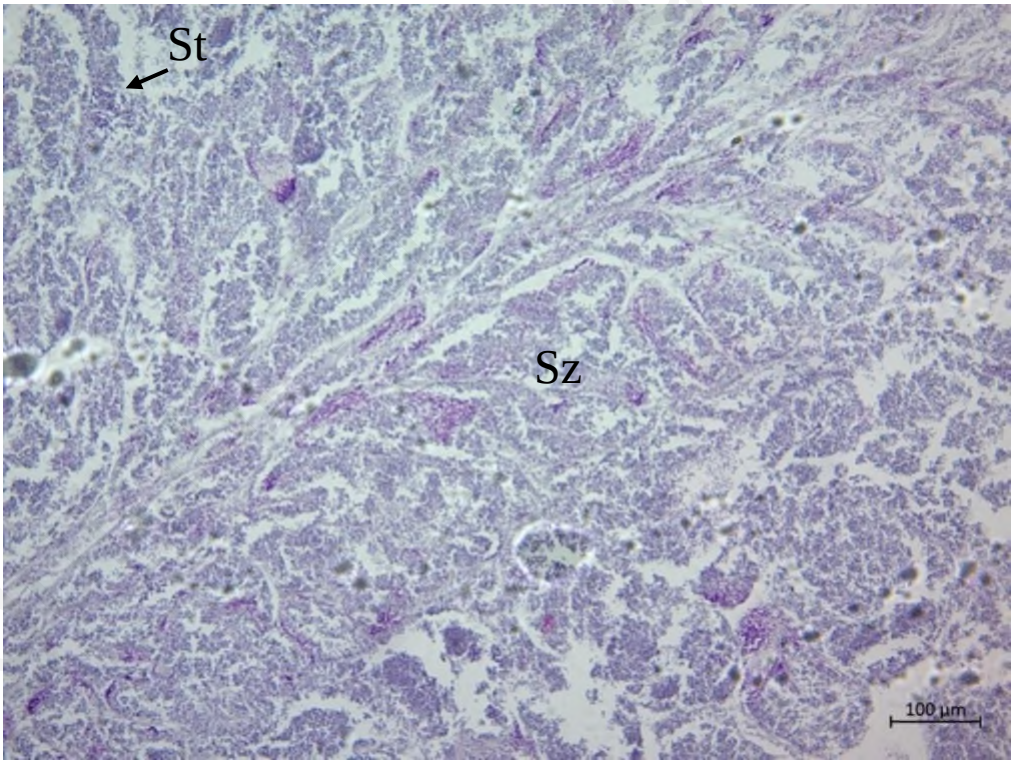


Figure 19. Histological appearance of mature testes.
St = spermatids; Sz = spermatozoa; bar = 100 µm.

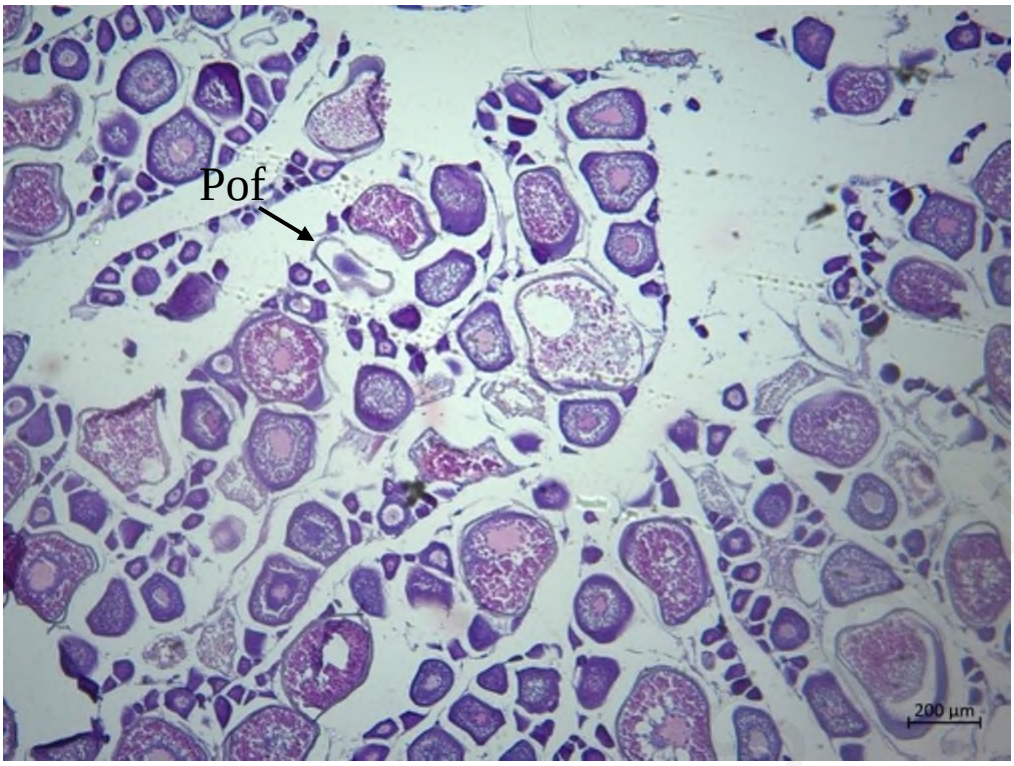


Figure 20. Histological appearance of spent ovaries.
Pof = Post-ovulatory follicle; bar = 200 µm.

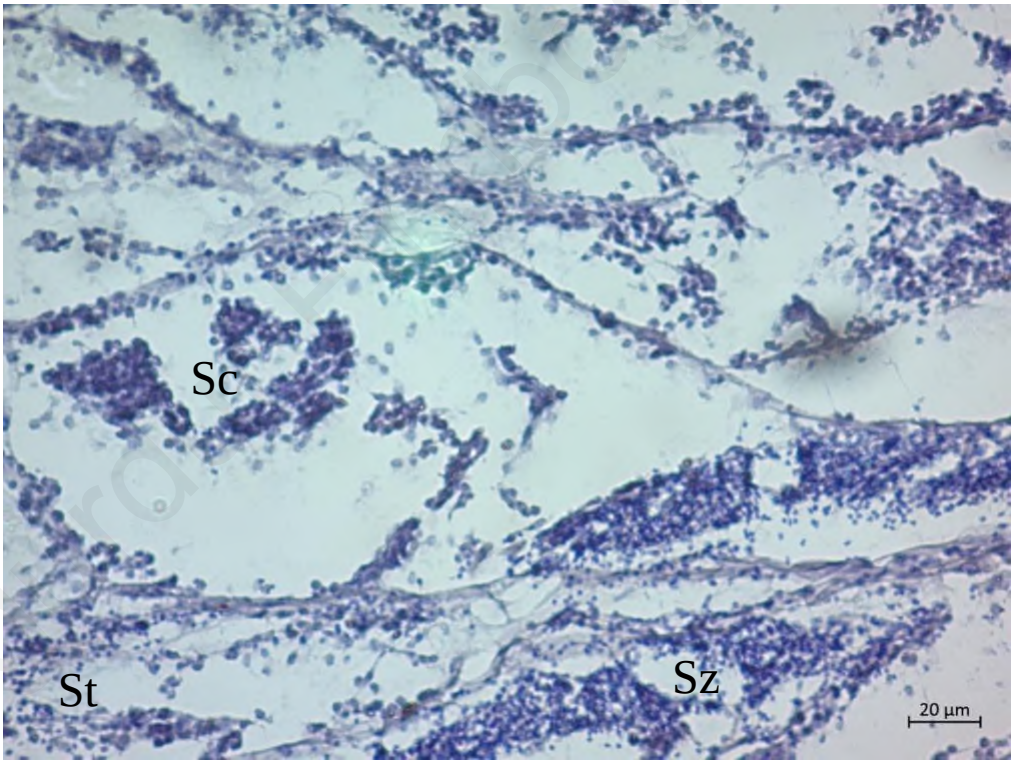


Figure 21. Histological appearance of spent testes.
Sc = spermatocytes; St = spermatids; Sz = spermatozoa; bar = 20 µm.

Spawning season

The monthly mean values of the gonadosomatic index peaked in July both for males (0.747 ± 0.109 SE) and females (4.791 ± 0.353 SE). The mean GSI value for females in July was significantly different from all the other values except for September. For males, no significant difference was detected between the mean GSI values in June and July (Figure 22). The GSI decreased in the autumn and reached its minimum monthly mean value in November for females (0.339 ± 0.043 SE) and in March for males (0.072 ± 0.012 SE). It remained low in the winter months but started to rise slowly in the spring for both sexes, and increased dramatically from May to June (Figure 22).

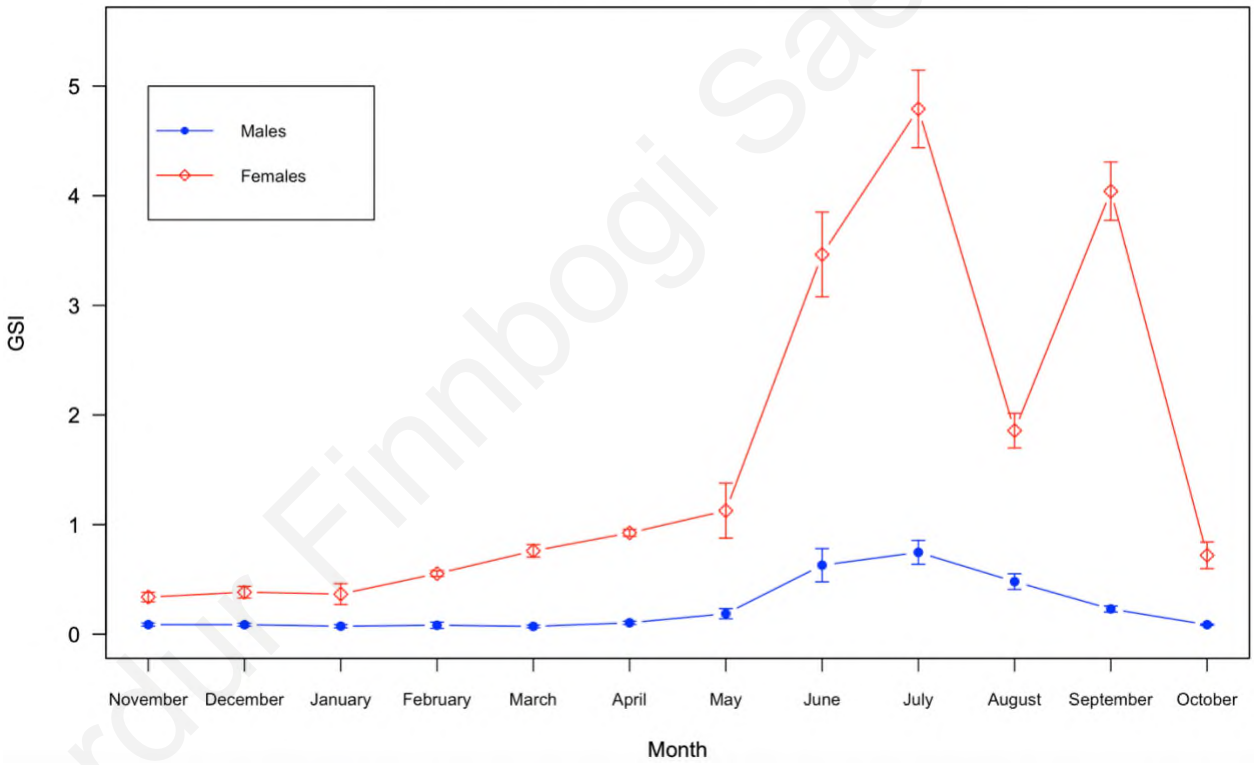
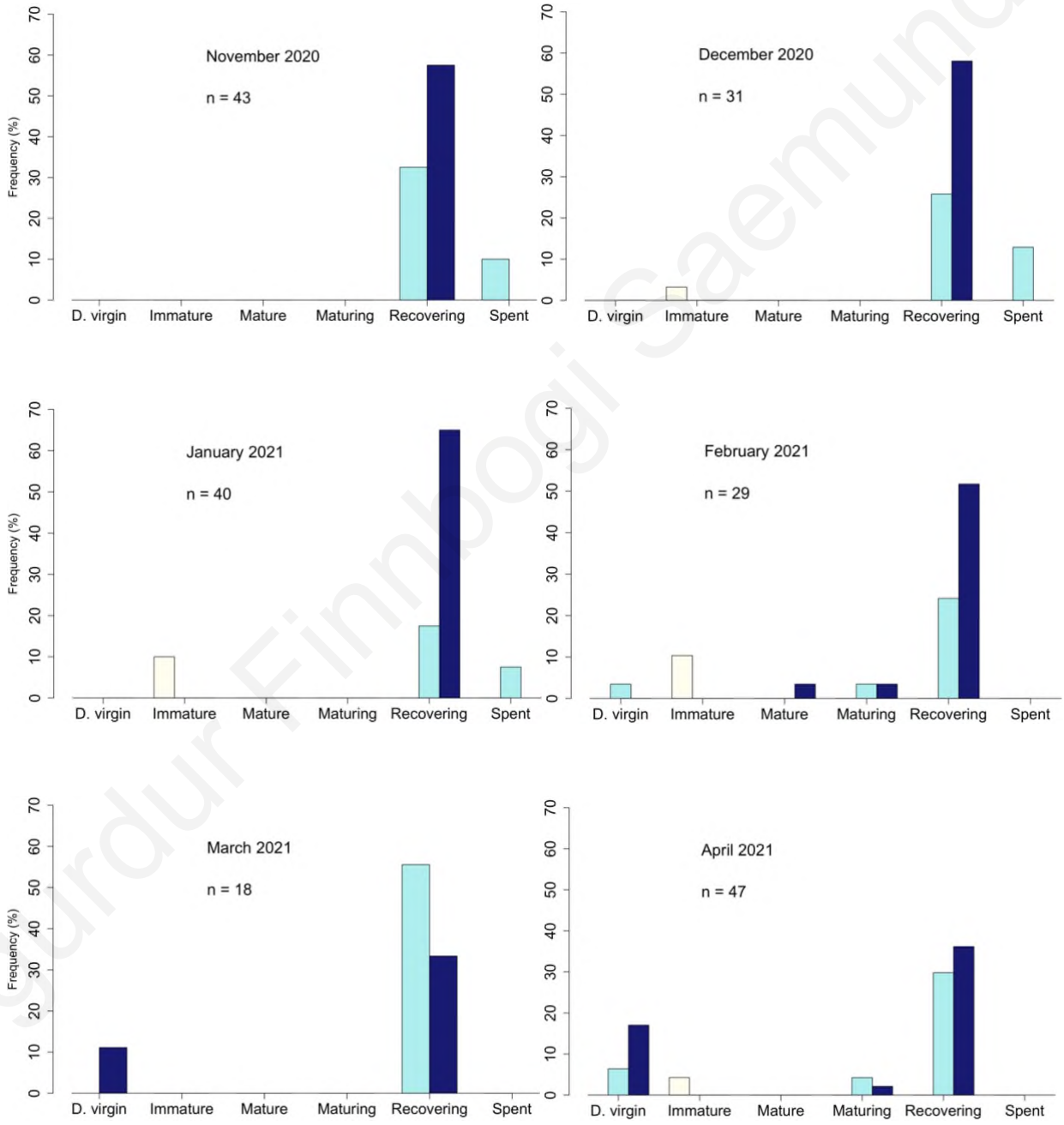


Figure 22. Monthly mean values of the gonadosomatic index for both males and females. Error bars indicate standard error.

The number of mature/spawning males and females were most abundant in July, followed by June for females and August for males (Figure 23). Males at all maturity stages were observed in August

and July, whereas in November, December, and January only recovering males were observed. No month included females at all stages, but in March they were all in the recovering stage, and in September they were all in the spent stage. A single mature/spawning male was observed in February, along with two maturing individuals, one of each sex (Figure 23). Immature individuals were observed in all seasons, but were most abundant in July.



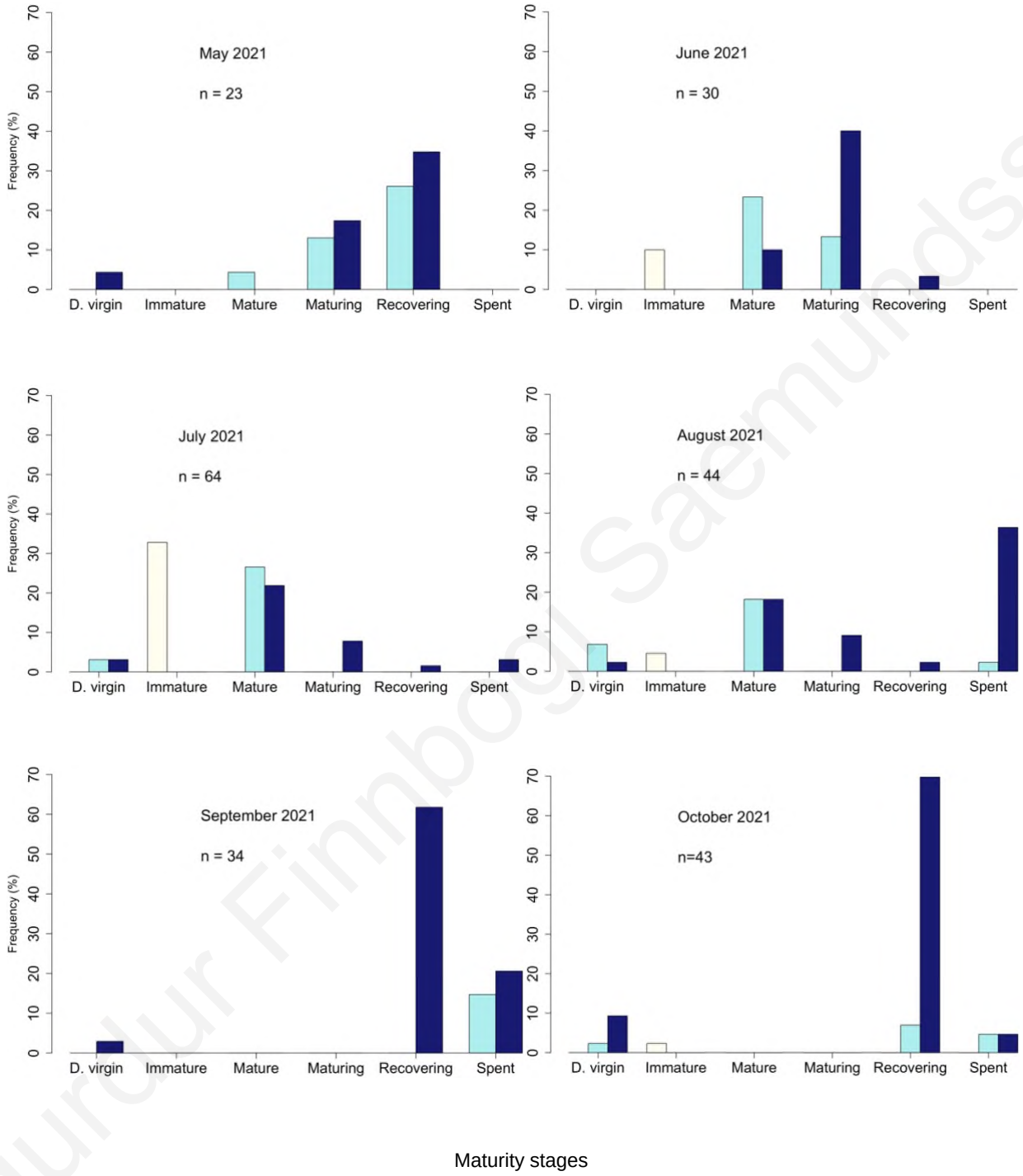


Figure 23. The relative frequency of maturity stages in each month.
■ = Males ■ = Females □ = Juveniles.

Size at first sexual maturity

The smallest sexually mature female was 10.4 cm (sampled in June) and the largest sexually immature female was 17.9 cm (sampled in April). The smallest sexually mature male was 14.3 cm (sampled in July) and the largest sexually immature male was 19.9 cm (sampled in April). For the combined data, where males, females, and juvenile fish were included, the estimated size (total length) at which 50% of fish were sexually mature was 14.3 cm (Figure 24). The estimated size at which 50% of males were sexually mature was 13.6 cm, and 11.9 cm for females (Figure 25). However, the likelihood ratio test indicated little evidence that the influence of TL on the probability of maturity differs between sexes (P = 0.72), that is, the size at first sexual maturity is not significantly different between males and females.

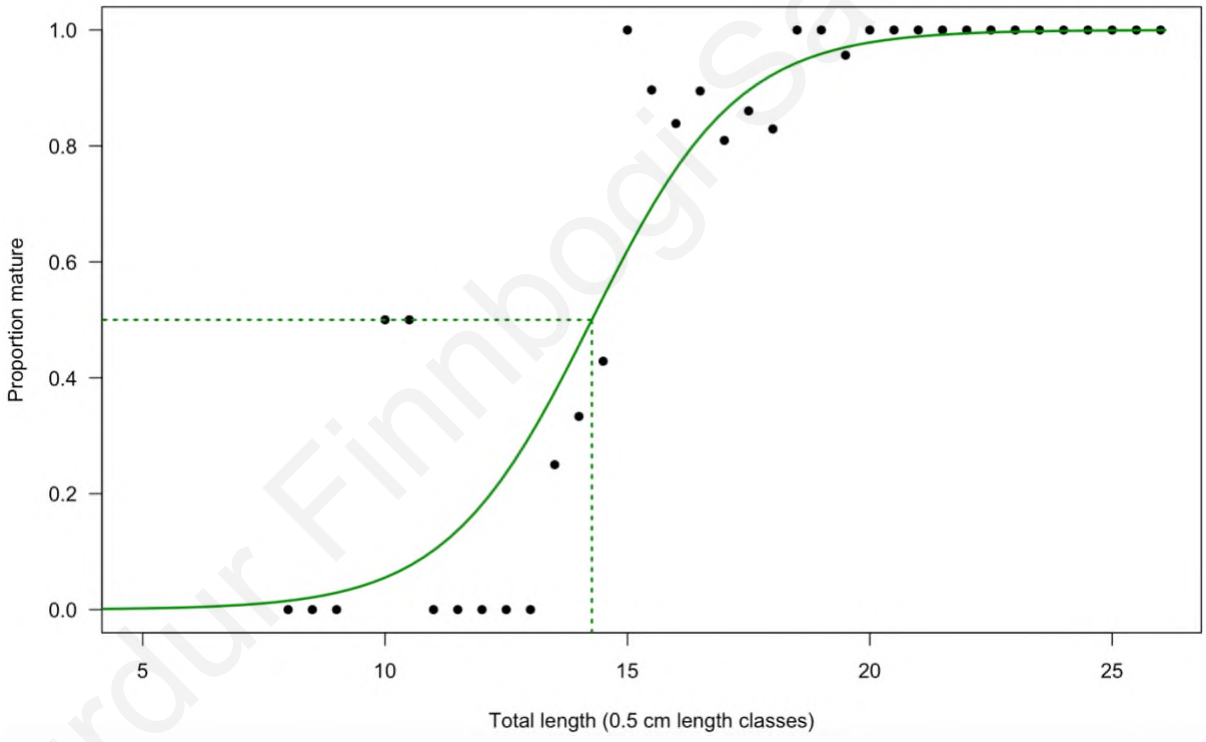


Figure 24. The size-at-maturity logistic curve for the combined data. The dotted lines indicate the size at which 50% of fish are mature (L_{50}).

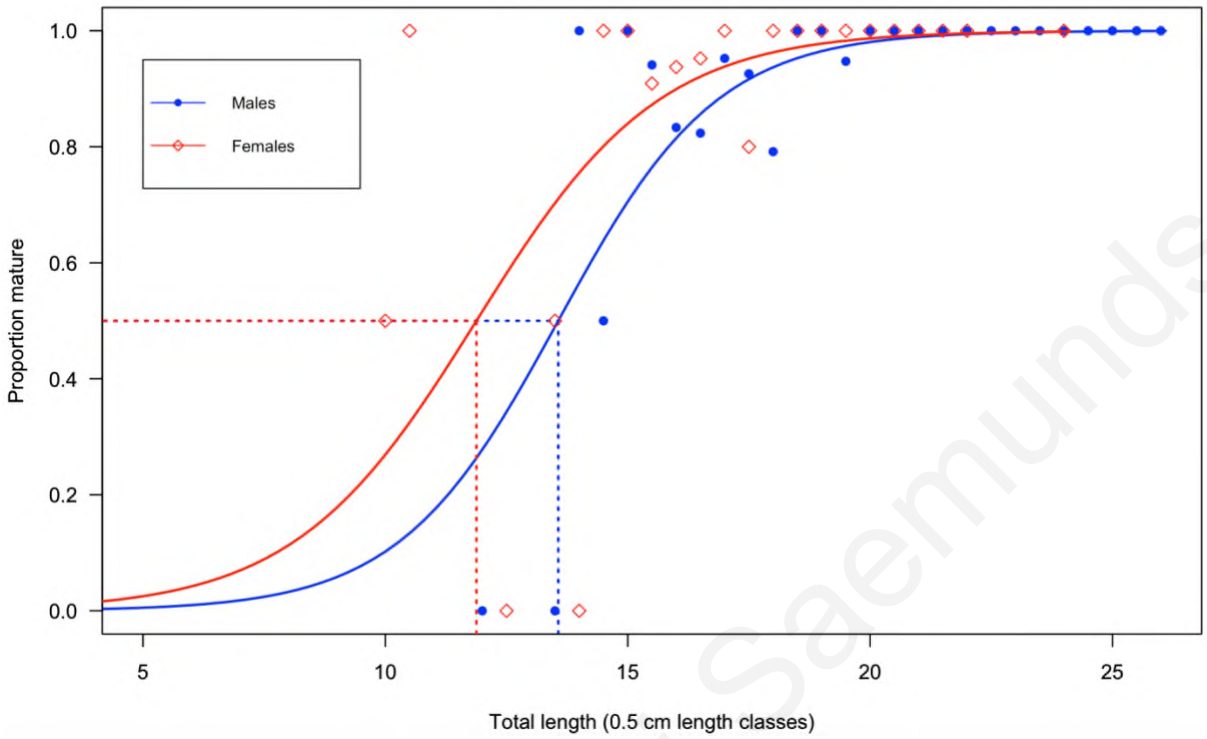


Figure 25. The size-at-maturity logistic curve for males and females. The dotted lines indicate the size at which 50% of fish are mature (L_{50}).

Length-weight relationship

The parameters of the length-weight relationship of *P. forsskali* are shown in table 4. When examining the whole dataset, the linear model exhibited a tight fit to the transformed data (R^2 = 0.99). The equation of the best fit line was log(TW) = -5.03 + 3.17 x log(TL), which is TW = 0.0065 x $TL^{3.17}$ for the original data (Figure 26). The t-test indicated that the slope of the regression line (3.17) was significantly different from the isometric value of 3 (t = 9.44, df = 464, P < 0.05).

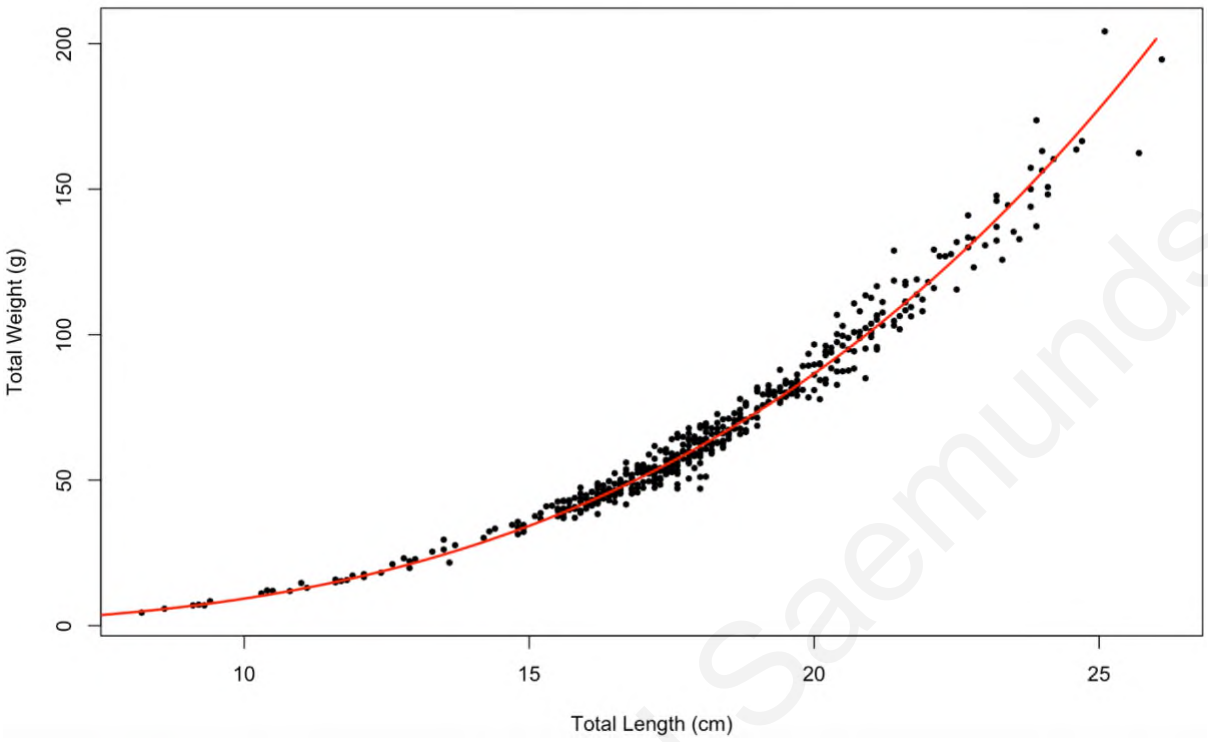


Figure 26. The length-weight relationship for all sampled fish.

For the male data, the linear model exhibited a tight fit to the transformed data (R^2 = 0.98). The equation of the best fit line was log(TW) = -5.04 + 3.18 x log(TL), which is TW = 0.0065 x $TL^{3.18}$ for the original data (Figure 27). The t-test indicated that the slope of the regression line (3.18) was significantly different from the isometric value of 3 (t = 6, df = 270, P < 0.05).

For the female data, the linear model also exhibited a tight fit to the transformed data (R^2 = 0.97). The equation of the best fit line was log(TW) = -4.76 + 3.07 x log(TL), which is TW = 0.0086 x $TL^{3.07}$ for the original data. The t-test indicated that the slope of the regression line (3.07) was not significantly different from the isometric value of 3 (t = 1.75, df = 138, P = 0.08).

No significant sex-based difference in the length-weight relationship was detected from the ANCOVA (F = 0.97, df = 409, P = 0.32).

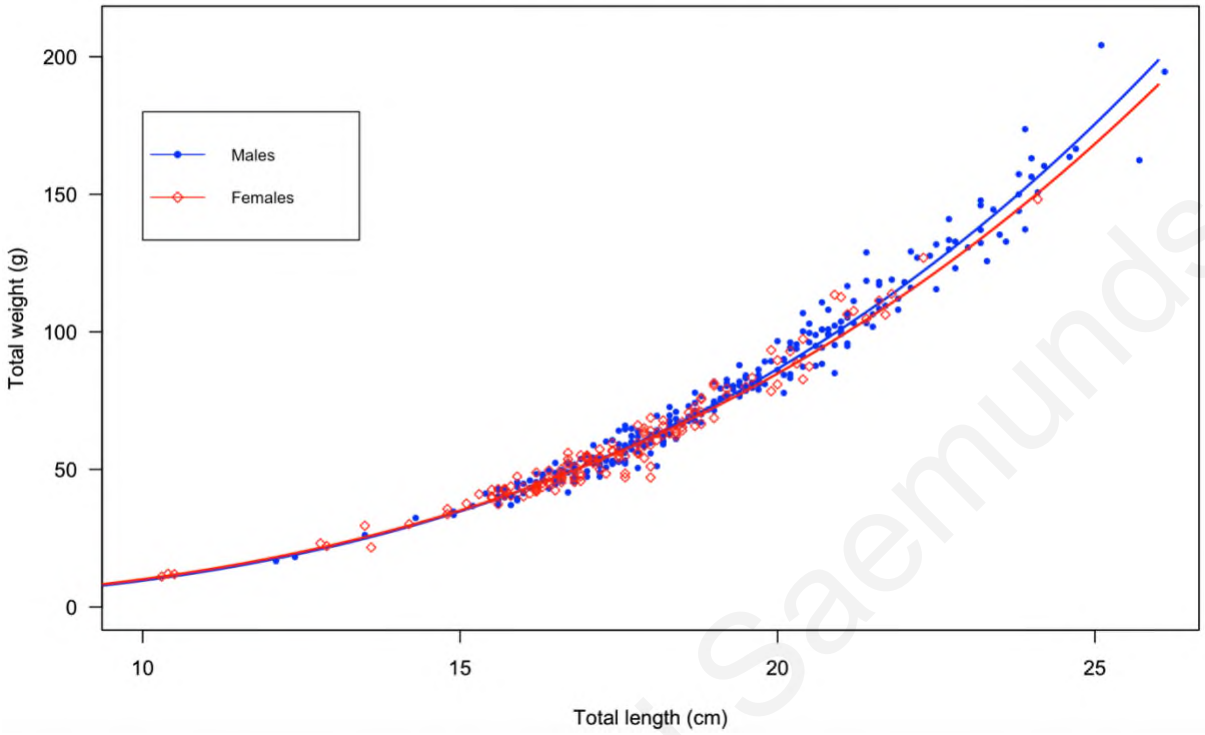


Figure 27. The length-weight relationship for males and females.

Table 4. The length-weight relationship parameters of *P. forsskali*.

Data	Number of individuals	*a*	*b*	95% CI of *b*	Type of growth
Male	272	0.0065	3.18	3.12 - 3.23	Allometric
Female	140	0.0086	3.07	2.98 - 3.16	Isometric/allometric
All	466	0.0065	3.17	3.14 - 3.21	Allometric

Note: CI = confidence interval.

Age and growth

Five age groups were identified from the length class-frequency distribution (Figure 28, Table 5), from age group 0 to age group 4 (0-4 years old). The most dominant age group was age group 1 with more than 53% of fish belonging to that group (Table 5).

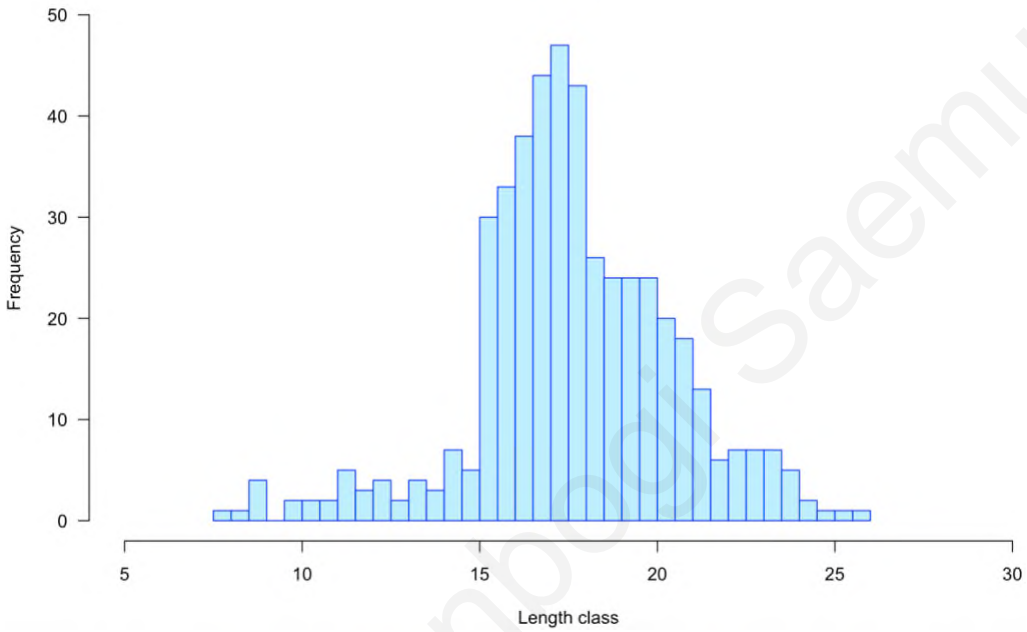


Figure 28. The length-frequency distribution using the 0.5 cm length classes.

Table 5. Length classes within age groups, and mean length and frequency of age groups.

Age group	Length classes	Mean length at age (cm) ± SE	Number of fish	Age group frequency (%)
0	8 - 13.5	11.5 ± 0.29	30	6.44
1	14 - 18	16.9 ± 0.06	250	53.65
2	18.5 - 21	19.8 ± 0.07	136	29.18
3	21.5 - 24	22.7 ± 0.13	45	9.66
4	24.5 - 26	25.2 ± 0.29	5	1.07

Note: SE = standard error.

The parameters of the von Bertalanffy growth function were L_∞ = 31.81 cm (SE = 5.5), K = 0.27 year^{-1} (SE = 0.12), and t_0 = -1.67 (SE = 0.47), giving the equation:

$$L_t = 31.8(1-e^{-0.27(t+1.67)})$$

The von Bertalanffy growth curve of *P. forsskali* is shown in figure 29.

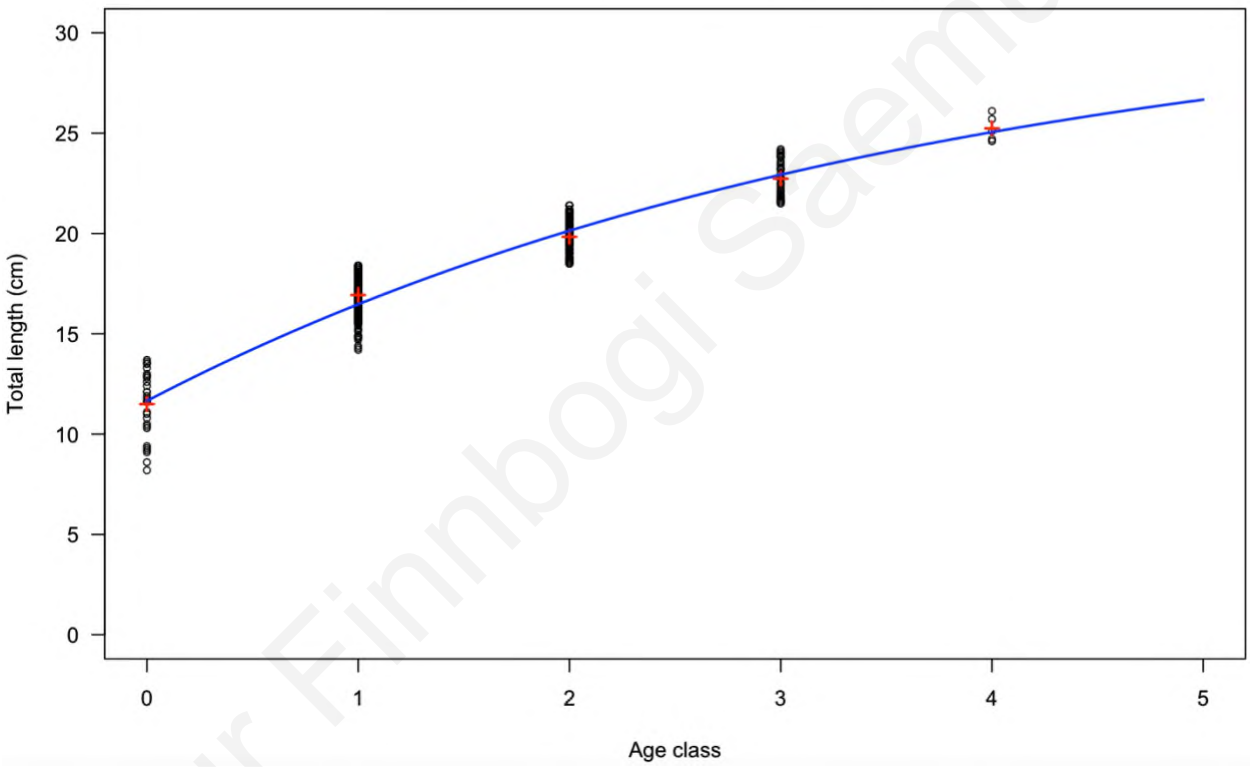


Figure 29. The von Bertalanffy growth curve of *P. forsskali*. The red crosses indicate the mean total length in each age class.

DISCUSSION

To the best of our knowledge, this is the first time the gonads of *Parupeneus forsskali* have been histologically analyzed in order to study the species' reproductive cycle and spawning season. Six maturity stages were identified in this study, setting the basis for future studies on gonadal maturity stages of this species.

Total length ranged from 8.2 cm to 26.1 cm, and total weight ranged from 4.49 g to 204.22 g. Sabrah (2015) studied *P. forsskali* in the Red Sea in a year long study and found that total length ranged from 11.1 cm to 28.5 cm (n = 456). Another year long study on *P. forsskali* in the Red Sea reported a total length of 11.5-27.9 cm, and total weight of 16.6-275.3 g (n = 375) (Mehanna et al. 2018). Neither of those studies fished specifically for juveniles like was done in the month of July in the present study, and they had nets with a larger mesh size (2-3 cm), which could explain why their smallest individuals are bigger than the smallest individuals sampled here. Conditions in the Red Sea, such as more stable temperatures and greater food availability, may explain why larger individuals are found there and not around Cyprus, as it is their native region. Males were on average heavier and bigger than females, however, the length-weight relationships of males and females were not significantly different from each other, meaning that males and females of the same length generally do not differ in weight. In most fish species, the females grow bigger than the males, despite them having to spend more energy on reproduction. Males usually spend more energy on courtship or fighting with competitors, limiting their growth (Pauly 2019). Studies on *Mullus barbatus* (Sieli et al. 2011) and *Upeneus pori* (Geraci et al. 2018), both off the coast of Italy, reported significantly larger females, but Sabrah (2015) reported slightly larger *P. forsskali* males than females in the northern Red Sea. This could mean that *P. forsskali* females are more active than males. The sex ratio showed that males were considerably more abundant than females (1.94:1). Sabrah (2015) also reported more *P. forsskali* males than females in the Red Sea. A study on other mullids (*Upeneus guttatus* and *U. pori*) in the Gulf of Suez showed different results, with females being more abundant, with almost a 1:2 ratio (Sabrah et al. 2017). Amin et al. (2016) reported a female dominance of *Mullus surmuletus* in the Egyptian Mediterranean. The reason for this high proportion of males in the present study is difficult to pinpoint, but the difference in the

sex ratio was by far greatest in the months of August, September, and October, so it could have something to do with post-spawning activities. Since females spend a lot of energy on reproduction, they might be more susceptible to injury, disease, or predation after spawning (Wootton and Smith 2015).

The monthly mean gonadosomatic index of *Parupeneus forsskali* peaked in July for both males and females. It was quite high in June and August as well, indicating a summer spawning season. The mean GSI of females rose sharply from August to September, becoming almost as high as the July value. This can be explained by a lack of samples, since only five females were obtained in September, all having a relatively high gonadosomatic index. The maturity stages also indicate that spawning activities take place in the summer, since the most advanced stages were mostly observed in those months. The high number of juvenile individuals observed in July was mainly due to the fact that a smaller net was used specifically to capture smaller fish. The GSI and maturity stages therefore show that *P. forsskali* around Cyprus typically spawns from May until August, and possibly in September as well. No other published studies (to our knowledge) have reported the spawning season of *P. forsskali*, but other mullids in the Mediterranean and the Gulf of Suez have been observed spawning in the spring and summer months, for example, the red mullet (*Mullus barbatus*, April - June in the northern Aegean Sea) (Kokokiris et al. 2014), *Upeneus guttatus* (May - September in the Gulf of Suez) (Sabrah et al. 2017), *Upeneus pori* (April - June in the Egyptian Mediterranean) (Ramadan and El-Halfawy 2014), and the golden-banded goatfish (*Upeneus moluccensis*, August - September off the southern coast of Turkey) (Kaya et al. 1999). The summer spawning season could emphasize their tropical origin since the last three species mentioned, as well as *P. forsskali*, originate from the Indian Ocean or the Red Sea, so they are well adapted to higher sea temperatures (Uiblein 2007). Since a mature male and a maturing male and female were observed in February, it is possible that some individuals could have been spawning outside of the typical spawning season. *Mullus surmuletus* males have been observed spawning in February around Majorca in the western Mediterranean, even though the species typically spawns in the spring or early summer (Reñones et al. 1995).

The size at first sexual maturity was not different between males and females, and was 14.3 cm for the pooled data. A study on *P. forsskali* conducted in the Red Sea reported an L_{50} of 15.38 cm, and also detected no significant difference in the size at first sexual maturity between males and females (Sabrah 2015). A net with a larger mesh size was used in that study, so smaller fish

were more likely to escape which could explain the difference between these two L_{50} values. The L_{50} of *P. forsskali* in the Mediterranean can be used in fisheries management to determine the optimum mesh size of the fishing nets to ensure that the spawning population is maintained, keeping the fishery sustainable. Although, since it is not a native species and can have a negative impact on native mullids, the L_{50} can also be used to try to eliminate the species, or at least reduce its numbers.

The ANCOVA indicated that sex does not affect the LWR of *P. forsskali*, meaning that the relationship is not significantly different between males and females. However, the *b*-value for males (3.18) was significantly different from 3, but the *b*-value for females (3.07) was not. This difference in the *b*-values could be explained by the fact that larger and heavier males were sampled. The *b*-value for the female data had a 95% confidence interval of 2.98 - 3.16, and the P-value of the t-test was relatively low (0.08), so the type of growth indicated by the data could either be isometric or allometric. Overall, the length-weight relationship of *P. forsskali* off the coast of Cyprus can be described with the equation $TW = 0.0065 \times TL^{3.17}$. The *b*-value of 3.17 was higher and significantly different from the isometric value of 3, indicating a positive allometric growth, meaning that the fish becomes rounder as it grows. This could indicate that larger individuals are in better nutritional condition than smaller ones. A study on *P. forsskali* by Sabrah (2015) conducted in the Red Sea (n = 456) reported a *b*-value of 2.80, which was also significantly different from 3, indicating a negative allometric growth. Mehanna et al. (2018) also studied *P. forsskali* in the Red Sea and reported a *b*-value of 3.17. Both studies were conducted in Hurghada, Egypt, and had a similar sampling size and total length range, so environmental conditions in the years of study, such as food availability, must have been different enough to result in greatly differing *b*-values. Studies on another mullid species (*Mullus barbatus*) in the eastern Mediterranean have reported a *b*-value ranging from 3.09-3.44 (İşmen et al. 2007; Cengiz 2013; Altın et al. 2015), which are in agreement with our results.

Five age groups were identified for *P. forsskali*. Mehanna et al. (2018) and Sabrah (2015) identified six age groups in the Red Sea using otoliths, scales, and length-frequency data for aging. However, the largest individuals sampled in those studies were 27.9 cm and 28.5 cm long, respectively, whereas the largest individual in the present study was only 26.1 cm long. Furthermore, in the study by Sabrah (2015), the mean length of age group 5 was 26.9 cm, which is considerably bigger than the largest fish sampled in the present study. It is possible that *P.*

forsskali does not get this big around Cyprus and only lives for four years because of less favorable conditions, as opposed to five years in its native region, or that larger individuals are present but were not sampled. The mean length of age group 4 was 25.2 cm, which is similar to the same age group reported by Sabrah (2015), or 25.5 cm. Age group 1 was the most dominant age group, which could indicate extensive targeting of larger individuals by fisheries. Mehanna et al. (2018) reported the same results, with 46.1% of individuals belonging to age group 1, whereas Sabrah (2015) 4.12.2021 09:22:00reported age group 2 to be most dominant, with 40.6% of individuals, and age group 1 being second most dominant with 25.2% of individuals. The equation of the von Bertalanffy growth function was $L_t = 31.8(1\text{-}e^{-0.27(t+1.67)})$. Similar results were presented by Mehanna et al. (2018), with $L_\infty = 31.62$ cm, $K = 0.32$ year^{-1}, and $t_0 = -1.27$, and by Sabrah (2015), with $L_\infty = 30.0$ cm, $K = 0.38$ year^{-1}, and $t_0 = -0.43$. The differences in the von Bertalanffy parameters are possibly explained by different maximum observed lengths in each study. Changes in the growth rate between years can indicate changes in the environment, such as in the temperature of the ocean, so this information on age compostition and growth rate can be used to predict the status of the population in the future. The size at first sexual maturity for *P. forsskali* was 14.3 cm, which is within age group 1 (14-18.4 cm TL). By analyzing the size-at-maturity logistic curve it is apparent that the majority of individuals becomes sexually mature at age one. Sexually mature individuals within age group 0 were sampled, and sexually immature ones within age group 2 were as well. This indicates that *P. forsskali* reaches maturity at ages 0-2. These results are in accordance with Sabrah (2015).

In conclusion, this research has identified the spawning season of *Parupeneus forsskali* in the eastern Mediterranean Sea and described the cycle of maturity stages the species goes through each year, as well as identified the size at first sexual maturity, the length-weight relationship, and growth rate parameters. The Red Sea goatfish has only recently entered the eastern part of the Mediterranean Sea but is migrating further and further west at a rapid rate, so research on this species will probably take place across the Mediterranean in the coming years. The information provided in this study will hopefully prove useful for other researchers studying this species.

ABBREVIATIONS

ABBREVIATION	MEANING
L_{50}	Size at first sexual maturity
LWR	Length-weight relationship
GSI	Gonadosomatic index
TL	Total length
SL	Standard length
TW	Total weight
GW	Gutted weight
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
SD	Standard deviation
SE	Standard error
df	Degrees of freedom
CI	Confidence interval

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