UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO DI SCIENZE DELLA VITA E DELL'AMBIENTE

Corso di Laurea Magistrale

Biologia Marina

Valutazione del ciclo riproduttivo e dello stato di salute della sogliola comune (*Solea solea*) in Adriatico centrale

Evaluation of the reproductive cycle and health status of common sole (S*olea solea*) in the central Adriatic Sea

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Sessione straordinaria di febbraio

Anno Accademico 2023/2024

ABSTRACT

The common sole (*Solea solea*, Linnaeus 1758) is a species of high ecological and commercial value in the Mediterranean area particularly in the Adriatic Sea. Although monitoring studies on annual catches have been conducted regularly, reproductive studies on this species in the Adriatic Sea remain scarce.

The current study investigated the reproductive biology of common sole investigating gonadal developmental stages through macroscopic and histological analyses focusing on female specimens. Data were collected during an annual sampling conducted in 2019 along the coast of Ancona (GSA 17). Macroscopic and histological examination were applied to determine the reproductive period, sex ratio and L_{50}.

The results indicated that the reproductive period of common sole in the central Adriatic Sea extends from October to January, with a histological L_{50} estimated at 20.67 cm. Macroscopic evaluation proved to be a valid method to characterize the spawning period. However it presented a crucial limitation represented by the difficulty to discerne between immature and mature females.

RIASSUNTO

La sogliola comune (*Solea solea,* Linnaeus 1758) è una specie di elevato valore ecologico e commerciale nell'area Mediterranea, in particolare nel Mar Adriatico. Sebbene siano stati condotti regolarmente monitoraggi sulle catture annuali, esistono pochi studi che interessano la riproduzione di questa specie nel Mare Adriatico.

Il presente studio ha indagato la biologia riproduttiva della sogliola comune, determinando i diversi stadi di sviluppo gonadico attraverso analisi macroscopiche e istologiche effettuate su esemplari femminili. I dati sono stati raccolti nel corso di un campionamento annuale condotto nel 2019 lungo la costa di Ancona (GSA 17).

L'esame macroscopico e quello istologico sono stati utilizzati per determinare il periodo riproduttivo, la sex ratio e L_{50}.

I risultati hanno indicato che il periodo riproduttivo della sogliola nell'Adriatico centrale si estende da ottobre a gennaio, con una L_{50} istologica stimata a 20.67 cm. La valutazione macroscopica si è dimostrata un metodo valido per caratterizzare il periodo riproduttivo. Tuttavia, ha presentato un limite cruciale rappresentato dalla difficoltà di distinguere tra femmine immature e mature.

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1. INTRODUCTION

1.1. Biology and ecology of the common sole, Solea solea (Linnaeus 1758)

Taxonomic classification:

Phylum: *Chordata*

Subphylum: *Vertebrata*

Class: *Actinopterygii*

Subclass: *Neopterygii*

Infraclass: *Teleostei*

Superorder: *Acanthopterygii*

Order: *Pleuronectiformes*

Family: *Soleidae*

Genus: *Solea*

Species: *Solea solea*

Figure 1. Adult specimen of common sole, *Solea solea.*

The common sole, *Solea solea (Linnaeus 1758),* is a demersal and sedentary flatfish belonging to the Soleidae family (Figure 1).

This species is characterized by a typically oval and asymmetric body with one dorsal fin extending along the whole body length while the anal fin is slightly shorter (FAO 2024). The caudal fin is rounded and not well developed, and it is connected to the other two fins by a membrane. The lateral line is covered by scales and its supratemporal prolongation describes a curve on the head (FAO 2024).

Like for most of the Pleuronectiformes, in adult specimens both eyes are located on the right side of the body, this arrangement is the result of different metamorphosis processes that occur during larval phases and consist of both internal and external anatomical modifications including the migration of the nose and one eye to the right side of the body (Figure 2) (FAO 2024; Kahraman et al., 2021).

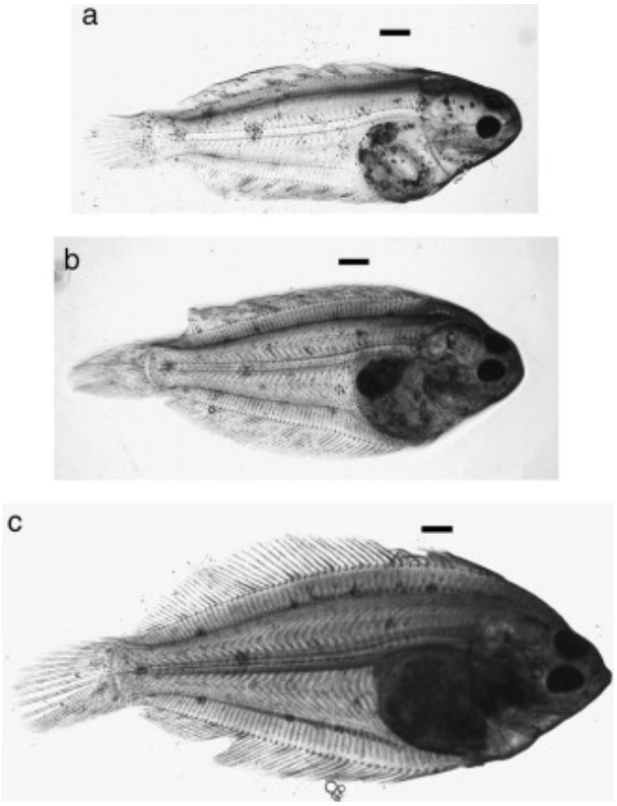


Figure 2. Different metamorphosis phases of a Pleuronectiform. (a) Pelagic larva in the early metamorphosis phase, (b) pelagic larva, during metamorphosis and start of settlement (c) demersal larva at the end of the metamorphosis phase (Geffen et al., 2007).

Adult specimens are colored, on the dorsal side, with a gradient of brown, varying from greyish to reddish brown, with small irregular dark spots. Sole can change their colouration to camouflage themselves and adapt to the environmental patterns of the seabed on which they live (Marshall and Johnsen, 2011). The blind side (the left/ventral one) appears white and covered with several small hair-like sensory fringes constantly in contact with the bottom and involved in tactile perception (FAO 2024; Kahraman et al., 2021).

The common sole is widely distributed in the eastern Atlantic Ocean (from Trondheim Fjord, including the North Sea and western Baltic to Senegal coast) and throughout the Mediterranean Sea, including the Gulf of Lion, Ligurian Sea, Ionian Sea, Tyrrhenian Sea, Aegean Sea and Adriatic Sea (Figure 3) (Fanelli et al., 2022; www.fishbase.se). The Atlantic Ocean is home to several sole species of the *Solea* genus, which preferentially inhabit its eastern part, while in the Mediterranean Sea, *Solea solea* is the only species that occupies the entire area of this basin (Ofelio et al., 2020; www.fishbase.se). In the Adriatic Sea, the common sole is mostly spread in the northern part where its spatial distribution is age-related. Juveniles are found along the Italian coast, especially near the mouth of the Po River while adults prefer the eastern Adriatic coasts (Fanelli et al., 2022; faoadriamed.org).

Figure 3. Global distribution of *Solea solea* (www.fishbase.se).

As mentioned before, this species lives on sandy and muddy bottoms of the continental shelf and in shallow lagoons, between 10 – 60 m and up to 200 m depth (Sardi et al., 2023; FAO, 2025). The common sole has nocturnal feeding habits typical of an opportunistic and generalist benthivore species, during night, it feeds mainly on benthic invertebrates such as Amphipods and Polychaetes, and bivalves while it spends the majority of the day in small niches on the bottom to hide itself from predators (Morat et al., 2014; Fanelli et al., 2022; Ao El-Aiatt et al., 2019; Link et al., 2014; Corti et al., 2024).

Larval and juvenile stages present different feeding strategies since they live in distinct habitats. Indeed, at the end of a pelagic larval phase, characterized by a diet based on zooplankton, they settle in coastal nurseries, where they go through the juvenile stage (Palazzi et al., 2006; Paoletti et al., 2021; Morat et al., 2014). Juveniles feed mainly on amphipods, due to the small size of these prey, and on errant polychaetes. With increasing size, the ability to prey and feed on larger prey and greater carapace thickness also increases. Adults feed on decapods, sedentary polychaetes and molluscs (Molinero and Flos, 1991). Before the spawning season it tends to prey on species that provide more energy, such as

crustaceans, to afford the highly cost-demanding process of spawning events and, in female specimens, of oogenesis (Fanelli et al., 2022).

The common sole, as a mesopredator that occupies the intermediate trophic levels, plays an important role within the marine food web. Species with this role are essential for maintaining ecological balance and facilitating energy transfer through marine food webs, controlling indirectly primary producers, by preying on benthic invertebrates, and acting as a link between them and apex predators that feed on the *Solea* genus (Tambling et al., 2018; Fanelli et al., 2022).

1.2. Life cycle and reproduction of Solea solea

Common sole is a gonochoric, dioecious species and a determinate batch spawner with one evident seasonal peak of spawning during the reproductive period (Ganias, 2013). Generally, each female can release between 8 and 12 batches per spawning season, at a rate of one batch released per week (Le Bec, 1983). Following the fertilization of the eggs, embryos develop in approximately 5 days (at 12°C), while after hatching, the larval phases usually last for around 35 days (at 18°C) (www.fishbase.se).

The passage from the larval stage to the juvenile phase is determined by consecutive metamorphosis events which lead to the final adult stage

(Geffen et al., 2007). Somatic growth rates can differ among geographical areas (Witthames and Walker, 1995) as well as the size and age at which adult specimens reach sexual maturity.

It was observed that the length at first maturity (L_m) varies among different regions (Table 1), presumably due to several factors such as food availability, size range and/or physiochemical parameters (Corti et al., 2024; Ao El-Aiatt et al., 2019). Based on Kahraman's study (2021), the range of L_m in the Atlantic Ocean is between 18.8 - 30 cm, while for the Mediterranean Sea it is around 18.6 - 21.5 cm. In the Adriatic Sea, the length at first maturity is 25 cm combined sexes (Follesa, M.C. and Carbonara, P., eds. 2019).

L_m	Sex	Country	Region	Study
18.8	M + F	Germany	North Sea	Froese & Sampang 2013
22.0	M	France	Bay of Biscay	Dorel 1986
24.8	M + F	UK	North Sea	Jennings et al. 1998
26.0	M + F		North Sea	Rijnsdorp & Vethaak 1997
27.0	M + F	Holland	Dutch ports	De Veen 1976
28.0	F	France	East and West Channel	Dorel 1986
30.0	M + F	Holland	Dutch ports	De Veen 1976
31.0	F	France	Bay of Biscay	Dorel 1986
32.0	F	France	Douarnenez Bay, Brittany	Deniel 1990
20.8	F	Turkey	Aegean Sea	Kinacigil et al. 2008
22.7	M			
20.4	F	Turkey	Güllük Bay	Cerim & Ateş 2019b
21.5	F	Turkey	Sea of Marmara	Kahraman et al., 2021
18.6	M			

Table 1. Length at first maturity (L_m) of *Solea solea* in different areas along the Mediterranean and Atlantic coasts (Kahraman et al., 2021).

Before the spawning season, adult specimens make spawning migrations, from the deeper areas where they usually live, to reach the spawning

grounds in shallower coastal waters (Koutsikopoulos et al., 1995; FAO 2024).

As for other teleost species, the dynamics of sole reproduction are mainly influenced by both biotic and abiotic parameters. Environmental factors such as salinity, temperature, habitat and diet could influence the beginning of the reproductive season among different regions and different years within the same area (Corti et al., 2024; Kahraman et al., 2021). Moreover, differences in the duration of the spawning season depend on the biological features of each specimen. Older and larger individuals tend to have a longer reproductive period, they begin to spawn earlier and cease later than younger/smaller ones (Ramsay and Witthames, 1996).

Considering the multiple factors involved in determining the reproductive season in sole, it is not surprising that different data have been collected over the years. Indeed, several studies analysed different aspects of the reproductive biology of this species, which differ within the Mediterranean Sea, depending on local characteristics and environmental factors (Corti et al., 2024; Ao El-Aiatt et al., 2019; Assem et al., 2019; Mehanna, 2014; Türkmen, 2003).

Considering the eastern-south part of the Mediterranean basin, the analysis of the frequency-distribution of maturation stages carried out in Abu Qir

Bay (Alexandria, Egypt), showed that the common sole has an extended spawning season, ranging from mid-November to early April (Follesa, M.C. and Carbonara, P., eds. 2019). The peak of the breeding season was observed in December and January (Assem et al., 2019).

In line with the above-mentioned study, in the Bardawil lagoon (North Sinai, Egypt), data on gonadosomatic index (GSI) and macroscopic analysis of gonadal developmental stages indicated that *S. solea* spawned from late autumn to early spring during 2011-2013 and 2016-2017 (Mehanna, 2014; Ao El-Aiatt et al., 2019). In addition, from the end of December, a decrease in GSI was observed concomitantly to the increase in spent stage frequency. Immature and mature stages were found throughout the year, with the highest percentage of immature and mature specimens in June and July respectively (Mehanna, 2014; Ao El-Aiatt et al., 2019).

Differently, data collected between 2000-2001 from İskenderun Bay (Turkey) suggested that the spawning period occurs between April and May, with higher values of GSI in April for both male and female specimens (Türkmen, 2003). In the Gulf of Biscay (Atlantic Ocean), the sole reproduces from January to mid-April, with a peak in January-February (Le Bec, 1983). It starts in February and from December with a peak in April-

May in the Baie de Douarenenez (Atlantic Ocean) and in the south-eastern North Sea respectively (FAO 2024; Bromley, 2003; Ramsay and Witthames, 1996).

A more constant situation has been observed in the Adriatic Sea over the years. In particular, in the central and northern Adriatic Sea, it was observed that spawning usually takes place between November and March (Scarcella, 2013; Froglia and Giannetti, 1985).

In the context of global warming, temperatures increasing may exert the most impactful effects on the reproductive biology of teleosts. Changes could occur in the regulation of the hypothalamic-pituitary-gonadal axis with changes also affecting the reproductive period (Alix et al., 2020).

1.2.1. Reproduction in teleost species

The complex process of sexual reproduction is regulated by the integrated control of environmental parameters and endocrine signals involved in the hypothalamus – pituitary – gonad axis (Figure 4) (Reinecke, 2010).

This neuroendocrine axis is silent till puberty, after which the secretory activity of the hypothalamus stimulates the pituitary secretory activity promoting the gonadal activity (Wootton and Smith, 2014).

Briefly, the hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the adenohypophysis to produce two kinds of

gonadotropins: luteinizing hormone – LH – and follicle-stimulating hormone – FSH. Moving downstream, thanks to the stimulation of these two hormones, gonads start biosynthesizing the steroid hormones – oestrogen, testosterone and progesterone. The last-mentioned triggers the onset of gonadal maturation and gametogenesis (Wootton and Smith, 2015). In addition, sex hormones are also responsible for the development of secondary sexual characteristics (Arcand-Hoy and Benson, 1998).

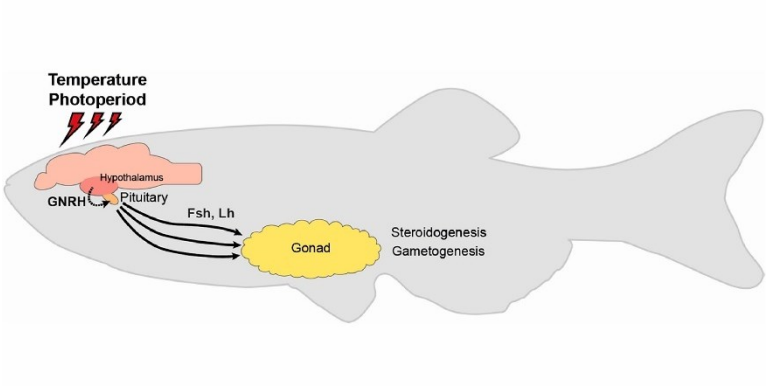


Figure 4. Graphical schematic representation of the hypothalamus-pituitary-gonad axis in teleost (Santhakumar, 2024).

Both female and male specimens secret the same sex steroid hormones: androgens, such as testosterone (T) and 11-ketotestosterone (11-KT), and oestrogens, such as 17β- estradiol and progesterone. However, the amount and processes activated and regulated by these hormones differ between sexes (Schulz et al., 2010; Wotton and Smith, 2014; Nagahama, 1994).

Gametogenesis is similar in all teleost species although it could present different temporal patterns in the timing of sexual maturation, reproductive cycle periodicity and the seasonality of spawning within the same species in different areas (Saborido-Rey, 2016).

Gametogenesis is the process that includes the formation, growth and maturation of gametes in females and males, named oogenesis and spermatogenesis respectively (Wotton and Smith, 2014).

The reproductive cycle of fish is characterized by different phases that mark different stages of gametes development and maturation (Figure 5) (Saborido-Rey, 2016; Brown-Peterson et al., 2011; Assem et al., 2019). The following description of gonadal maturation stages is based on Brown-Peterson macroscopic and histological criteria (Brown-Peterson et al., 2011):

- Immature. In juveniles gonads are small. The ovary has no developed oocytes while the testes are filiform and clear/translucent. We can't distinguish blood vessels. Blood vessels are not visible yet.
- Mature. In mature specimens the HPG axis is completely active and functioning, the individuals can be considered sexually mature and ready to reproduce.

  In mature specimens, it can be distinguished 4 different stages.

- Developing: fish at this stage are characterized by gonadal growth and gametes maturation. Females present ovaries that increase in size and blood vessels become more evident. This stage is also represented by the presence of primary growth oocytes, cortical alveolar, primary and secondary vitellogenic oocytes and no evidence of POF. Males present testis with visible spermatocysts along the lobules. At this stage secondary spermatogonia, primary spermatocyte, Secondary spermatocyte, spermatid and spermatozoa can be present.
- Spawning capable: the main features, in females, are the presence of large ovaries in advanced vitellogenesis, at the tertiary stage (Vtg3), and blood vessels prominent. Moreover, oocytes are visible also macroscopically. Finally, atresia processes of vitellogenic and/or hydrated oocytes may be present. Males present large, firm testes and spermatozoa in the lumen of the lobules and/or sperm ducts. All stages of spermatogenesis can be found (secondary spermatogonia, spermatocytes, spermatids, spermatozoa), spermatocysts are located throughout the testis and the germinal epithelium may be continuous or discontinuous.
- Regressing: females have un-ovulated eggs that undergo the atretic process to be reabsorbed. Ovaries are flaccid, the blood vessels are

prominent, presence of POF and atresia. In males, the remaining spermatozoa are present in the lumen and sperm ducts. Spermatogonial proliferation and germinal epithelium regeneration are common in the periphery of the testis. Testis are flaccid and no milt emission occurs with pressure.

- Regenerating: in iteroparous species, during this time, the gonads go through an important reorganization and preparation for the next cycle. Fish are no more in the reproductive season. Ovaries are small and so blood vessels are reduced. Only oogonia and primary growth oocytes with thick ovarian walls can be observed. Testes are small and threadlike; the lumen of the lobule often does not exist and no spermatocysts are present. Only spermatogonial proliferation occurs.

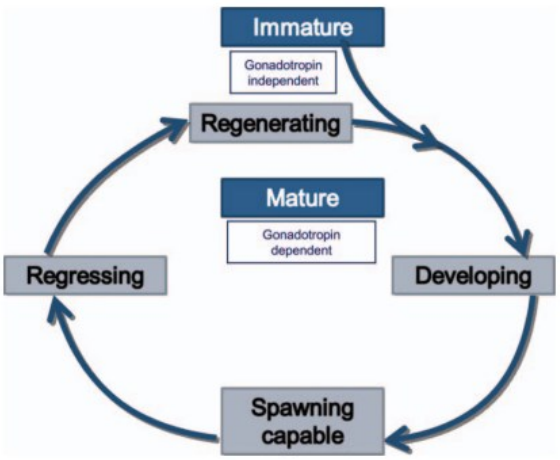


Figure 5. Model of fish reproductive cycle (Brown-Peterson et al., 2011).

1.2.2. Spermatogenesis

Spermatogenesis occurs in testis which usually are paired organs and consist of two compartments: a germinal and an interstitial one which are separated by a basement membrane (Schulz et al., 2010).

Testis can be of two types, lobular and tubular (Figures 6) (Billard, 1986; Bobe and Labbé, 2010). In the lobular type (Figure 6A), the germinal compartment consists of seminiferous lobules that end blindly at the edges, directed towards the vas deferens. Lobules show a progression in the maturation of germ cells, with spermatogonia confined to the testicular periphery and spermatozoa close to the lumen (restricted lobular testis) or spermatogonia may be distributed in the length of the lobules (unrestricted lobular testis) (Bobe and Labbé, 2010; Wootton and Smith, 2014).

In the tubular-type testis (Figure 6B) the germinal compartment is branched and does not conclude at the edges of the tubule. In the internal part, there is a large cavity (lumen) where the spermatozoa are stored (Bobe and Labbé, 2010).

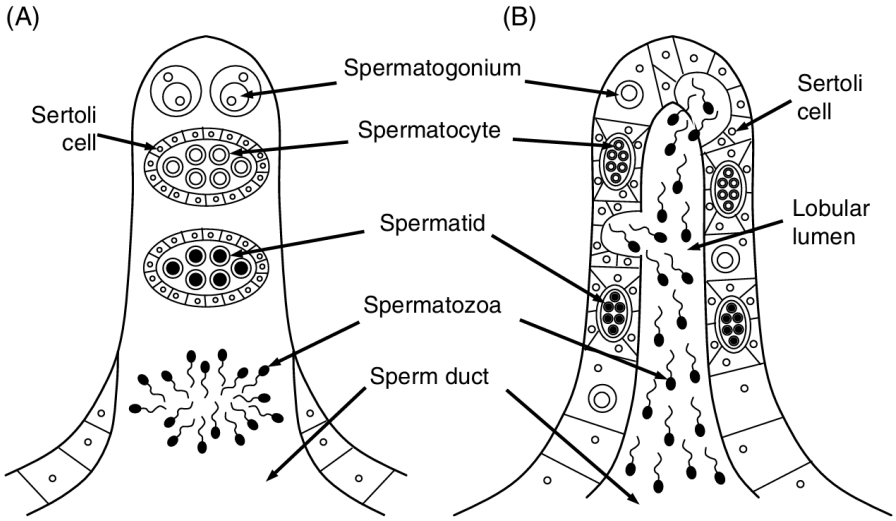


Figure 6. Two most common testicular conformations in teleost. (A) Tubular structure, (B) lobular structure (Wootton and Smith, 2014).

Spermatogenesis in fishes occurs in testicular cysts enveloped by Sertoli cells. The last ones represent one of the somatic cell types involved in the spermatogenesis. In each cyst, the development of germ cells is synchronous (Nagahama, 1994). The developmental process starts from a small number of spermatogonia stem cells that proliferate and generate many differentiated spermatozoa, each containing a haploid, recombined genome (Schulz et al., 2010).

This process has different stages (Figure 7):

1. Proliferation (mitosis): spermatogonial stem cell undergo mitotic divisions to produce spermatogonia. There are two types of these diploid cells, type A and type B. The first one will continue to

proliferate through mitosis, providing a continuous supply of germ cells (Schulz et al., 2010).

2. Growth and maturation (meiosis): spermatogonia type B differentiate into primary (preleptotene) spermatocytes following mitotic division (Schulz et al., 2010). These new cells undergo the first meiotic division resulting in secondary spermatocytes. At this point, the second meiotic division occurs producing spermatids which represent the immature sperm cells.
3. Differentiation (spermiogenesis): in the last phase of spermatogenesis, haploid spermatids go through morphological and structural changes becoming flagellated, motile spermatozoa (Schulz et al., 2010). Indeed, these last transformations include the development of a tail, chromatin condensation and formation of the acrosome essential for fertilization (Schulz and Miura, 2002). Mature sperm cells are finally released into the cyst lumen and eventually into the ducts to reach the external environment in species characterized by an external fertilization process.

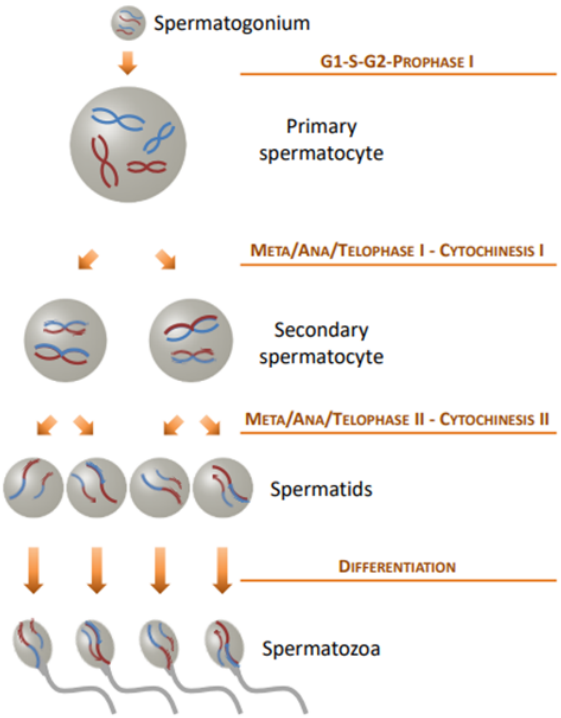


Figure 7. Scheme of spermatogenesis phases (Saborido-Rey, 2016).

During the different phases of spermatogenesis, sex steroid hormones production is regulated by the presence of FSH receptors located on Sertoli cells and LH receptors in Leydig cells which are somatic components of the male apparatus as well (Schulz et al., 2010). Sertoli cells play an important role in functional and structural support to the germ cells during spermatogenesis. They are activated by the FSH hormone. Leydig cells are mainly responsible for androgen (11-ketotestosterone) production, which occurs once they are activated by the hormone LH (Schulz et al., 2010; Zhang et al., 2015).

1.2.3. Oogenesis

The oogenesis represents the series of cytoplasmic and nuclear processes, which lead oogonia to differentiate into egg cells (Lubzens et al., 2010).

Oogenesis takes place in the ovary, which in teleost is a paired organ located within the abdominal cavity, on either side of the mesentery. It is typically elongated and sac-like in shape. It is composed of numerous lamellae containing oocytes, each lamella is suspended within the ovarian cavity which extends to the oviduct (Brow-Peterson, 2011).

After each reproductive cycle, in this area, the mitotic division is stimulated to guarantee reproduction in the following years. Indeed, oogonia, in iteroparous teleost, maintain the capacity to proliferate for the entire life (Menn et al., 2007).

After the initial proliferation, oogonia interacts with the somatic cells of the gonads, specifically the granulosa and theca cells, to form the ovarian follicle (Nagahama et al., 2008). Granulosa cells are the first to interact with oogonia, followed by the formation of the outermost layer composed of theca cells (Reading and Sullivan, 2011).

Ovarian follicles are characterized by two growth phases (Figure 8) (Reading and Sullivan, 2011). The primary growth, or previtellogenic phase, has a slow rate and at the expense of the oocyte itself. It is a

gonadotropin-independent pre-puberty phase where the oocyte is responsible for the synthesis of macromolecules, such as RNA, and proteins (Lubzens et al., 2010; Alix et al., 2020). On the other hand, the secondary growth, also called vitellogenic phase, is faster and characterized by the achievement of a much larger size. The oocyte uptake macromolecules produced by the liver, such as vitellogenin and fatty acids. Vitellogenin is a dimeric phospholipoglycoprotein. All these components will be necessary for embryonic development (Reading and Sullivan, 2011).

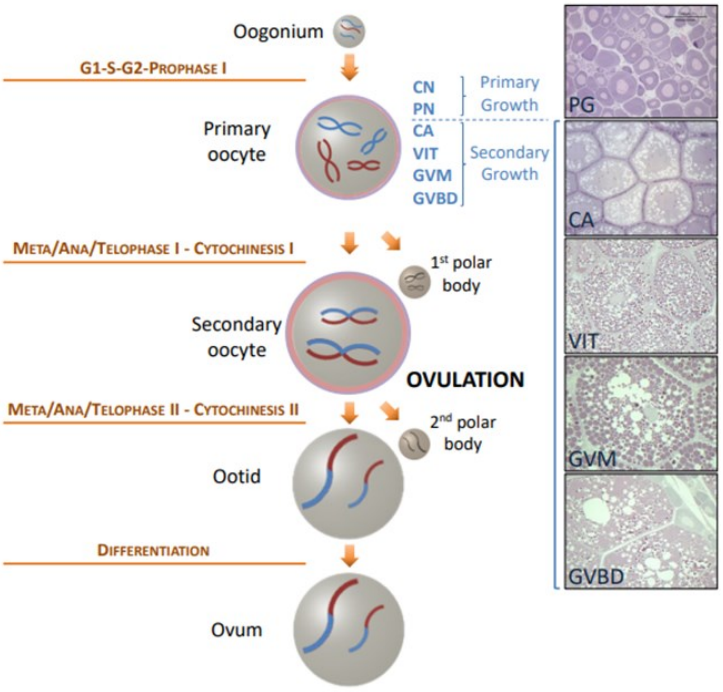


Figure 8. Scheme of oogenesis phases (Saborido-Rey, 2016).

Oogenesis consists, at the first stage, of consecutive mitotic divisions of primordial germ cells (PGCs), which undergo structural changes forming oogonia and nests of oogonia. Within these nests, the cells are connected by cytoplasmatic links and are surrounded by a layer of somatic pre-granulosa cells (Lubzens et al., 2010; Wootton and Smith, 2014).

The differentiation of PGCs into oogonia is gradual and consists of a decrease in cell electron density; the size of the cell and nucleus increases, and the nucleolus assumes a central fibrilar structure (Babin et al., 2007).

Oogonia enter the first meiotic phase and differentiate into primary oocytes (Wootton and Smith, 2014).

The first meiotic division arrests at prophase, after reaching the diplotene stage, in which the formation of the initial follicle takes place.

The primary growth phase is gonadotropins-independent, and it's also called the "perinucleolar stage" and it is characterized by a scarce cytoplasm and a centrally located nucleus, or germinal vesicle, containing a single, large basophilic nucleolus (Wallace and Selman, 1981). The organelles involved are mitochondria, Golgi bodies and smooth endoplasmic reticulum (Wootton and Smith, 2014). Primary growth consists of the accumulation of mRNAs and proteins (Lubzens, 2010) and the formation of brush-like and non-condensed chromosomes (Wallace and

Selman, 1981). In this phase, the synthesis of substances and molecules, fundamental in further development events, occurs. The nucleus increases in size and multiple nucleoli arrange themselves around it. Balbiani bodies (ribosome accumulation zones) and early cortical alveoli also form and, in some species, the oil droplets appear (Wallace and Selman, 1981; Wootton and Smith, 2014). Primary oocytes are characterized by evident nucleoli, highlighting the high protein synthesis activity, rarely observed in other cells, and by the presence of associated theca and granulosa cells, forming a follicular complex (Wootton and Smith, 2014; Babin et al., 2007). The layer of granulosa (GCs) and theca cells (TCs) is thin at this stage (Reading and Sullivan, 2011).

The oocyte extends microvilli towards the granulosa cells to remain in contact and the zona pellucida proteins (ZPs) are synthesized between the oocyte and GCs. This group of proteins will form the protective acellular envelope known as zona pellucida, around the oocyte. The zona pellucida begins to lay down in the space between the oocyte and the granulosa cells, providing a protective barrier for the oocyte (Wootton and Smith, 2014).

Once a specific size is reached, the oocyte goes into secondary growth (gonadotropins-dependent growth) and vitellogenesis begins. Total oocyte volume at spawning mostly depends on the contribution of this phase. The

first structures to appear in the cytoplasm are the yolk vesicles, which progressively fuse centripetally or remain independent (Wallace and Selman, 1981).

Ions and small molecules originating in the liver, pass between the granulosa cells and the oocyte, allowing the important transfer of factors regulating vitellogenesis and maturation. This exchange occurs through gap junctions formed between the microvilli of the cells (oocyte and granulosa cells). Vitellogenin (VTG), the main yolk precursor, is synthesized by the liver, under the control of oestrogen produced by the granulosa cells, and the oocytes uptake and process it into yolk globules (Wootton and Smith, 2014).

This molecule is the major nutritional maternal contribution that guarantees resources during embryo development till the initiation of exogenous feeding (Wootton and Smith, 2014). During the vitellogenesis phase, additional maternal substances, such as lipid-soluble vitamins and immunologically active molecules are stored by the oocyte and also play a vital role. During this stage, the follicular complex undergoes a significant development: the zona radiata increase in thickness, and the accumulation of oil droplets persists.

Another feature typical of this phase is the development of polarity in the oocyte along an animal-vegetal axis. The animal pole is defined by the micropyle and at this point, fertilization will occur, while the vegetal pole may contain the yolk mass during embryo development.

The secondary growth phase represents a massive allocation of resources away from the somatic body to the gonads; after this phase, in some species, gonads represent even 20% of the total body mass. Moreover, this stage is characterized by the highest rate of protein increase during the whole oogenesis (Babin et al., 2007).

Maturation starts when the absorption of VTG ends. This phenomenon is associated with a reduction in the number of nucleoli (Wootton and Smith, 2014). The maturation phase is characterized by the migration of the oocyte nucleus towards the egg's animal pole and the resumption of meiosis, including the rupture of the nuclear membrane of the oocyte, together with the production of the first polar body. Meiosis I is now concluded, and the oocyte enters meiosis II. The nucleus of the oocyte acquires its position at the animal pole of the oocyte and stops at the metaphase of meiosis II. The oocyte keeps enlarging by about 10-15% due to proteolysis of yolk proteins and hydration, microvilli retract, and the egg is ready to be ovulated (Wootton and Smith, 2014).

The end of this process is characterized by the mature oocytes' reaching a large size, 100-200 times larger than the initial cell (Wootton and Smith, 2014). Egg ovulation consists of the release of the oocyte in the ovary lumen, and it is triggered by hormonal stimulation provoked by mating (Babin et al., 2007). In the ovary, the somatic components of the empty follicle are now regarded as post-ovulatory follicles (POFs) which are heading towards their degenerative fate (Wootton and Smith, 2014; Alix et al., 2020).

Based on the species, the ovary of teleost can be characterized by varying numbers of germ cells at different stages of maturation during specific periods (Murua and Saborido-Rey, 2003). According to this criterion, ovaries may exhibit synchronous, group-synchronous, or asynchronous patterns of development.

- Synchronous: all the oocytes develop and ovulate at once. This kind of ovaries is found mainly in semelparous teleost, such as anadromous and catadromous species.
- Group-synchronous: a minimum of two populations of oocytes are recognizable, a larger one and a mixed population of smaller oocytes. The larger oocytes are going to be spawned during the ongoing breeding season, while the latter will be spawned in future

breeding seasons. Such kind of ovary may be found in iteroparous species with a relatively short spawning period during which more ovulations occur.

- Asynchronous: presence of oocytes at different developmental stages. Such ovaries are typical of iteroparous species with an extended spawning season. Yolk accumulation mostly depends on food availability at that moment in the environment.

1.3. Fishing activity

Common sole is one of the most valuable flatfish species and therefore a resource of major commercial interest (Cerim et al., 2019; Sabatini et al., 2018).

In the Adriatic Sea, it is mostly fished in winter and autumn, when the new year class enters the catches, even though its fishing has no proper seasonality (faoadriamed.org). The minimum catch size permitted by law is 20 cm (Regolamento UE 2019/1241).

This species is a target for the "rapido" fishing gear, a bottom-trawl net used solely in the Adriatic Sea (figure 9A), and the "sfogliara" fishing gear (figure 9B). The small-scale artisanal fishery, on the other hand, employs gillnets specifically rigged to catch this species by weighting the sinker line, the lower part of the net (ittico.bmti.it).

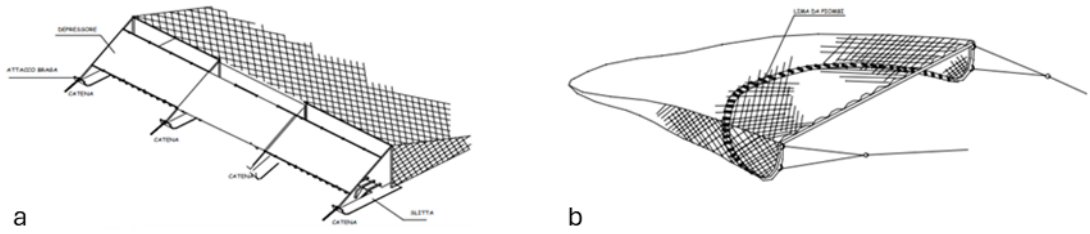


Figure 9. Example of fishing gear traditionally used in sole fishing. (A) Rapido; (B) Sfogliara (isprambiente.gov.it).

This species is one of the most fished species throughout the northern Adriatic Sea (Mediterranean Sea, FAO subdivision GSA 17), accounting for 23% of the landings in FAO-GFCM Area 37, which includes the Mediterranean and Black Sea. This is a factor of particular importance in this basin considering the high fishing pressure on the species, the production of which has been about 2000 tons/year in the last decade in Italy (Sabatini et al., 2018; Fanelli et al., 2022).

This species is subjected to a constant demand from global industries and markets and the amount of the catches appears regular although in recent years it seems to be characterized by a slightly declining trend (figure 10).

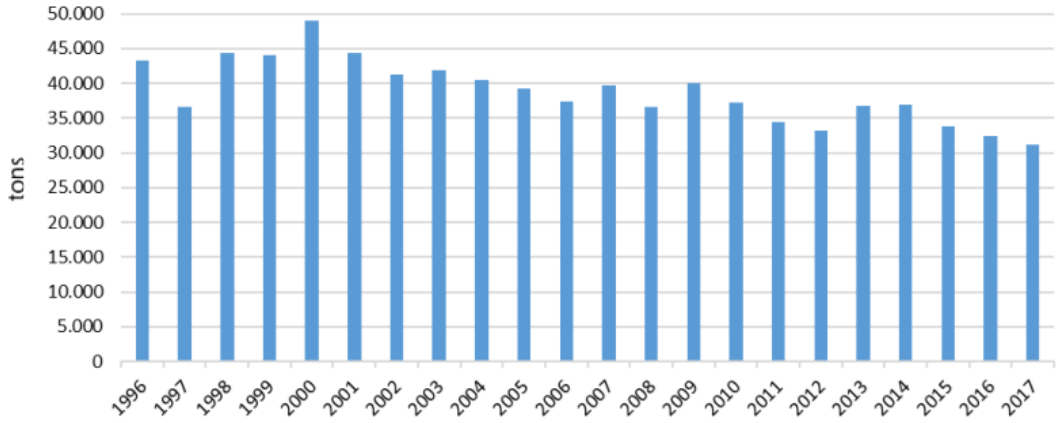


Figure 10. Global sole catches trend from 1996 to 2017 (ittico.bmti.it).

On the other hand, in Italy, the catch shows various fluctuations (figure 11).

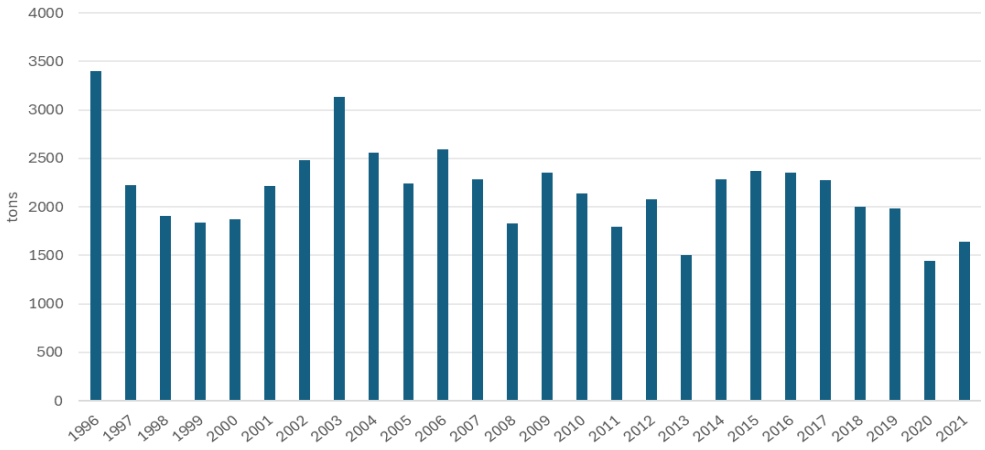


Figure 11. National sole catches trend from 1996 to 2021 (FishStatJ FAO, 2023).

2. AIM OF THE STUDY

The common sole, *Solea solea*, represents a resource of high economic and commercial value in the Mediterranean area, particularly along the Adriatic coasts. Therefore, the sustainable management of sole stock is crucial to minimize the impact of fishing efforts on reproductive stocks and to preserve the population's regeneration potential.

Despite its key role, studies on the sole reproductive cycle focusing on the Adriatic region are still scarce. This gap needs to be filled, especially when considering the actual context of global warming and climate change, which are impacting sea temperatures, seasonal cycles, and habitats' structure. In the Adriatic Sea, which is particularly susceptible to environmental fluctuations, monitoring these variables appears crucial to assess both direct and indirect effects of environmental changes on the biological parameters of fish species.

The present study aims to characterize the reproductive biology aspects of *Solea solea* in the central Adriatic Sea (GSA 17) to contribute in completing the knowledge of this species. Histological analysis of the ovary was used to determine the immature and different mature ovarian stages, while simultaneously testing the accuracy of ovarian stage determination based on macroscopic parameters.

Reproductive parameters, such as sex ratio, macroscopic maturity staging, and somatic indices were assessed to obtain a comprehensive overview of common sole females' reproductive traits. Furthermore, macroscopic and histological size at first maturity were evaluated and compared.

The results obtained characterized the reproductive biology of the common sole providing both valuable information on possible adaptative responses of this species to changing environmental conditions and scientific information to guarantee sustainable exploitation of this fishery resource.

3. MATERIALS AND METHODS

3.1. Sampling activity

Samples collection was performed in cooperation with the Institute for Marine Biological Resources and Biotechnology of the National Research Council IRBIM-CNR of Ancona (Italy), in the context of the Data Collection Framework—Biological Sampling of Commercial Catches (DCF, EU Regulation 2017/1004).

Adult specimens of common sole were collected in the northern part of the Adriatic Sea off the coast of Ancona (GSA 17) (Figure 12) on a monthly basis, from January 2019 to December 2019. All specimens were caught from a professional "rapido" of the fishing fleet in the landing harbour of Ancona.

Successively, samples were processed at the laboratory of the CNR-IRBIM (Ancona) for tissue collection and biometric parameters recording. Samples were not collected in August due to the fish ban imposed by the Italian law.

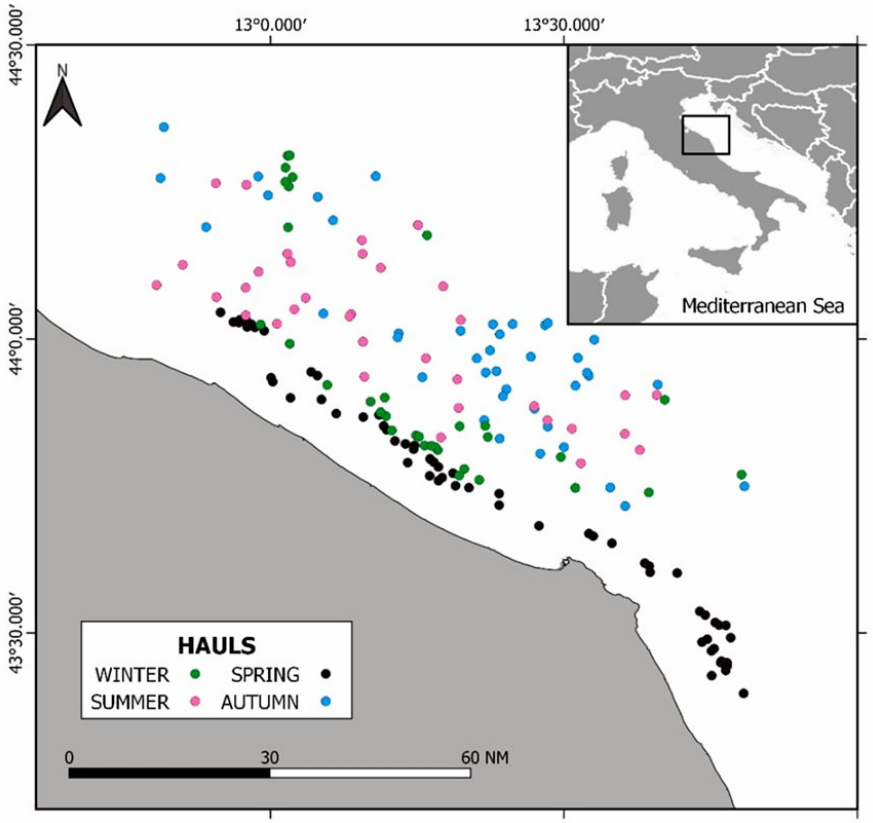


Figure 12. Sampling sites for the study of *Solea solea* in the Central Adriatic Sea in 2019 (Fanelli et al., 2022).

3.2. Collection of biometric data

Female and male specimens (N=311, N=166 respectively) were identified through macroscopic examination of the gonads following the characterization proposed by Brown-Peterson (2011) (Figure 13).

At each sampling time, sole (both females and males) were individually weighted (g) and the total length (TL) (cm) measured from the mouth to the end of the caudal fin, at the nearest cm below, using an ichthyometer (Figure 14). For the purpose of the present study, only

female specimens were further processed and analysed, while males' frequency was recorded to calculate the sex ratio.

Figure 13. Sex identification of sampled Common sole specimens. (a) immature female, (b) mature female and (c) male.

Figure 14. Measurement of sole total length (TL) using the ichthyometer.

Liver and ovaries were sampled from each female and individually weighted (g). From a subsample of females, at each sampling time, (total n=99), a portion of the gonads was collected and stored in Dietrich fixative solution (95% ethanol 30 mL; formalin 10 mL;

glacial acetic acid 2 mL, distilled water 58 mL) to perform histological analysis.

3.3. Indexes calculation

Biometric data were used to calculate the gonadosomatic index (GSI), the hepatosomatic index (HSI) and Fulton's condition factor (K) with the following formula respectively:

$GSI = GW / TW \cdot 100$

where GW represents the gonad weight (g) and TW the total weight (g).

$HSI = LW / TW \cdot 100$

where LW is the liver weight (g) and TW the total weight (g)

$K = TW/TL^3 \cdot 100$

where TW represents the total weight (g) and TL the total length (cm).

3.4. Sex ratio

The sex ratio was determined using the following formula:

$SR = F/F+M$

where F is the total number of females and M is the total number of males.

3.5. Length at first maturity (L_{50})

The length of first maturity (L_{50}) of female specimens was calculated twice, based on maturity determined by both macroscopic and histological analysis.

L_{50} was calculated through the logistic equation (Prager et al., 1989):

$p = [1 + e - r (x - x 50)]-1$

where p denotes the proportion of mature females in each length class, r is the parameter calculated by the software, x denotes the total length, x50 is the total length at which 50 % of the females are mature.

3.6. Histological analyses

Histological analyses were performed at the Laboratory of Developmental and Reproductive Biology at the Department of Life and Environmental Science of the Polytechnic University of Marche (Ancona, Italy). Ovary samples stored in the fixative solution were washed in 70% ethanol and successively dehydrated in ethanol solutions at increasing concentrations (80%, 95%, 100%) and washed in xylene before the inclusion in paraffin.

Samples, embedded in paraffin blocks, were cut in sections of 5 μm in thickness, using a microtome (Leica, RM2125 RTS, Nussloch, Germany) and positioned on glass slides.

Finally, sections were stained with hematoxylin and eosin Y stain (MerckKGaA) and then observed using an optical microscope (Zeiss Axio Imager.A2, Oberkochen, Germany). Images at different magnifications were acquired with a combined color digital camera Axiocam 503 (Zeiss, Oberkochen, Germany).

3.6.1. Identification of gonadal maturation stage

Ovary sections were analysed to establish females' maturity and maturation stages following the criteria proposed by Brown-Peterson (2011) (Table 2).

Phase	Macroscopic features	Histological features
Immature	Small ovaries, often clear and blood vessels indistinct.	Only oogonia and PG oocytes present. Thin ovarian wall and compact ovary.
Developing	Enlarging ovaries, blood vessels becoming more distinct.	PG, CA, Vtg1 and Vtg2 oocytes present. No POFs or Vtg3 oocytes.
Spawning capable	Large ovaries, blood vessels prominent. Individual oocytes visible macroscopically.	Vtg3 oocytes present or POFs present in bach spawners. Atresia of vitellogenic and/or hydrated oocytes may be present.
Regressing	Flaccid ovaries, blood vessels prominent.	Atresia and POFs present. Some CA and/or vitellogenic (Vtg1, Vtg2) oocytes present.
Regenerating	Small ovaries, blood vessels reduced but present.	Only oogonia and PG oocytes present. Muscle bundles, enlarged blood vessels, thick ovarian wall and/or advanced atresia or old, degenerating POFs may be present.

Table 2. Macroscopic and microscopic descriptions of different phases of female teleost reproductive cycle (Brown-Peterson et al., 2011).

3.7. Percentage of agreement between macroscopic and histological methods

The level of agreement (%) between the macroscopic and histological methods to determine ovarian maturation stages into the four stages (developing, spawning capable, regressing and regenerating) was quantified through Choen's kappa (k) statistical measure.

The calculation of the weighted kappa coefficient was performed assuming the four stages were ordered. The interpretation of Choen's kappa coefficient was performed following the criteria proposed by Landis and Koch (1977).

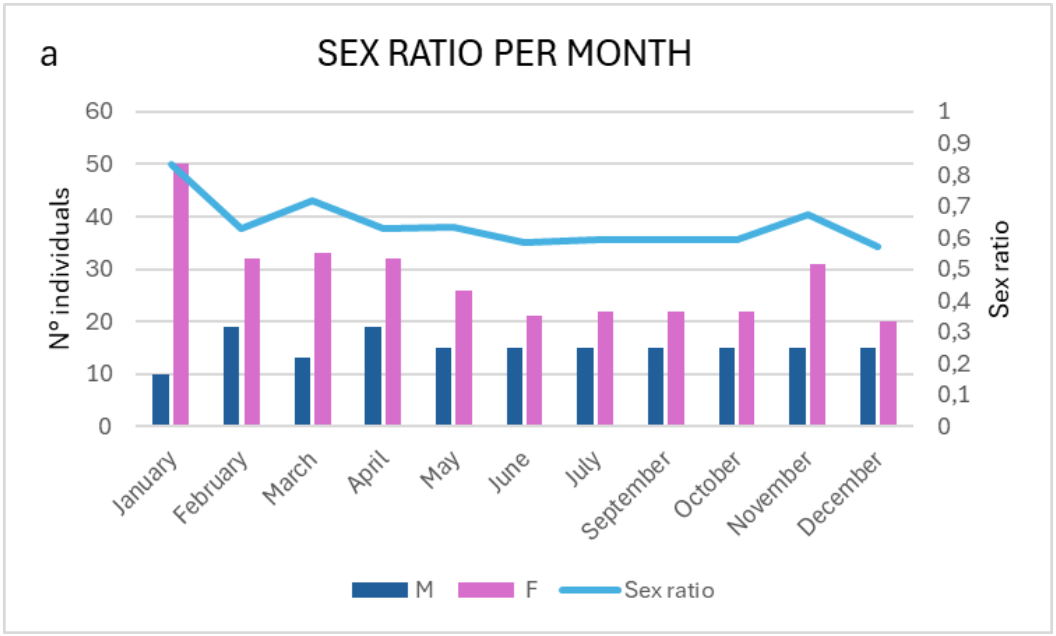
3.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism 8 software. Data collected from biometric measurements (GSI, HSI and K factor) were analysed by one-way ANOVA analysis of variance followed by Tukey's post-test after assessing the normal distribution of data.

4. RESULTS

4.1. Sex ratio

Figure 15 represents the sex ratio for each sampling month (Figure 15a) and length class (Figure 15b) based on the macroscopic observation of gonads.



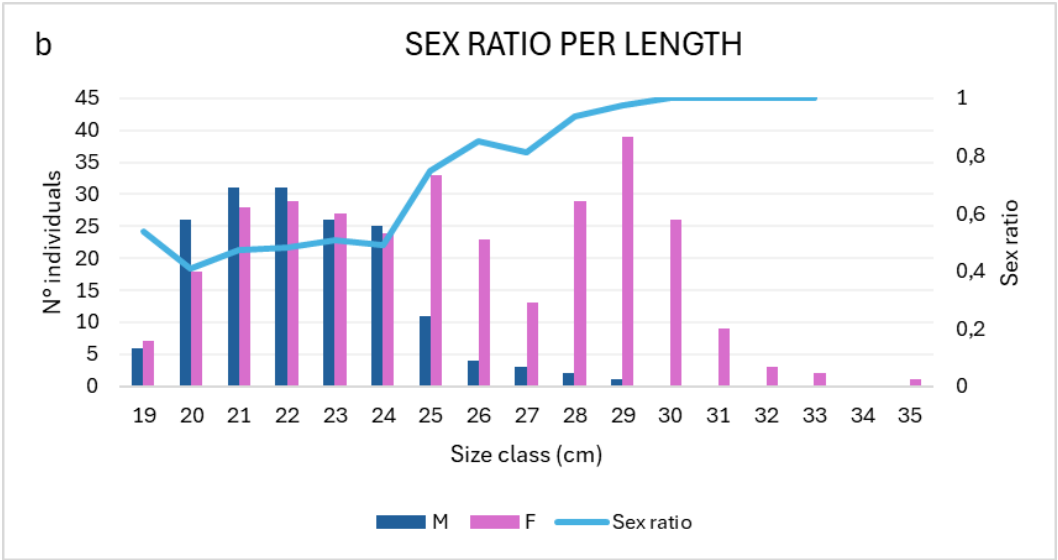


Figure 15. (a) Sex ratio per month, (b) sex ratio per length class (cm).

The monthly trend (Figure 15a) indicated that the sex ratio was unbalanced in favor of females throughout the entire year.

Regarding the sex ratio per length class (Figure 15b), males prevailed in the shorter-length classes while females prevailed in the longer-length classes.

Male specimens were observed in all length classes between 19 to 29 cm while from the 30 cm class to the maximum length observed (35 cm) only females were collected.

4.2. Characterization of the reproductive period of females

4.2.1. Gonadosomatic index (GSI)

The gonadosomatic index (Figure 16) showed significantly higher values in November, December and January compared to the other months ($p \leq 0.05$). From February to September the GSI values remained close to zero while increased from October reaching the highest value in December.

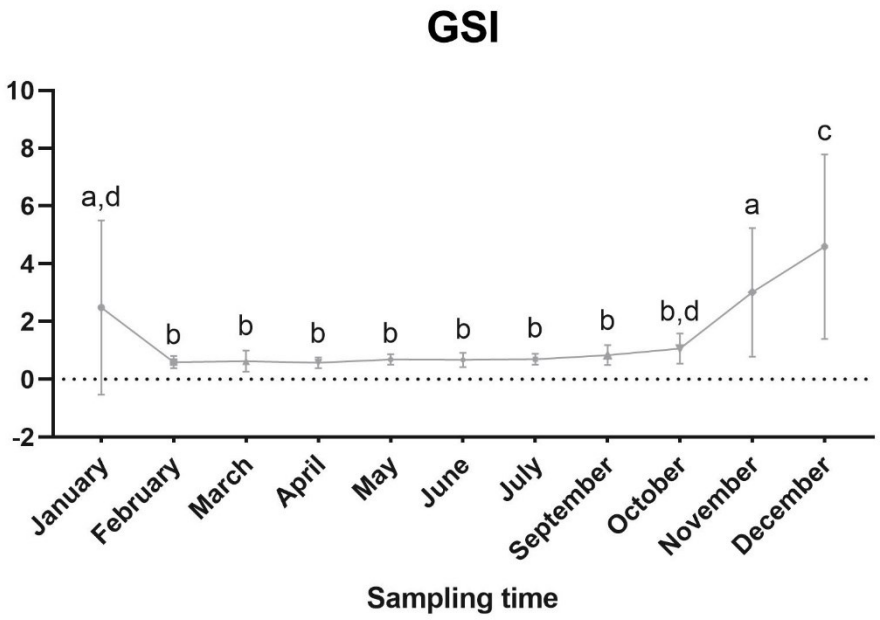


Figure 16. Gonadosomatic index (GSI) values over the sampling months.

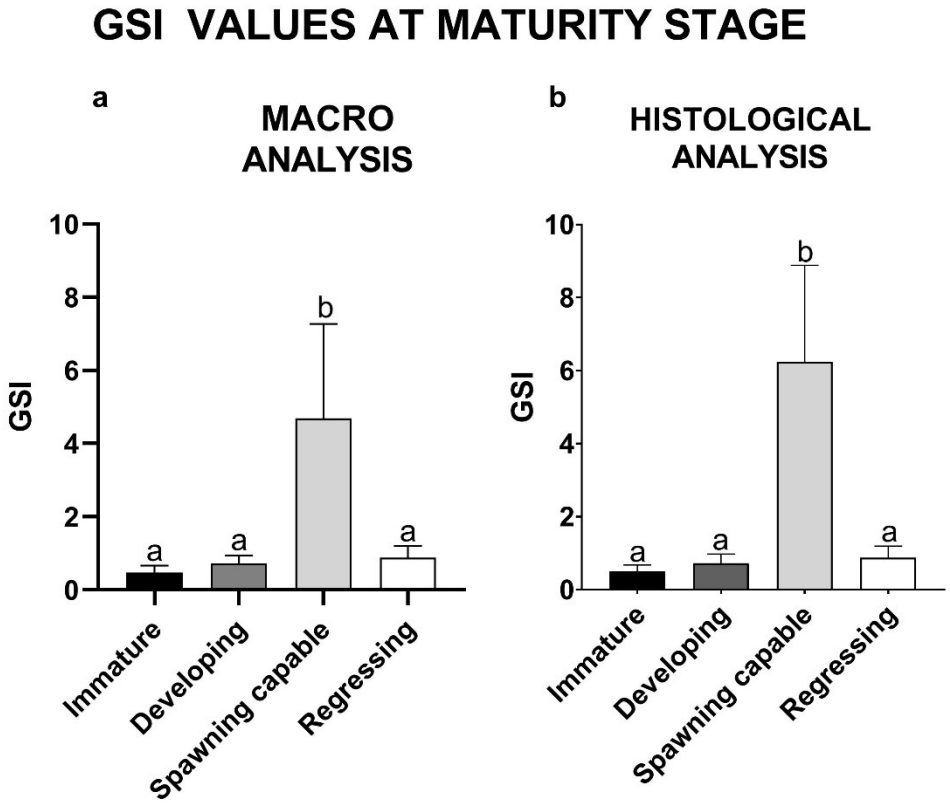


Figure 17. GSI values at each maturity stage. (a) GSI values for samples analysed through macroscopic analysis and (b) GSI values for samples analysed through histological analysis.

GSI values, reported for each maturation stage, showed the same trend between histologically (Figure 17a) and macroscopically (Figure 17b) analysed samples.

The GSI value for the "spawning capable" stage was significantly higher ($p \leq 0.05$) than all other stages (immature, developing and regressing) in both figure 17a and b.

4.2.2. Hepatosomatic index (HSI)

The HSI analysis showed similar values throughout the year (Figure 18). From January to October the values ranged between 1 and 1.5. Differently, in November and December HSI had the highest values significantly different ($p \leq 0.05$) compared to other months.

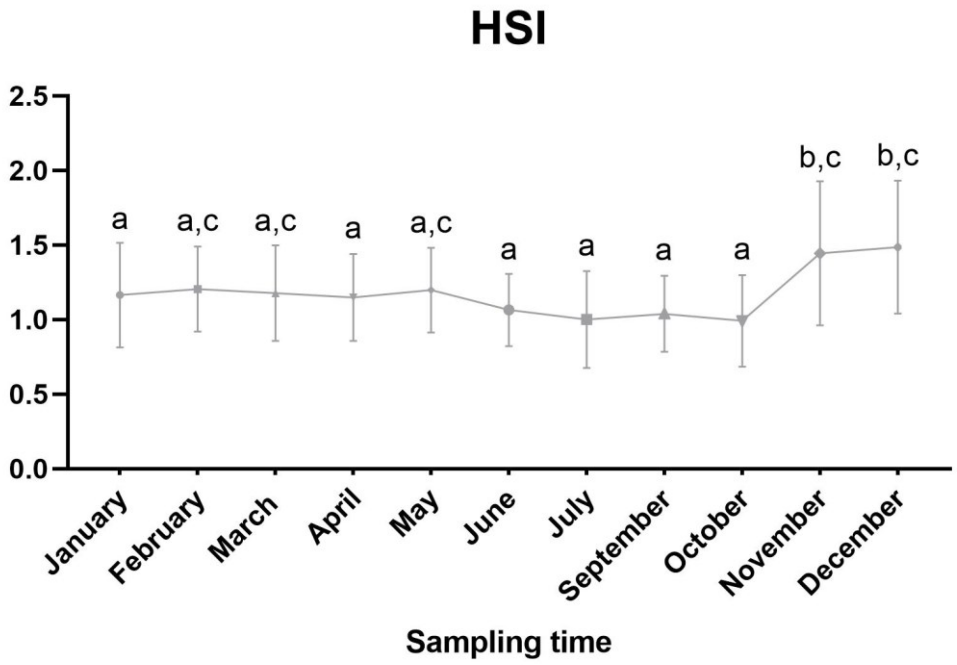


Figure 18. Hepatosomatic index (HSI) values over the sampling months.

4.2.3. Fulton's condition factor (K)

Fulton's condition factor values did not show any evident trend, they fluctuated between values of 0.8 and 0.9 during all the sampling months (Figure 19).

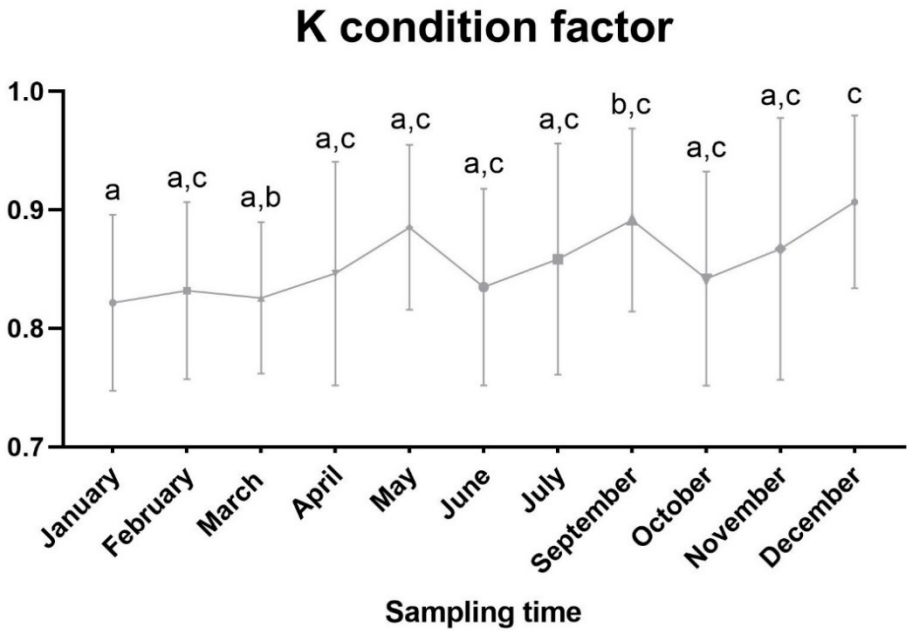


Figure 19. Fulton's condition factor values over the sampling months.

4.3. Histological analysis

4.3.1. Identification of gonadal maturation stage

Ovary maturation stages were identified within the following categories: immature, developing, spawning capable and regressing (Figures 20 and 21).

Figure 20. Representative *Solea solea* ovary images at different reproductive stages macroscopically determined following the Brown-Peterson criteria. (a) immature; (b) developing and regenerating; (c) spawning capable; (d) regressing. Black arrow = onset of vascularisation; green arrow = individual oocyte; red arrow = blood vessel prominent. Image b shows a gonad potentially in the developing or regressing stages which do not exhibit any distinctive macroscopic characteristics (Image by S. Colella).

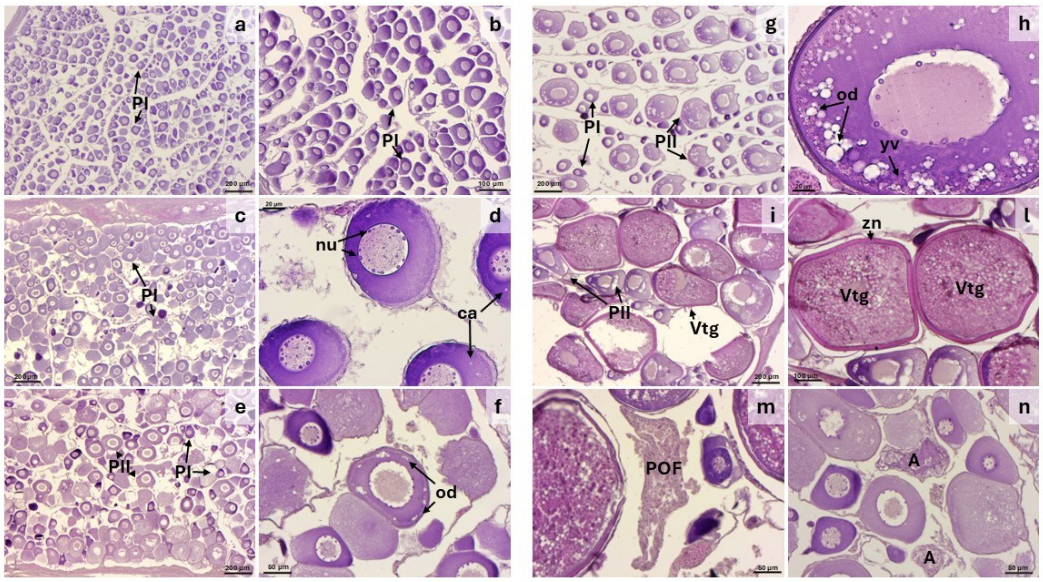


Figure 21. Representative *Solea solea* ovary histological images at different reproductive stages determined following the Brown-Peterson criteria. (a, b) immature; (c, d) early developing; (e, f) late developing; (g, h) early vitellogenic; (i, l) spawning capable; (m, n) regressing; PI = primary oocyte; PII = secondary oocyte; ca = cortical alveoli; nu = nucleolus; od = oil droplet; yv = yolk vesicle; Vtg = vitellogenic oocyte; POF = postovulatory follicle; A = atretic oocyte. Images a, c, e, g, i: scale bar 200 µm; b, l: scale bar 100 µm; f, m, n scale bar: 50 µm; d, h scale bar: 20 µm.

Considering the distribution of reproductive stages throughout the year, a comparison was made between macroscopically and histologically classified samples.

Figure 22a illustrates the distribution of gonadal development stages over the sampling months classified according to the macroscopic observation. Immature females were present in all the sampling months except September and December. Females at the developing stage were present in all the months while spawning capable females were recorded only from September to January.

Figure 22b presents the distribution of gonadal development stages over

the sampling months based on histological analysis. Females in the immature stage were present in January, March, May and July. Similarly to Figure 22a, the developing stage was observed in all sampling months while the spawning capable stage was present from September to January.

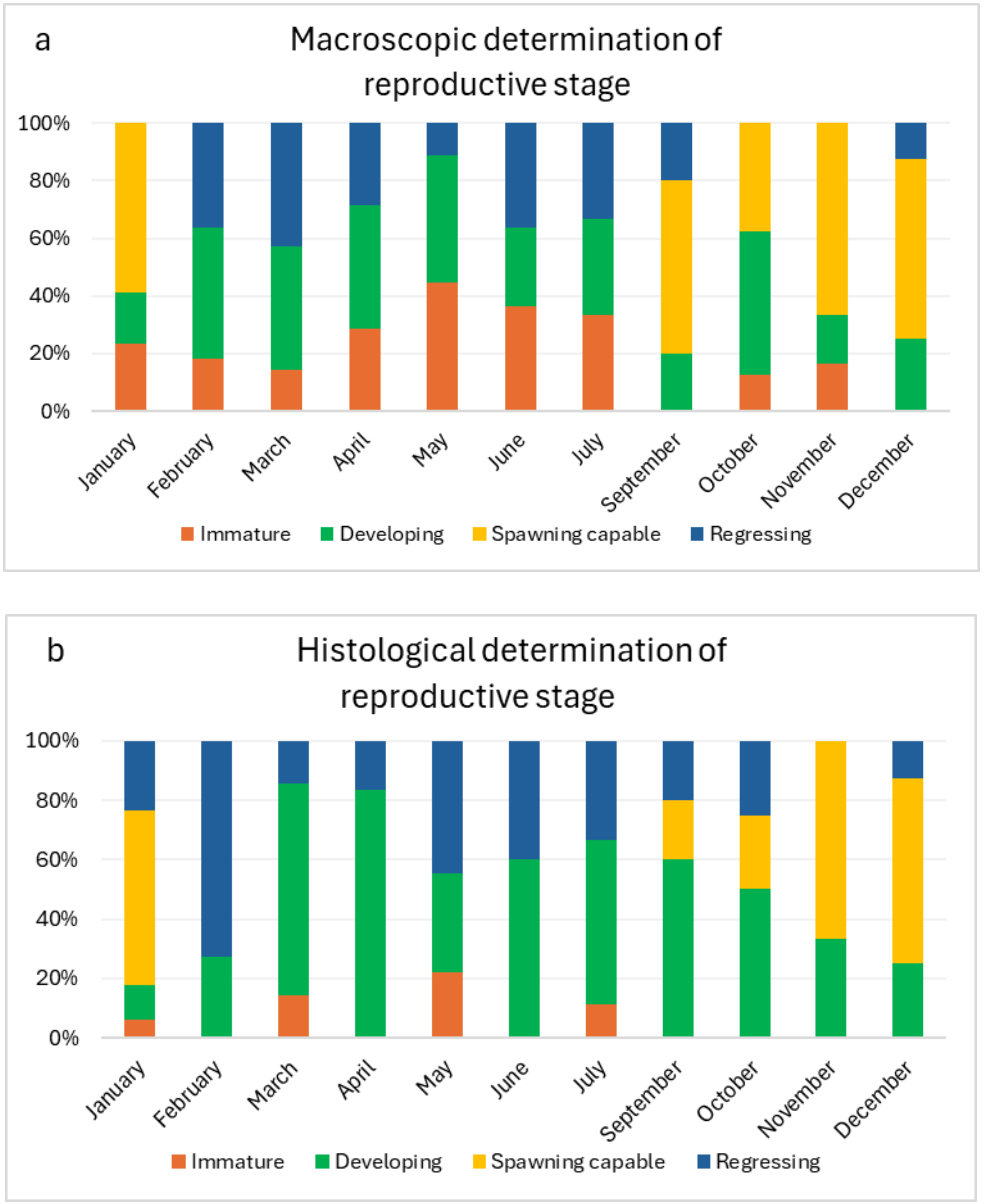


Figure 22. Comparison of the distribution of reproductive stages over different months classified by (a) macroscopic criteria and (b) histological criteria.

4.3.2. Percentage of agreement between macroscopic and histological methods

The percentage of agreement, in gonadal stages identification, between the two different approaches (macroscopic and histological) was 72.63%. Considering the histological classification as the reference baseline, the percentage changed according to the maturity stage: the highest similarity percentage was registered for spawning capable stage (88.0%), conversely, the lowest values were recorded for immature stage (26.3%). In the cases of developing and regressing, the similarity percentage was 81.3% and 84.2%, respectively. Cohen's k was 0.62 (95% confidence interval:−0.505–0.06), which corresponds to a "substantial" level of agreement.

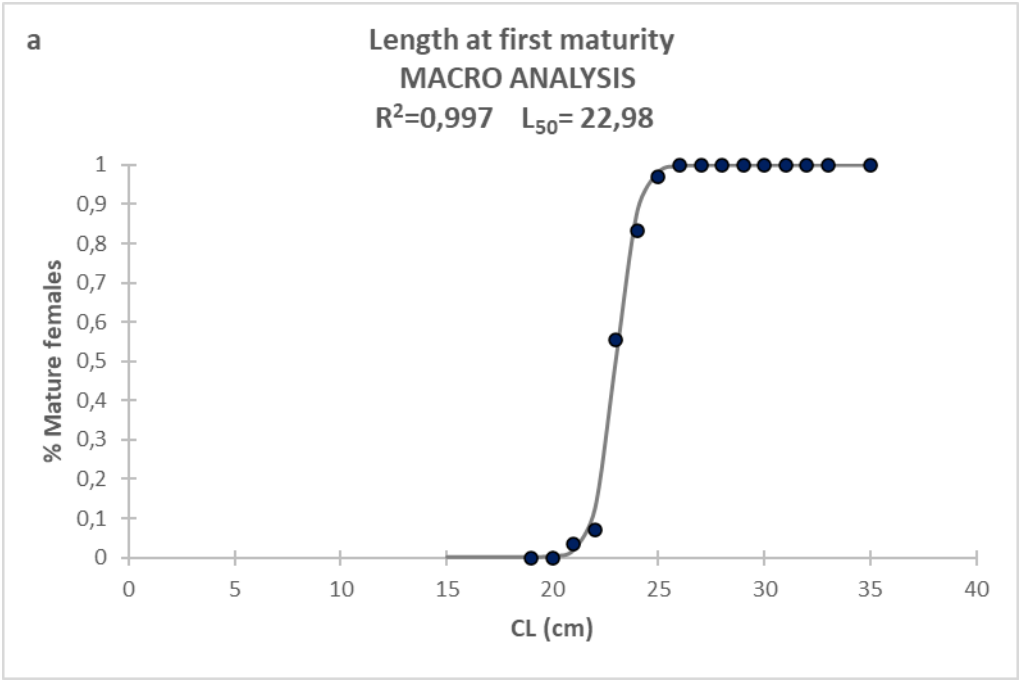
4.4. Length at first maturity (L_{50})

The length at first maturity (L_{50}) was calculated based on females mature, and immature identified using both macroscopic and histological approaches (Figure 23). Mature specimens included developing, spawning capable, regenerating and regressing stages.

The length at first maturity by macroscopic analyses was calculated on a total number of 311 individuals. As shown in the graphical

representation (Figure 23a), the length at which 50% of the population reached sexual maturity was 22.98 cm.

A different result was obtained with the histological analyses of the gonads of 96 females. Figure 23b indicates that the size of first maturity based on the histological evaluation of maturity was reduced to 20.67 cm.



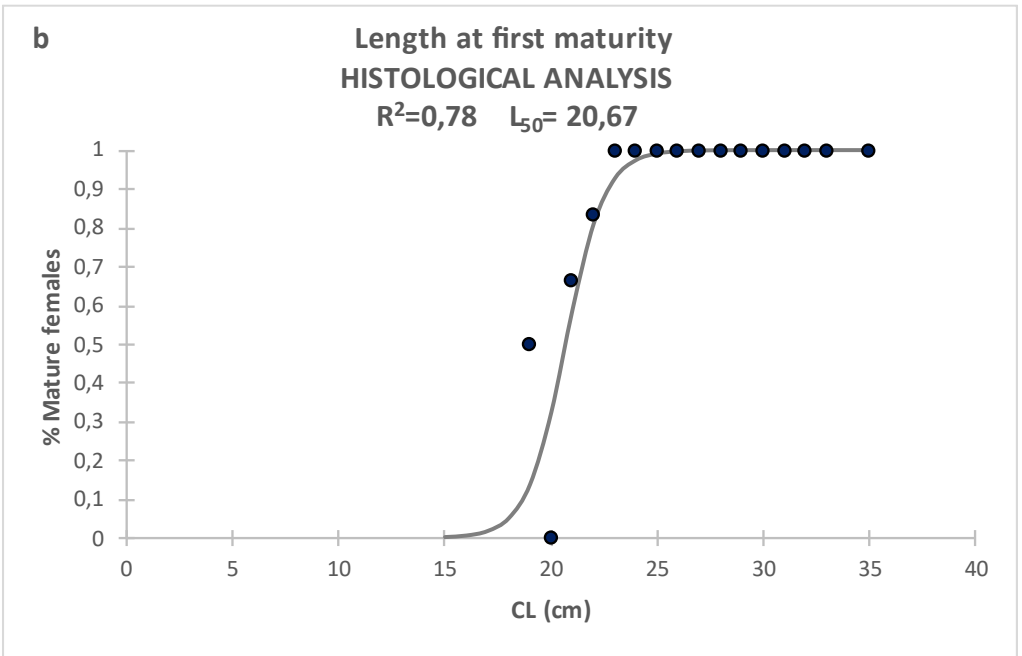


Figure 23. Length at first maturity calculated based on gonadal macroscopic (a) and histological (b) analyses, (N=311, N=96 respectively).

5. DISCUSSION

Effective management of fishery activity requires reliable and in-depth knowledge of the reproductive biology of the species. These information are essential in particular in this historical period characterized by commercial species that are subjected to the dual pressure exerted by the ever-increasing fishing effort and the irreversible climate changes affecting the marine environments.

Due to its commercial relevance as fishery resource, the common sole, *Solea solea,* is included in stock assessment surveys carried out in different areas (FAO-AdriaMed project, 2025; Scarcella, 2013). Over the years, several data have been collected on the reproductive biology of the common sole within the Mediterranean Sea, differently, only a few were focused on the Adriatic Sea (Türkmen, 2003; Kahraman et al., 2021; Cerim and Ates, 2020; Daban et al., 2021).

This thesis aimed to describe the annual reproductive cycle and the L_{50} of the common sole as a key strategy for monitoring the population dynamics and gaining a deeper understanding of the species' reproductive biology.

The determination of the correct gonadal maturation stage in females allows the identification of the reproductive period, estimation of the L_{50}, assessing

the reproductive potential, and consequently plan sustainable management and conservation strategies based on reliable biological informations.

Histological analyses were associated with the macroscopical identification of gonadal stages to verify its validity in identifying the actual stage based on morphological features of the ovary.

A first sign that provides information on both the health status and the reproductive success of a population is the sexes distribution. The sex ratio observed in this study was in favor of female specimens (1:1.87, males:females). In general, a sex ratio with a predominance of females over males is associated to a stable population, since the predominance of female specimens lead to a high fecundity with the potential production of a high number of eggs (Richter and Goos, 1982).

Over the years, the existing literature on the common sole revealed various situations among different areas of the Mediterranean Sea, the predominance of both males (Kahraman et al., 2021), females (Cerim and Ates, 2020), or a balanced ratio between sexes was recorded (Türkmen, 2003; Scarcella, 2013; FAO-AdriaMed project, 2025). However, to understand how the sex ratio will influence the reproductive pattern of a population, it should be analyzed considering the sex distribution of different size-class range and over the year.

Data collected in the present study, indicated that females were predominant in the bigger length classes and during all the year. Differently, males reach the maximum size of 29 cm. This population structure may be related to both a difference between sexes' lifespan and growth rates.

Past studies supported the difference in growth between sexes, it is noted that males grow relatively faster than females in the first years of life (Türkmen, 2003; Ofelio et al., 2020) but reach a shorter length (Türkmen, 2003; Kahraman et al., 2021; Cerim and Ates, 2020; Daban et al., 2021; Vallisneri et al., 2000). Indeed, in the study conducted by Türkmen (2003) in the İskenderun Bay, sole males and females showed a total length range of 19-29.5 cm and 19-35 cm respectively. In the Sea of Marmara the highest length reached by males and females was 27 cm and 31 cm respectively (Daban et al., 2021).

Concerning the possible age gap between sexes, literature data highlighted the males absence in older age class. According to the study conducted in the Southern Aegean Sea, females were observed to reach the 8 years old while oldest males have 7 years (Cerim and Ates, 2020), while in Ismir Bay (Turkey) an absence of male individuals was observed in the older age group (Hoşsucu et al., 1999). Considering the possible errors in age determination, a one-year difference may not be significant. Therefore,

literature data support the assumption of a reduced growth in males compared to females.

Indipendenlty of different growth rates, both sexes have been characterized by a decrease of size over the years within the Mediterranean area at least (Daban et al., 2021; Türkmen, 2003; Cerim and Ates, 2020). In the upper Adriatic Sea, for example, during 1996-1997 the largest female and male measured 37 cm and 34 cm respectively (Vallisneri et al., 2000). These values are significantly higher than those observed in the following years (Kahraman et al., 2021; Cerim and Ates, 2020; Türkmen, 2003) and in the present study where the longhest females reached 35 cm while the longest males measured 29 cm.

This reduction in sole size can be associated to the pressure exerted by commercial fishing. Intensive fishing practices target larger individuals, leading to a decrease in the average body size over time. This phenomenon, known as “fishing-induced evolution”, has been documented in various species (Law, 2000; Kuparinen et al., 2016).

Gonadal development, other than structural and functional parameters, usually involved the growth of gonad size and weight especially in female specimens. For this reason GSI values are used as a valid index to identify

the spawning season, since it is positive correlated with the gonadal weight (Ungaro, 2008).

Based on the GSI values, the present study identified the reproductive period of common sole in the mid Adriatic from October to January, with a peak in December which corresponded to the highest GSI values. This result partially differed from what observed from the past literature. More extended spawning seasons were reported for the common sole within the same Ariatic area (GSA 17) in different years. Indeed, based on the macroscopic evaluation of the gonads Scarcella (2013) and Maiorano et al, (2019) indicated sole's reproductive period between November and March, and from September to April repesctively.

The shortening of the spawning period within the same area, could be related to changes of the marine environment related to global climate change, since long-term temperature variations can have a significant impact on spawning areas or the timing of spawning events (Alix et al., 2020). In marine environments the optimum temperature for spawning ranges from 8 to 12 °C (FAO 2025), suggesting that the differences recorded in the spawning times may be attributable to climate change-induced variations in water temperature. Due to rising temperatures, the period of the year in which optimal conditions for spawning are reached

has changed. This explanation is particularly plausible considering that the Adriatic Sea is particularly susceptible to environmental fluctuations (Moulin et al., 2024).

However, data collected in the past could be altered due to the evaluation method used to define the ovary maturation stages and the reproductive season. Macroscopic identification of gonadal stages represents a commonly used procedure based on the evaluation of gonad size and colour as discrimination criteria. Moreover, it represents a rapid, low cost procedure that allow the examination of a large number of samples. However, it is affected by the subjectivity of the operators and the limitation of the method itself that can provide altered results.

The present study highligheted the limitation of the macroscopic method compared to histological analyses in gonadal stage classification. In particular, the lowest percentage of accordance between the two methods was observed for the discrimination of immature females from mature developing/regressing speciemens.

Females classified as immature and/or in the regressing stage using the macroscopic protocol were identified in developing stage after the histological analysis. This divergence occured since size and color of the gonads are not real a discriminating features between an immature and

developing ovary which are mainly characterized by functional and structural changes, rather than an actual increase in size.

This error of judgement is crucial, since a wrong classification of immature speciemens influences the calculation of the L_{50} (the length at which 50 % of specimens is mature) on which conservation measures and fishing regulations are based (Mehanna, 2014). In the present study the L_{50} was calculated based on both macroscopic and histological evaluation of the ovary. Macroscopic analysis indicated a 22.98 cm L_{50}, while the microscopic analysis led to a L_{50} of 20.67 cm. As expected, the higher value obtained from the macroscopic observation is related to an overestimation of individuals defined macroscopically as "immature".

Unfortunately, data relative to the L_{50} of female common sole are scarce for the Mediterranean population. Studies conducted over different years in the Sea of Marmara, in Bardawil Lagoon and in Alexandria coasts (Egypt) determined female L_{50} values of 21.9 cm, 20.1 cm and 15.87 cm, respectively (Daban et al., 2021; AO El-Aiatt et al., 2019; Mehanna et al., 2015). In the Adriatic Sea, Follesa and Carbonara (2019) reported a L_{50} of 25.8 cm for females. The comparison between both the histological and macroscopical L_{50} values obtained in the present study with the L_{50} reported by Follesa and Carbonara (2019), suggested a possible reduction of the

length at first maturity of common sole from the central Adriatic area. This trend usually reflects a common strategy adopted by different species to face different external threats ensuring the production of a sufficient amount of eggs and potential offspring which will represent the next generation. These mechanism could be triggered by several factors, including the increased fishing pressure, which may favor individuals that mature earlier to ensure reproduction before being caught (Kuparinen et al., 2016).

The current minimum catch size for this species in the Adriatic Sea is 20 cm which still allow the limitation of catching immature female specimens. However, considering the past decreasing trend of L_{50}, and the actual values observed, this limit has to be constantly monitored over time.

Finally, the monitoring of reproductive patterns should be associated to the evaluation of sole health condition. The simplest and most direct way to gather information on individuals' energy reserves, welfare status and a species' reproductive biology is through the analysis of somatic indexes.

In the present study, HSI values collected over the year, gave important information on the hepatic energy storage (lipid accumulation).

In female specimens energy reservoir also give information on the reproductive success, related to lipid mobilization during vitellogenesis

(Lloret et al., 2013; Rizzo and Bazzoli, 2020). In the present study similar values of HSI were observed during the sampling year suggesting that sole may accumulate energy reserves independently of the reproductive cycle. In fact, sole, as an “income breeder” species, feeds throughout the year and use their current intake to pay for a reproductive attempt (Stephens et al., 2009). The higher values of HSI at the beginning of the reproductive period was related to a higher lipid accumulation related to the higher energy request for vitellogenin synthesis (Rizzo and Bazzoli, 2020).

Similar to HSI, Fulton’s condition factor is another parameter used to evaluate the general well-being of a species. (Rizzo and Bazzoli, 2020; Lloret et al., 2013). Although this index does not appear to be associated with reproduction, its constant trend, observed in the present study, suggested the maintenance of a good health status throughout the year. This is a condition required to successfully complete the reproductive activity.

6. CONCLUSION

The present study provided the first histological characterization of the common sole reproduction in the central Adriatic Sea. Due to the high commercial interest on this species, studies on the length at first maturity and the reproductive cycle are fundamental for both short- and long-term management perspectives.

Based on the results collected, long-term monitoring program are still necessary to determine whether the spawning strategy of this species are changing in response to the present context of increasing fishing effort and environmntal changes.

In conclusion, it would be necessary to integrate traditional methods of macroscopic monitoring with more specific analyses, such as histological examination.

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