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Reproduction of *Merluccius merluccius* (Actinopterygii: Merlucciidae) from the northern Atlantic coasts of Morocco based on histological analysis of gonads

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Summary: The hake (*Merluccius merluccius*) fishery occupies an important place in the Moroccan trawl fishery. Despite the ecological and commercial value of this species, the reproductive biology of European hake populations in Moroccan coastal waters has been little studied. Here, we describe the seasonal variations of gonad histology of hake collected from August 2017 to August 2018 along the northern Atlantic coast of Morocco. The histological changes were compared with macroscopical changes, and the body length at first maturity (L50) was calculated. The results revealed differences between macroscopic and histological maturity staging of the gonads. The L50 was estimated to be 34.7 cm for females and 28.6 cm for males (histology-based) and 30.6 cm for females and 26.8 cm for males (macroscopy-based). In addition, the observations of ovarian maturity indicated the presence of a protracted spawning season throughout the year with a peak around January and in midsummer.

Keywords: *Merluccius merluccius*; Atlantic; Morocco; histology; hermaphrodite; reproduction; gonad.

Reproducción de *Merluccius merluccius* (Actinopterygii: Merlucciidae) de las costas atlánticas norte de Marruecos a partir del análisis histológico de gónadas

Resumen: La merluza (*Merluccius merluccius*) ocupa un lugar importante en la pesca de arrastre marroquí. A pesar del valor ecológico y comercial de esta especie, la biología reproductiva de las poblaciones de merluza europea en las aguas costeras marroquíes ha sido poco estudiada. Aquí, describimos las variaciones estacionales de la histología de las gónadas de la merluza recolectada desde agosto de 2017 hasta agosto de 2018 a lo largo de la costa atlántica norte de Marruecos. Los cambios histológicos se compararon con los cambios macroscópicos y se calculó la longitud corporal en la primera madurez (L50). Los resultados revelaron diferencias entre los estadios de madurez macroscópica e histológica de las gónadas. El L50 se estimó en 34,7 cm para las hembras y 28,6 cm para los machos (basado en la histología) y 30,6 cm para las hembras y 26,8 cm para los machos (basado en la macroscopía). Además, las observaciones de madurez ovárica indicaron la presencia de una estación de desove prolongada a lo largo del año, con un pico alrededor de enero y en pleno verano.

Palabras clave: *Merluccius merluccius*; Atlántico; Marruecos; histología; hermafrodita; reproducción; gónada.

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INTRODUCTION

The European hake (*Merluccius merluccius,* Linnaeus, 1758) is the economically most important demersal fish species in the Atlantic and the Mediterranean Sea (Murua 2010). Its geographical distribution extends along the Atlantic coasts of Europe and Africa, from the northwest of Iceland-Norway in the north to Mauritania in the south, and in the western Mediterranean sea (Lloris et al. 2005). In Morocco, the European hake is found on sandy, sandy-muddy and muddy bottoms from the Strait of Gibraltar to Sidi Ifni (29°N) (INRH 2019).

The results of an FAO/CECAF (2017) survey showed that the stock of European hake in Morocco is overexploited due to increasing fishing mortality of juveniles. In fact, abundance indices decreased by 50% from 2009 to 2019 (INRH 2019). To develop a sustainable strategy of stock management, more information regarding the reproductive biology of common hake living along the Moroccan coasts is needed.

The reproductive biology of *M. merluccius* has been studied in several regions along the Atlantic coast (Domínguez-Petit et al. 2008, Costa 2013). However, for the populations of *M. merluccius* living along the Moroccan coast, to date almost no information is available (El Habouz et al. 2011). Since the distribution of the species can influence the reproductive biology, especially the timing and duration of the breeding season (Domínguez-Petit et al. 2008, Khoufi et al. 2014), findings obtained from other regions may not apply to the Moroccan hake populations. In particular, detailed knowledge on the maturity size and annual breeding cycle of Moroccan hake would help to improve the management of this valuable resource.

To investigate the reproductive biology of fish species, a histological analysis of gonad development is the approach most commonly used. It is universally known and consolidated (Tyler and Sumpter 1996). For Moroccan *M. merluccius*, however, a histological examination of the gonads over the annual cycle is not available to date.

The present study aimed to investigate the reproductive biology of *M. merluccius* on the northern Atlantic coasts of Morocco. More specifically, the study aims (i) to describe the seasonal changes of gonad histological and macroscopical appearance, (ii) to assess differences between males and females in gonad maturation, (iii) to relate gonad seasonality to changes in somatic growth and condition, and (iiii) to determine body length at first maturity.

MATERIALS AND METHODS

Biological material

The fish used in the present study were collected from the area between Asilah (35°28'10.387"N, 6°2'19.111"W) and El Jadida (33°15'14.321"N, 8°30›2.401»W) (Fig. 1), where the stock of *M. merluccius* is exploited (El Bouzidi et al. 2022). The fishes were collected monthly from the catch of local trawler fisheries. A total of 1780 fishes were collected between 2017 and 2018 (23 months). For each individual, total length (TL) to the lowest half cm, total weight (TW) to 0.1 g and gonad and liver weight to 0.01 g were recorded. In addition, sex and stage of maturity were estimated from macroscopic examination of the gonads.

Histological techniques

374 gonads were fixed immediately after dissection. The gonads were fixed for at least 24 h in Davidson solution (Fournie et al. 2000). Then, the tissues were dehydrated by a gradual series of ethanol (ethanol 70°, ethanol 95°, absolute ethanol), moved to xylene, and then embodied in low-melting kerosene (raised to 56°C-58°C). Two 2 µm sections were prepared from each gonad and stained with haematoxylin-eosin using an automated stainer of the brand MEDIZINTECHNIK MEDITE.

Histological examination of gonads

Histological slides were examined under an optical microscope. Readings were taken three times: twice by the same person followed by an independent examination by a second person.

Microscopic classification of *Merluccius merluccius* ovaries and testes

The germ cell stages and the general state of the gonadal maturity were classified using the criteria of Dietrich et al. (2009) and Murua and Motos (2006).

M. merluccius is a partial spawner with asynchronous germ cell development (Murua 2010). This means that the gonads contain all germ cell maturation stages; only their relative frequency differs between the various gonad maturation stages (Table S1). In the ovaries, in addition to the maturing oocytes, postovulatory follicles and atretic oocytes can also be observed.

Macroscopic classification of *Merluccius merluccius* ovaries and testes

For the macroscopic staging of the gonads, the criteria established by Holden and Raitt (1974), of fractional spawners were applied (Table S2). Since the resting stage is macroscopically similar to the maturing stage, the two stages were grouped together. From macroscopic stage 3 on, both sexes were considered to be mature.

Sex ratio

The sex ratio was calculated for the whole sample pool (n=1780) as well as per length class (1 cm). The variation of the sex ratio according to the length was defined as the proportion of males and females by length class in the whole sampled population and the monthly sex ratio was obtained by dividing the number

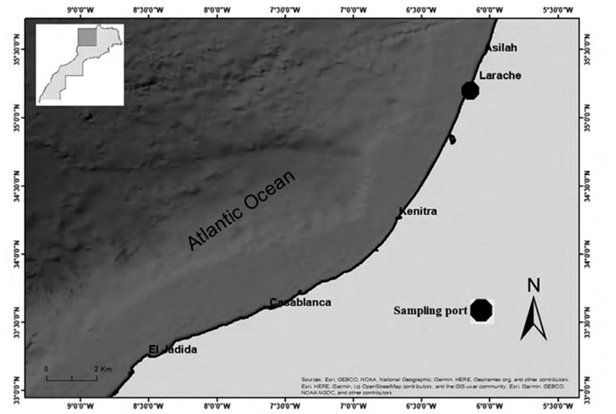

Fig. 1. – Map showing the study area between Asilah (35°28’10.387”N, 6°2’19.111”W) and El Jadida (33°15’14.321”N, 8°30’2.401”W).

of females and males by the total number of individuals per month.

Gonadosomatic index (GSI)

The gonadosomatic index of the whole samples (n=1780), was calculated as a percentage of the gonad weight in relation to the TW of the fish, $GSI = [\frac{GW}{TW}] \times 100$ (Barber and Blake 2006).

Body length at first maturity

To estimate body length at 50% maturity (L_{50}) (i.e. the length at which 50% of the individuals are mature), the observed proportion of mature specimens was adapted to a logistic curve (Roa et al. 1999). The proportion of mature individuals in each length class was calculated by setting the maturity threshold as follows; (i) stage 3 and higher (advanced maturation) in the macroscopic scale; (ii) stage IIC and higher (late development) of the microscopic staging of the female gonads; and (iii) stage IIB (mid-spermatogenic) and higher of the microscopic staging of the males gonads. We opted for a linear regression method to estimate the coefficient of the regression line β_1 and the constant β_0 (intercept). These parameters are necessary for the calculation of the equations $PCS = \frac{1}{1+e^{-(\beta_0 + \beta_1 \times X)}}$ and $L_{50} = -\beta_0/\beta_1$, where PCS is the probability for an individual to be mature at a given length X (Roa et al. 1999).

RESULTS

A total of 1780 *M. merluccius* individuals were sampled during two years, including 737 females and 1043 males. Each month, 42 to 115 fish were sampled for the analysis of sex ratio and gonadosomatic index. In parallel, 374 individuals were taken for the histology analysis and comparison with the macroscopic approach to maturity stages and L50. Among these 374 fishes, 193 fishes had female gonads, 176 had male gonads, and five individuals had gonads with both male and female germ cells (see below). The detailed numbers per month and the size range of the sampled fishes are shown in the supplement (Table S3).

Season- and length-related variation of sex ratio and gonadosomatic index

Fishes displaying gonads that were macroscopically classified as ovaries ranged between 16 and 73 cm TL. Fishes macroscopically classified as males ranged between 16 and 44 cm TL. Females were generally predominant in the larger size groups (>27 cm), and the length groups greater than 44 cm were composed exclusively of females. Males dominated in the length groups smaller than 28 cm TL (Fig. 2A). Accordingly, there was a significant difference in sex ratio between size groups (t-test=12.8; $p<0.05$). The overall sex ratio of the total sample displayed a significantly higher percentage of males (59%) than females (41%) ($p=0.002$).

The GSI values of females varied over the 23 months (Fig. 2B). In 2017 the highest values were recorded in January-February, then again in May-July and in September. In 2018, the variation of the female GSI was less strongly expressed than in 2017, but January and midsummer were still the periods with high GSI values. The GSI values of males generally showed less variation.

Gonad histological status of male and female European hake

The stages of germ cell development of females and males are listed in Table S1 and in Figure 3. A large number of females had immature ovaries (n=94). Females with developing ovaries were the second most frequent group (n=70); their ovaries were dominated by perinuclear and cortical alveolar oocytes (stage IIa, Table S1) Females with mature ovaries accounted for only 4% (n=7).

Among the males, a high number (n=68) of individuals showed immature testes, while males with mature gonads were rare (only 10% of the 176 males examined).

Season- and length-related variation of the gonad histological status

The annual variation of ovarian maturation stages is shown in Figure 4A: Females with immature ovaries were most abundant in September 2017 (75%), February 2018 (92%) and June 2018 (75%). Mature females were found only in July, January and March and accounted for a small percentage.

Immature males were abundant in February 2018 (86%), December 2017 (67%) and June 2018 (63%). Males with mature testes were observed mainly in January 2018, August 2017 and October 2017 (Fig. 4B).

All females smaller than 21 cm were immature, while mature females were observed only in fish of ≥34 cm (Fig. 4C). Small males (≤18 cm) were immature, while maturing individuals appeared at 19 cm TL and mature males at 23 cm and more. At a body length of 34 cm, all males displayed spent testes (Fig. 4D). Pearson’s correlation tests showed a significant correlation between TL and gonad maturity stage for both sexes (p value <0.01, <0.05, Table 1A).

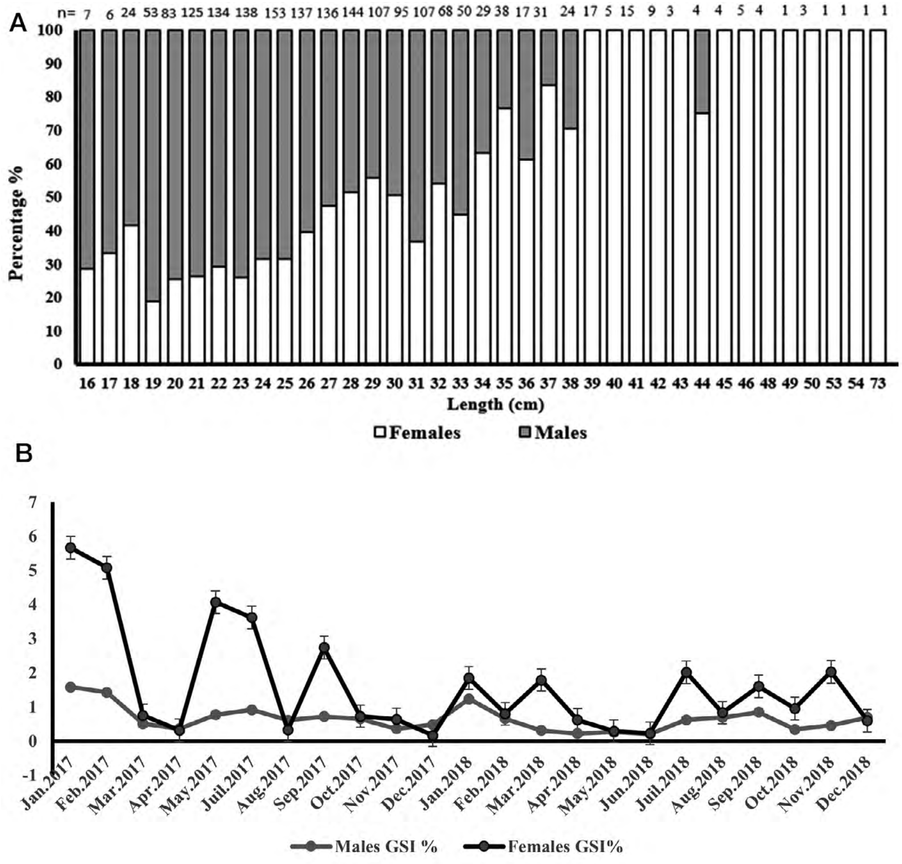

Fig. 2. – A, sex ratio according to length, and B, monthly variation of gonadosomatic index (GSI) for females and males (n=1780, with 737 females and 1043 males).

Table 1. – Summary of statistical analysis (n=369): (A) Pearson correlation between TL and microscopical maturity stages of M. merluccius. (B) Paired sample statistics (Student t-test) of the main stages of maturity.

A.	Length	Immature micro	Maturing micro	Mature micro	Spent micro
Pearson Correlation	Females	-0.802**	0.053	0.441*	0.671**
	Males	-0.910**	0.005	0.401	0.702**
Sig. (bilateral)	Females	0.000	0.811	0.035	0.000
	Males	0.000	0.982	0.089	0.001
B.	Mean	Standard deviation	t	F	Sig. (bilateral)
Immature-micro Immature-macro	4.846	6.427	2.719		0.019 **
Maturing-micro Maturing-macro	-6.385	6.371	-3.613		0.004 **
Mature-micro Mature-macro	-0.769	3.166	-0.876		0.398
Spent-micro Spent-macro	-0.615	1.938	-1.145		0.275

** The correlation is significant at the 0.01 level.
* The correlation is significant at the 0.05 level.

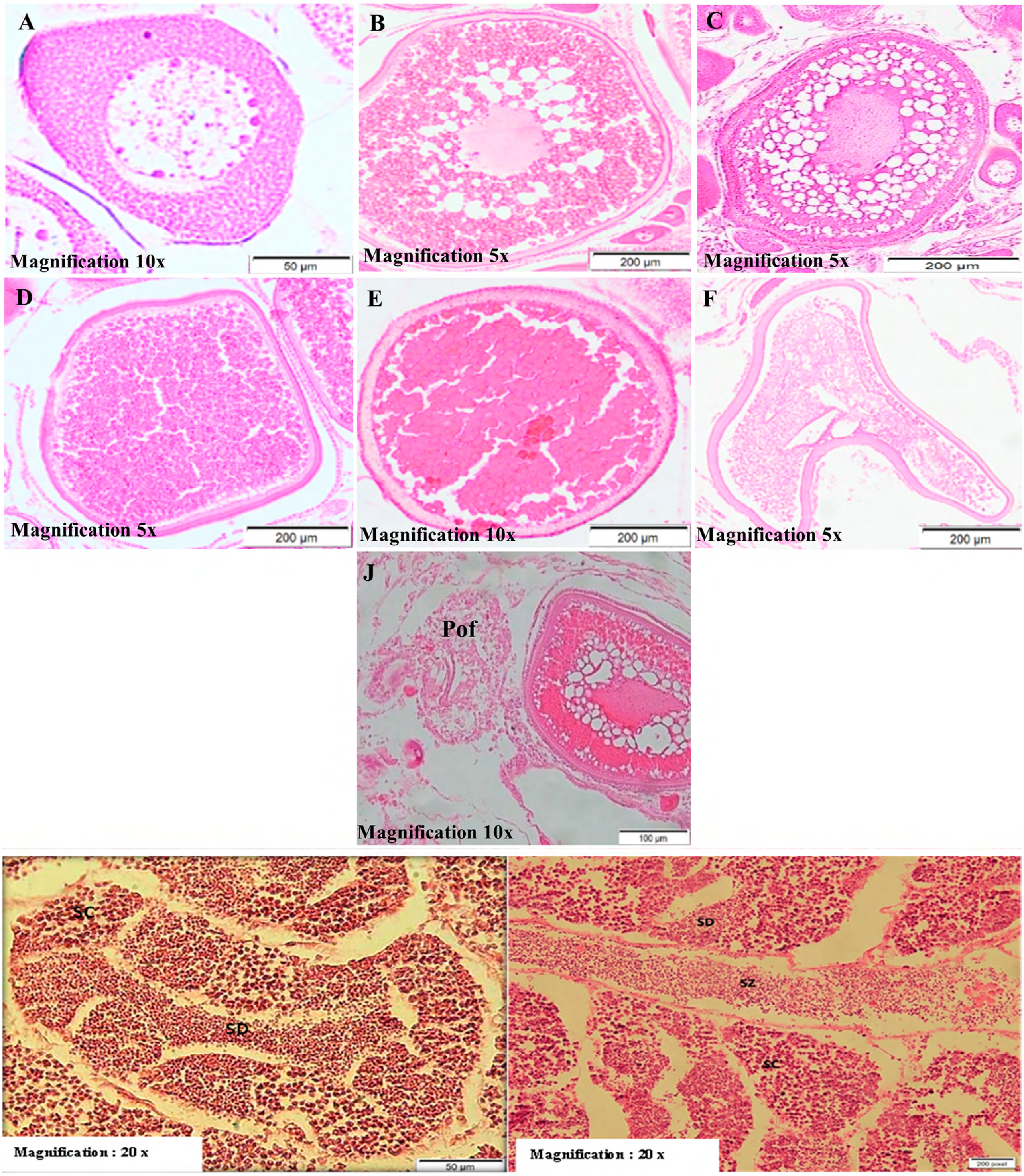

Fig. 3. – Germ cell phases of *M. merluccius.* Oogenesis: A, immature: perinucleolar oocyte with a large nucleus; B, in maturation: cortical alveolar oocyte with accumulation of oil droplets in the cytoplasm; C, in maturation: mid-vitellogenic, oocyte with vitellogenic yolk granules; D, maturing: late vitellogenic, oocyte with abundance of vitellogenic granules; E, mature stage: migration of the nucleus and absence of cell structure; F, atretic follicle with a shapeless ovary, chorion and yolk resorbed; J, postovulatory follicle. H and I, spermatogenesis: SG, spermatogonia; SC, spermatocytes; SD, spermatid; SZ, spermatozoa.

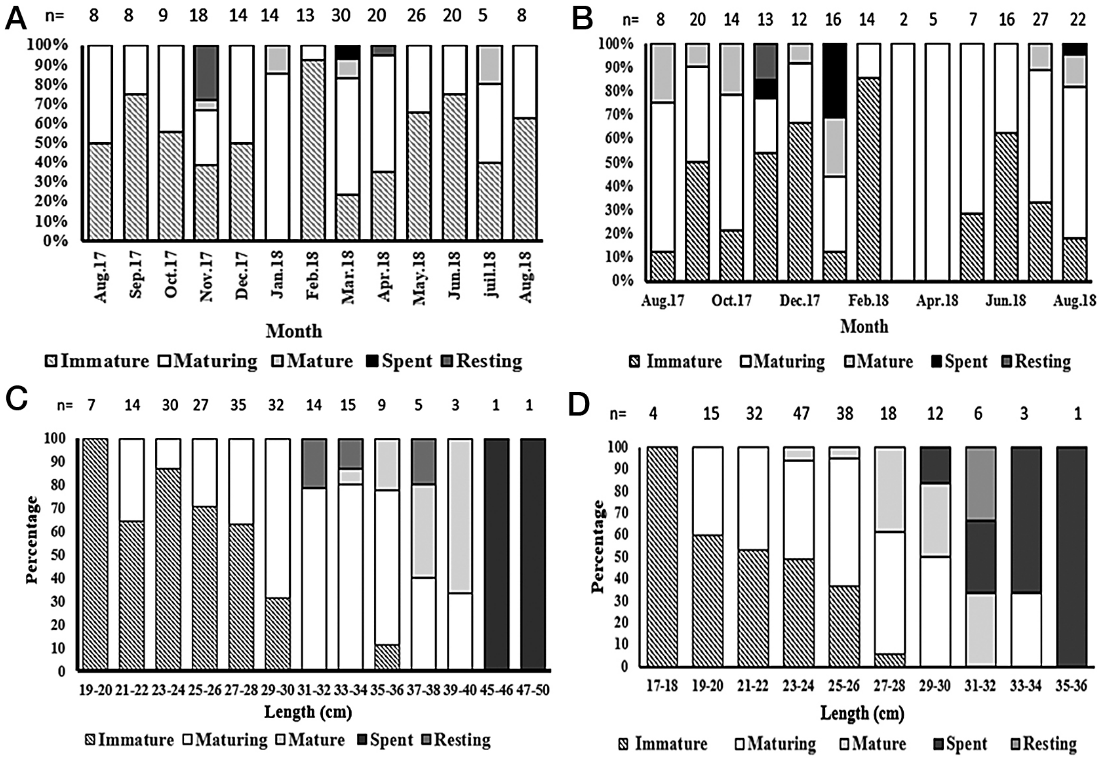

Fig. 4. – Maturation stage assessed histologically by month for females (A) and males (B) and frequency of *M. merluccius* maturation per total length for females (C) and males (D) during 2017-2018. Females (n=193); males (n=176).

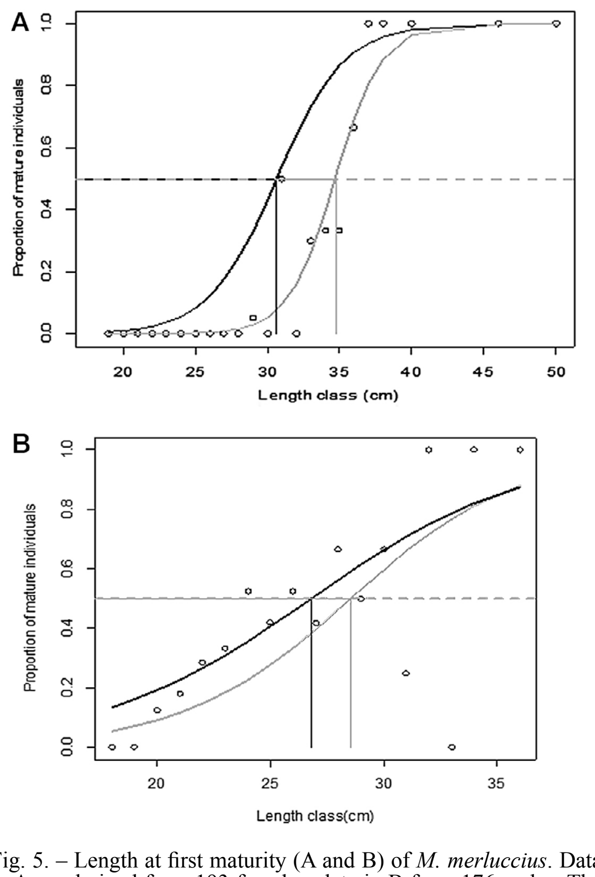

Fig. 5. – Length at first maturity (A and B) of *M. merluccius*. Data in A are derived from 193 females, data in B from 176 males. The grey line represents the histological estimate, and the black line represents the macroscopic estimate.

Difference between macroscopic and histological gonad staging

Histologically, 66% of the investigated fishes were classified to be immature. Macroscopically, however, only 34% of the examined gonads were classified as immature. This difference might be due to the fact that individuals classified macroscopically as mature were sometimes classified as immature individuals in the histological analysis. Differences between the microscopic and macroscopic gonad classifications were also found for spent gonads: while according to the histological examination, individuals with spent gonads were found in January, March and August 2018 and November 2017, macroscopically, fish with spent gonads were observed only in November 2017. Overall, the difference between macroscopic and microscopic staging was significant (Table 2B).

Length at first maturity (L_{50})

The length at first sexual maturity (L_{50}) based on histological data was estimated to be 34.7 cm for females and 28.6 cm for males. Based on the macroscopic data the L_{50} was found to be 30.6 cm for females and 26.8 cm for males (Fig. 5B, D).

Intersex cases

A small number of the histologically examined fish (4 cases) displayed intersex gonads, i.e. the gonads appeared either as testes but also contained a small number of fe-

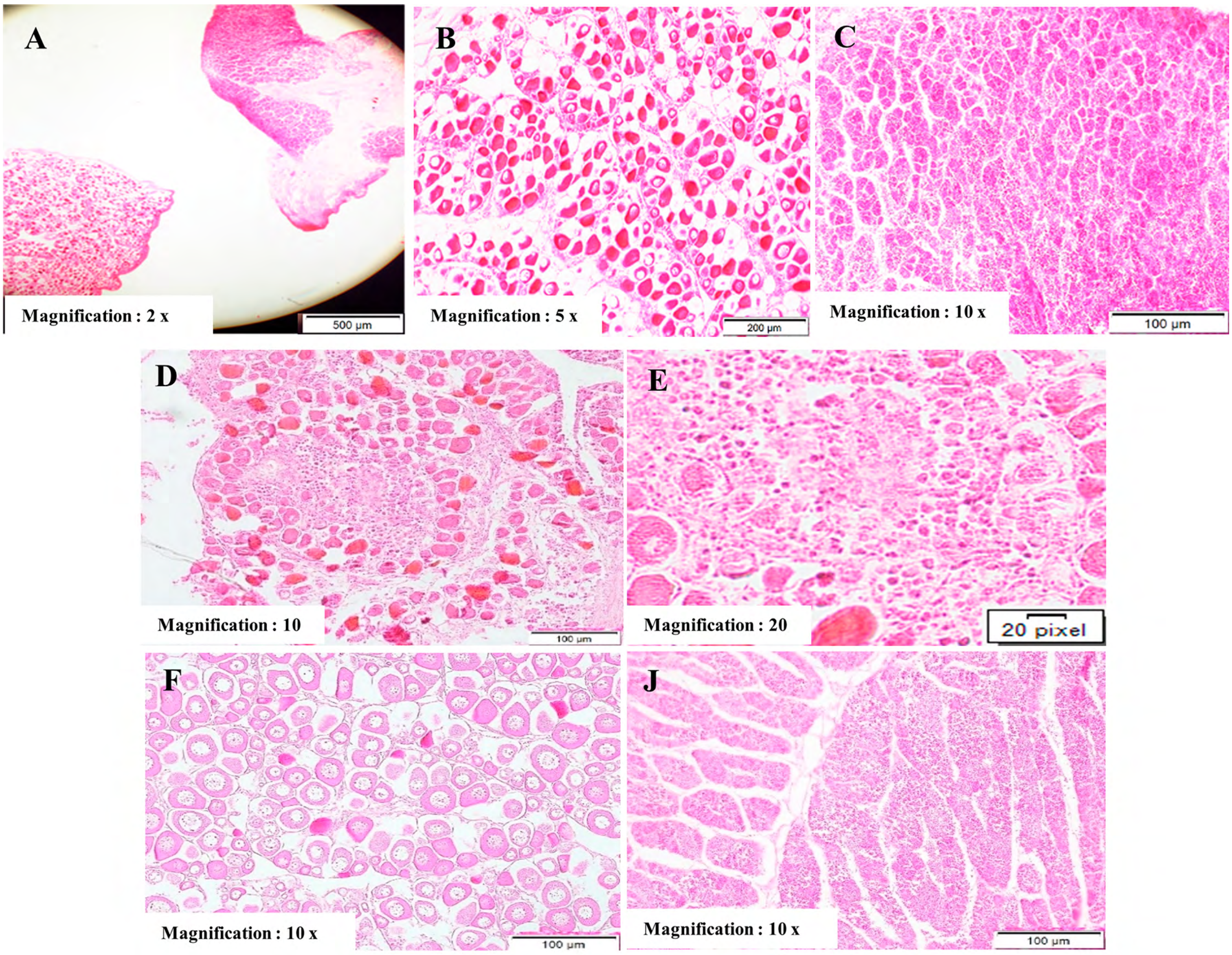

Fig. 6. – Different structures of *M. merluccius* gonads. A, intersex gonad containing both differentiated ovarian and testicular tissue. B and C, parts of image A showing a tissue of male and female, respectively. D, an unidentified structure, oocytes with strange cells inside (not inflammatory cells). E, part of image A with high magnification. F and J, normal gonads of females and males, respectively.

male germ cells, or the gonads displayed an ovarian histology but also contained a small fraction of male germ cells.

In addition, we observed a single intersex case in which the gonads were composed of well-differentiated but anatomically separated ovarian and testicular tissues within the same individual (Fig. 6).

DISCUSSION

In this paper, we present the first attempt at a histological description of the gonads of *M. merluccius* collected from fishing grounds in Morocco. The aim of this study was to provide baseline information on the maturation of male and female gonads, to describe the seasonal changes in gonad histology, and to determine the length at first maturity of females and males. In the following, we discuss our findings with respect to four questions:

Question 1: Does the sex ratio of *M. merluccius* change with body length?

The sex ratio varied with body length: males were predominant in size classes below 27 cm (TL), while females were predominant among the larger individuals. Similar findings have been reported for *M. merluccius* populations from other regions: Khoufi et al. (2014) observed for the Mediterranean Sea that males were predominant in size classes of less than 40 cm (TL) and females in the larger size classes. For hake populations of Atlantic Ocean, the critical body length for the sex ratio shift was 45 and 50 cm (El Habouz et al. 2011, Costa 2013). On the Portuguese coasts, a dominance of males in small size classes and of females in large size classes of hake populations has also been reported (ICES 1988).

This size-related shift of the sex ratio could have two explanations: protandric sexual development (Todd et al. 2016); or sex-specific differences in growth, as occurs in a number of fish species (Cervino 2014). If the European hake were a protandric species, one might expect the occurrence of transition stages that display intersex gonads with morphological characteristics of males and females. This was not observed in the present study. What we found was a very low number of sexually mixed gonads (<1%). The morphology of these gonads does not resemble the gonadal morphologies of protandric transition stages as described in the literature (e.g. Shihab et al. 2017). Thus, the second possible explanation, i.e. the differential growth of male and female fish, appears to be responsible for the size-related shift of the sex ratio. The available literature reports a clear difference in growth rate and L infinity between males and females (Murua 2010, El Bouzidi et al. 2022). Overall, our findings argue against protandric development and in favour of gonochoristic development of hake in Moroccan waters, with a low level of natural or environmental intersex cases.

The occurrence of a low number of individuals with intersex gonads has been reported for various gonochoristic fish species, such as European whitefish (Bittner et al. 2009) and Japanese Medaka (Grim et al. 2007). These gonadal abnormalities are usually interpreted as a spontaneous natural phenomenon (PA Bahamonde 2013). Intersex gonads in fish can also be environmentally induced, for instance, by exposure to endocrine-disrupting compounds (Jobling et al. 1998).

Apart from the sexual size dimorphism, both male and female gonad maturity increased with body length: the longer the fish was, the more mature the gonad was. This is indicated from the significant correlation between the stages of maturity and the body of European hake (Pearson test, $p<0.01$) (Table 1). Importantly, the size maturity relationship applies for both male and female hake. Congruent findings were recorded by Murua (2010).

Question 2: Is there a seasonal variation in European hake maturity?

The variation in gonad maturity stages of *M. merluccius* over the years observed in this study shows limited interannual variation. Although values differed slightly between years [for instance, the percentage of immature females accounted for 50% in August 2017 compared with 60% in August 2018 (Fig. 4A)], the overall pattern appears similar between the two study years. This is in line with the reports in the literature (see El Habouz et al. 2011).

The results must be interpreted with caution because several factors influence the seasonal variation. Both the timing and duration of the maturation and spawning cycle can be seasonally variable due to changing environmental conditions, notably temperature and food access, which influence available energy resources for the reproductive investment (Khoufi et al. 2014). Also, sample size and sample composition can influence the annual variation of reproductive parameters. Nevertheless, with all necessary caution, it appears that around January and in midsummer, a high percentage of fish with mature gonads are present, suggesting that these are periods of enhanced spawning activity. This interpretation is in line with the published literature: El Habouz et al. (2011) found that males and females were involved in the spawning process all year around, but a peak occurred in winter (January-February) and a secondary one in summer (July–August). In contrast, for hake population on the Portuguese coast, Costa (2013) reported three breeding peaks: in January-March, May-June and August. On the Mediterranean coast, Khoufi et al. (2014) also observed three spawning peaks in January, April and August. Murua (2010) in the Atlantic Ocean, showed a definite seasonal peak of GSI or gonad maturity for *M. merluccius*. The disparity in spawning periods in the European hake populations across the regions may be explained by the latitude factor (Khoufi et al. 2014) or by biological and environmental effects (Murua and Moto 2006).

Question 3: Differences between the macroscopical or histological staging of the gonads.

The classification of maturation stages differed between the two methods. This may be partly explained by the asynchronous oocyte development, which can be detected only by histology and not by macroscopy. Additionally, at the macroscopic scale, it is not possible to distinguish between immature/maturing and resting gonads or between spent and immature ones (Costa and Carmo Silva 2016). With such differences in resolution between the two methods, the macroscopic method appears to be less reliable than the histological one. Congruent conclusions were drawn by (Costa and Carmo Silva 2016).

Question 4: Is there any difference between the L_{50} obtained macroscopically and microscopically?

The L_{50} is an important parameter in fisheries science. It provides indications for the minimum size for sustainable management of fish populations, although the choice of the legal minimum size for commercial fishing depends on other factors as well. The information needed for the L_{50} is the gonad maturity stage: at what size does the fish reach gonad maturity for the first-time? In the present study the L_{50} analysis yielded different values when based on microscopic or macroscopic gonad examination of the same samples (n=369). The L_{50} was 35 cm (histology-based) and 31 cm (macroscopy-based) for females, while for males it was 29 cm (histology-based) and 27 cm (macroscopy-based). Thus, the L_{50} increased by almost 4 cm for females and by 2 cm for males when assessed by histology rather than by macroscopy. Given the difference between microscopical and macroscopical gonad classifications, as discussed above, it is not surprising that the two methods also yield different L_{50} values.

The length at first maturity reported by Lahrizi (1996) (41.1 cm for females; 37.8 cm for males) in the northern Atlantic coast of Morocco and by El Habouz et al. (2011) (33.8 cm for females; 28.6 for males) in the southern Atlantic coast of Morocco showed higher values than the macroscopical estimation obtained in the present study (31 cm for females; 27 cm for males). This may result from overexploitation of the European hake in the northern Atlantic coast of Morocco (El Bouzidi et al. 2022). Given that fishing pressure can lead to the elimination of large and older hake, this could decrease the size at maturity as a compensatory response for the recovery of the stock (Domínguez-Petit et al. 2008). However, overexploitation may not be the only explanation for size changes at maturity; environmental conditions such as temperature, upwelling and North Atlantic Oscillation could play an important role in influencing size at maturity (Domínguez-Petit et al. 2008). For example, the histological L_{50} found in this study was higher than that reported by El Habouz et al. (2011). This difference could be due to the upwelling activity that characterizes the southern Atlantic coast of Morocco (INRH 2019).

With respect to the histology-based L50, we found slightly higher values than Kahraman et al. (2017) in their study on hake: 28.6 cm and 33.8 cm for males and females from the Sea of Marmara in Turkey. For females hake sampled along the Tunisian coast, the L_{50} was 29.0 cm (Khoufi et al. 2014) while on the Galician Shelf the values obtained from L_{50} females sampled in 2003 and 2004 were 44.45 cm and 42.97 cm, respectively. The differences in size at maturity reported from different regions may depend on parameters such as fishing pressure, temperature, food, genetic factors (Engelhard et Heino 2006), overexploitation, latitudinal variation (Khoufi 2014), fishing mortality and upwelling (Domínguez-Petit et al. 2008).

CONCLUSIONS

The results are consistent with those described in other studies conducted in Atlantic and Mediterranean waters. Furthermore, our data suggest that gonad histological analysis is more reliable than macroscopic analysis for gonad staging. Finally, the findings from this study provide arguments that the minimum landing size of *M. merluccius* could be revised. Currently, this size is 20 cm TL, whereas the L_{50} found in the present study, both histologically and macroscopically, was clearly higher. This means that with the current reference size, hakes that have not yet reached sexual maturity will be caught. Such a fishing practice would not support a sustainable exploitation of the fishing resources.

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REFERENCES

Bahamonde P.A., Munkittrick K.R., Martyniuk C.J. 2013. Intersex in teleost fish: are we distinguishing endocrine disruption from natural phenomena? Gen. Comp. Endocr. 192: 25-35.
https://doi.org/10.1016/j.ygcen.2013.04.005

Barber B.J., Blake N.J. 2006. Reproductive physiology. In: Shumway S. E., Parsons G. J. (eds): Scallops: biology, ecology, and aquaculture. Elsevier. Amsterdam, pp. 357-406.
https://doi.org/10.1016/S0167-9309(06)80033-5

Bittner D., Bernet D., Wahli T., et al. 2009. How normal is abnormal? Discrimination between deformations and natural variation in gonad morphology of European whitefish *Coregonus lavaretus*. J. Fish Biol. 74: 1594-1614.
https://doi.org/10.1111/j.1095-8649.2009.02233.x

Cervino S. 2014. Estimating growth from sex ratio-at-length data in species with sexual size dimorphism Fish. Res. 160: 112-119.
https://doi.org/10.1016/j.fishres.2013.11.010

Costa A.M. 2013. Somatic condition, growth and reproduction of hake, *Merluccius merluccius* L., in the Portuguese coast. Open J. Mar. Sci. 13: 12-30.
https://doi.org/10.4236/ojms.2013.31002

Costa A.M., Silva M.C. 2016. Why do we need histology of hake (*Merluccius merluccius*) gonads? The Portuguese case. Relat. Cient. Téc. Portugal, 14 pp.

Dietrich D., Krieger H., Rumpf S., et al. 2009. Histological Analysis of Endocrine Disruptive Effects in Small Laboratory Fish. Wiley-Interscience. 369pp.
https://doi.org/10.1002/9780470431795

Dominguez-Petit R., Alonso-Fernández A., Saborido-Rey F. 2008. Reproductive strategy and oocyte recruitment process of European hake (*Merluccius merluccius*) in Galician shelf waters. Cybium. 32: 317-318.

El Bouzidi C., Abid N., Awadh H., et al. 2022. Growth and mortality of the European hake *Merluccius merluccius* (Linnaeus, 1758) from the North of Moroccan Atlantic coasts, Egypt. J. Aquat. Res. 48: 233-239.
https://doi.org/10.1016/j.ejar.2021.12.003

El Habouz H., Recasens L., Kifani S., et al. 2011. Maturity and batch fecundity of the European hake (*Merluccius merluccius*, Linnaeus, 1758) in the eastern central Atlantic. Sci. Mar. 75: 447-454.
https://doi.org/10.3989/scimar.2011.75n3447

Engelhard G. H., Heino M. 2006. Climate change and condition of herring (*Clupea harengus*) explain long- term trends in extent of skipped reproduction. Oecologia 149: 593-603.
https://doi.org/10.1007/s00442-006-0483-3

FAO/CECAF. 2017. Working Group on the Assessment of Demersal Resources. Subgroup North. Tenerife, Spain, pp. 6-15.

Fournie J.W., Krol R.M., Hawkins W.E. 2000. Fixation of fish tissues. In The laboratory fish. Academic Press, 2000. pp. 569-578.
https://doi.org/10.1016/B978-012529650-2/50043-3

Grim K.C., Wolfe M., Hawkins W., et al. 2007. Intersex in Japanese medaka (*Corsivo*) used as negative controls in toxicologic bioassays: a review of 54 cases from 41 studies. Environ. Toxicol. Chem. 26: 1636-1643.
https://doi.org/10.1897/06-494R.1

Holden M.J., Raitt D.F.S. 1974. Manuel des sciences halieutiques. Deuxième partie. Méthodes et recherches sur les ressources et leur application. Doc. Tech. FAO, 223 pp.

ICES. 1988. Working Group on Assessment of Hake Stocks. Copenaghen, 51 pp.

Institut National de Recherche Halieutique (INRH). 2019. Rapport annuel d'Etat des stocks et des pêcheries Marocaines au Maroc, Depmt. Des Péches, Maroc, Casablanca, ISSN : 2509-1727, 565pp.

Jobling S., Nolan M., Tyler CR., et al. 1998. Widespread sexual disruption in wild fish. Environ Sci Tech. 32: 2498-2506.
https://doi.org/10.1021/es9710870

Kahraman A.E., Yıldız T., Uzer U., Karakulak F.S. 2017. Age composition, growth and mortality of European hake *Merluccius merluccius* (Linnaeus, 1758) from the Sea of Marmara, Turkey. Acta Zool. Bulg. 69: 377-384.

Khoufi W., Ferreri R., Jaziri H., et al. 2014. Reproductive traits and seasonal variability of *Merluccius merluccius* from the Tunisian coast. J. Mar. Biol. Ass. UK. 94: 1545-1556.
https://doi.org/10.1017/S0025315414000356

Lahrizi H. 1996. Etude de la biologie de croissance et de reproduction du merlu blanc *Merluccius merluccius* (L. 1758) débarqué par les chalutiers au port de Casablanca. PhD thesis, Mohamed 7. Univ. Rabat.

Lloris D., Matallanas J., Oliver P. 2005. Hakes of the world (Family Merlucciidae). An annotated and illustrated catalogue of hake species known to date. FAO Species Catalogue for Fishery Purposes. Rome, FAO. 57pp.

Murua H. 2010. The Biology and Fisheries of European Hake, *Merluccius merluccius*, in the North-East Atlantic. Adv. Mar. Biol. 58: 97-154.
https://doi.org/10.1016/B978-0-12-381015-1.00002-2

Murua H., Motos L. 2006. Reproductive strategy and spawning activity of the European hake *Merluccius merluccius* (L.) in the Bay of Biscay. J. Fish Biology. 69: 1288-1303.
https://doi.org/10.1111/j.1095-8649.2006.01169.x

Roa R., Ernst B., Tapia. F. 1999. Estimation of size at sexual maturity: an evaluation of analytical and resampling procedures, Fish. Bull. 97: 570-580.

Shihab I., Gopalakrishnan A., Vinesh N., et al. 2017. Histological profiling of gonads depicting protandrous hermaphrodistism in *Eleutheronema tetradactylus*. J. Fish. Biol. 90: 202-211
https://doi.org/10.1111/jfb.13324

Todd E.V., Liu H., Muncaster S., Gemmell N.J. 2016. Bending Genders: The Biology of Natural Sex Change in Fish. Sex Dev. 10: 223-241.
https://doi.org/10.1159/000449297

SUPPLEMENTARY MATERIAL

Table S1. – Histological classification of *Merluccius merluccius* ovaries and testes.

State	Histological criteria of ovaries	Histological criteria of testes
I. Immature	The ovary contains oogonia and immature oocytes of the perinucleolar stage. Absence of cortical alveolar oocytes. Entirely immature stages from oogony to perinucleolar oocytes, without cortical alveoli.	The immature testicles are formed into nests of spermatogonia with a spherical or slightly oval-shaped nucleus. The large spermatogonia display a pale nucleus. The gonad contains >90% of spermatogonia nests and primary stages of spermatocytes. Spermatozoa are absent. Cytoplasm and the nucleus contain a dark nucleolus.
II. Developing/ Maturing	Appearance of cortical alveoli and/or vitellogenic oocytes phases. This stage is characterized by a thick ovary wall, high vascularization of gonads and/or disorganization of lamellae. No or few signs of postovulatory follicles. We observed three sub-stages: a) Early development: (ovary has >90% of pre-vitellogenic follicles), predominantly perinucleolar over cortical alveolar. b) Mid-development: at least 50% of the follicles are at the early or mid-vitellogenic stage. c) Late development: the majority of follicles are late vitellogenic.	This stage is marked by the start of active spermatogenesis, the presence of spermatogonia and spermatocytes is the main feature. They are recognized by their complex structure: they contain a voluminous nucleus and a mass of chromatin in the centre. However, the spermatids and spermatozoids can be found at the end of this phase of development. We observed two sub-stages: a) Early spermatogenic: predominantly of the spermatogonia and secondary spermatocytes (>90); the germinal epithelium is thinner than it is during the next stage II. b) Mid-spermatogenic: presence of spermato-cytes, spermatids and spermatozoa in equal proportions; the germinal epithelium is thinner than in Stage I, but thicker than in Stage III.
III. Spawning (late maturation/ hydrated)	Late vitellogenic follicles and mature/spawning follicles are present in the ovary. At this final stage of development, the follicles display a continued increase in size through hydration and the central nucleus migrates towards the micropyle. The majority of follicles (>90%) are late vitellogenic and mature/spawning follicles; the follicles are larger than late vitellogenic follicles	Contains predominantly mature sperm cells (>90%), but all sperm maturation stages can be observed. The germinal epithelium is thinner than it is during Stage II. The seminiferous lobules contain the mature spermatozoa and a low quantity of spermatids (they are spherical and the volume of the nucleus is reduced and contains condensed chromatin). The spermatids are then transformed into spermatozoa and lose the interlobular connective tissue that marked the spawning phase. Young spermatozoa are characterized by a centric nucleus and a mass of chromatin.
IV. Post-spawning	This stage is marked by a higher number of blood vessels, a thick ovary wall and disorganization of ovary structures, and a predominance of spent (postovulatory) follicles which consist of remnants of theca externa and granulosa.	This stage is characterized by loose connective tissue; the testis contains distorted empty lobules, vacuolated with the residue of the spermatozoa. The development of spermatogenesis is finished. At the end of this period, new generations of spermatogonia and captured spermatozoa are formed to prepare for the next spawning season.
V. Resting	A part of the eggs is released. The resting ovary is small, has a large wall and an abundance of blood vessels. Resting ovary is present in mature females with absence of mature oocytes. The lamellae are not as compact as those of immature ovaries.	Resting testicles are present in mature males with absence of mature testes but signs of maturation such as residual bodies of spermatids or spermatozoa.

Table S2. – Maturity scale for partial spawners (Holden and Raitt 1974) based on macroscopical staging.

Stage	State	Macroscopic staging of the gonads
I	Immature	Ovaries and testes are about one third the length of the body cavity. Ovaries pinkish, translucent; testes whitish. Eggs indistinguishable to naked eye.
II	Maturation and resting	Ovaries and testes about half the length of the body cavity. Ovaries pinkish, translucent; testes whitish, more or less symmetrical. Macroscopically, resting and maturing stage ovaries cannot be distinguished.
III	Advanced maturing	Ovaries and testes are about two thirds the length of the body cavity. Ovaries pinkish-yellow with grainy texture. Testes white to creamy. No transparent or translucent eggs visible.
IV	Mature (Ripe)	Ovaries and testes are about two thirds the length of the body cavity. Ovaries orange pink, with appearance of superficially visible blood vessels. Ovaries largely mature and transparent, with ripe ova visible. Testes whitish- creamy, soft.
I	Post-spawning	Ovaries and testes shrunken to about half the length of the body cavity. Walls loose. Ovaries may contain remnants of opaque, darkened or translucent eggs. Testes flabby and bloodshot.

Table S3. – Monthly summary table with the number and length range of samples collected for histological analysis.

Month	Females	Males	Total	Range length (cm)
Aug. 2017	8	8	16	21-35
Sep. 2017	8	20	28	17-34
Oct. 2017	9	14	23	21-36
Nov. 2017	18	13	31	24-36
Dec. 2017	14	12	26	19-29
Jan. 2018	14	16	30	23-38
Feb. 2018	13	14	27	20-33
Mar. 2018	30	2	32	22-50
Apr. 2018	20	5	25	22-40
May 2018	26	7	33	24-34
Jun. 2018	20	16	36	21-31
Jul. 2018	5	27	32	19-40
Aug. 2018	8	22	30	18-30
Total	193	176	369	18-50