IPMA
Instituto Português
do Mar e da Atmosfera

RELATÓRIOS CIENTÍFICOS E TÉCNICOS

SÉRIE DIGITAL

VALIDATION OF THE MACROSCOPIC MATURITY STAGES FOR BLUE JACK MACKEREL, *Trachurus picturises* (Bowdich, 1825), ACCORDING TO HISTOLOGICAL EXAMINATION

Diana Feijó, Carmo Nunes Silva, Georgina Correia, Mónica Inácio, Paula Abreu e Ana Maria Costa

2023

40

Relatórios Científicos e Técnicos do IPMA - Série Digital

Destinam-se a promover de forma simples e acessível a divulgação de resultados de carácter científico e técnico, resultantes das atividades de investigação e do desenvolvimento e inovação tecnológica nas áreas de investigação do mar e da atmosfera. Esta publicação é aberta à comunidade científica e tem, para além desta, como público-alvo, as diversas fileiras de atividade económica e ainda a sociedade em geral, podendo os trabalhos serem escritos em português, francês ou inglês**.**

Temas e Corpo Editorial:

Meteorologia

Diamantino Henriques, Lourdes Bugalho

Sismicidade, Geomagnetismo e Tsunamis

Célia Marreiros, Jorge Cruz

Clima, Variabilidade e Alterações Climáticas

Emília Salgueiro, Sílvia Antunes

Biogeoquímica e Geologia Marinha

Mário Mil-Homens, Vítor Magalhães

Estrutura e Dinâmica dos Ecossistemas Marinhos e de Águas de Transição

André Gonçalves, Teresa Moura, Victor Henriques

Biologia, Ecologia e Exploração de Recursos Vivos Marinhos

Rogélia Martins, Rui Coelho

Aquacultura

Laura Ribeiro, Paula Ramos

Qualidade e Valorização do Pescado e outros Produtos Marinhos

Amparo Gonçalves, Carla Pires

Contaminação, Salubridade e Segurança Alimentar

Helena Silva, Miguel Caetano

Desenvolvimentos Tecnológicos Laboratoriais, de Análise e de Monitorização

Corina Chaves, Susana Rodrigues

Coordenação: Maria Manuel Angélico, Francisco Ruano, Irineu Batista

Edição digital e capa: Conceição Almeida

ISSN: 2183-2900

Instruções para os autores

http://ipma.pt ou através do endereço rct@ipma.pt

Edição

IPMA - Instituto Português do Mar e da Atmosfera; Rua C, Aeroporto de Lisboa; 1749-007 Lisboa, Portugal

Todos os direitos reservados

Validation of the macroscopic maturity stages for blue jack mackerel, *Trachurus picturatus* (Bowdich, 1825), according to histological examination

Diana Feijó[1], Carmo Nunes Silva[2], Georgina Correia[1], Mónica Inácio[2], Paula Abreu[2] e Ana Maria Costa[2]

1 - Instituto Português do Mar e da Atmosfera, Av. General Norton Matos, nº 4, 4450-208 Matosinhos

2 - Instituto Português do Mar e da Atmosfera, Av. Alfredo Magalhães Ramalho, nº 6, 1495-165 Algés

Recebido em: 06/04/2022 Aceite em: 13/02/2023

ABSTRACT

The assessment of fisheries resources requires the correct identification of fish maturity stages. The methodologies applied should be standardised and all uncertainty regarding macroscopic identification should be validated by microscopic observation. This work proposes a microscopic maturity scale for the blue jack mackerel (*Trachurus picturatus*, Bowdich, 1825), based on individuals collected by commercial bottom trawl in northern Portugal. After assigning the macroscopic maturity stage, the gonads were processed for histological observation. The slides were observed and the gonads were classified into 6 stages according to Walsh et al. (1990). The corresponding macroscopic and microscopic maturity stages were described. The correspondence to the new maturity scale presented at WKASMSF (ICES, 2018) is also presented, which is mandatory when reporting maturity data to ICES databases (since the 1st. January 2020).

Keywords*: Trachurus picturatus*, histology, macroscopic identification, microscopic maturity scale.

RESUMO

Título: Validação dos estados de maturação macroscópicos de carapau negrão, *Trachurus picturatus* (Bowdich, 1825), de acordo com a identificação microscópica

A gestão das pescarias requer a correta identificação dos estados de maturação dos peixes. As metodologias aplicadas devem ser padronizadas e todas as incertezas quanto à identificação macroscópica devem ser validadas por observação microscópica. Este trabalho propõe uma escala microscópica de maturação para o carapau-negrão (*Trachurus picturatus*, Bowdich, 1825), baseada em indivíduos recolhidos por arrastões comerciais no norte de Portugal. Após a atribuição do estado de maturação macroscópico, as gónadas foram processadas para observação histológica. As lâminas foram observadas e as gónadas classificadas em 6 estados de acordo com a escala de maturação de Walsh et al. (1990). Os correspondentes estados de maturação macroscópicos e microscópicos foram descritos. É também apresentada a correspondência com a nova escala de maturação apresentada no WKASMSF (ICES, 2018), que é obrigatória no reporte dos dados de maturação às bases de dados do ICES (desde o dia 1 de Janeiro de 2020).

Palavras chave: *Trachurus picturatus*, histologia, identificação macroscópica, escala de maturação microscópica.

REFERÊNCIA BIBLIOGRÁFICA: Feijó, D.; Nunes Silva, C.; Correia, G.; Inácio, M.; Abreu, P.; Costa, A.M. 2023. Validation of the macroscopic maturity stages for blue jack mackerel, *Trachurus picturatus* (Bowdich, 1825), according to histological examination. **Relatórios Científicos e Técnicos do IPMA (http://ipma.pt), nº 40, 34 pp.**

INTRODUCTION

Small pelagic species are an important resource worldwide (Claramunt et al., 2019). The assessment of the status of living marine resources is based on the joint modelling of resource dynamics and fishing fleets thus taking into account biological (growth, reproduction, feeding habits, etc.) and fishing information (fishing effort, catches, landings, discards, etc.) (Rothschild et al., 1987; Sparre et al., 1989; Shepherd, 1993).

Several assessment models (for example, Stock Synthesis - SS3, Methot and Wetzel, 2013, or Assessment for all - A4A, Scott et al., 2016) use information regarding maturation states of the individuals (Rothschild et al., 1987; Sparre et al., 1989), and the knowledge of the species reproduction to be evaluated is essential to know the spawning time and the proportion of mature and immature individuals. For example, category 1 stocks (such as hake, sardine and horse-mackerel) have quantitative and full analytical assessments and forecasts that are either age/length-structured or based on production models. Such stocks also require assessing whether spawning-stock size is above levels where recruitment is not impaired, since the stock is highly dependent on annual recruitment (ICES, 2022). Like all other vertebrates, fish have sexual reproduction and in most cases, fertilisation is external and takes place in an aquatic environment. The reproduction of teleosts is complex, which is reflected in the great variety of structures constituent of the gonads; however, the germ cell morphology and the somatic elements constituting the gonad tissue are similar across species. Estimates of reproductive parameters such as fecundity, maturity ogives, spawning times and spawning biomass require the distinction between active and inactive mature individuals. In each phase of the reproductive cycle, the gonads show a specific development resulting from the maturation stage of the oocytes in the case of the female gonads and the spermatozoids in the male gonads. The attribution of maturity stages is based on the sexual development of the gonads and is performed by the observation of the external morphology of the gonad. However, the categorization of a continuous process is often difficult to achieve. An incorrect attribution of maturation state can lead to problems in fisheries management. Managers need to know the actual number of spawning fish in the population as well as the length at first maturity to effectively manage the fish stock. Because certain characters cannot be identified macroscopically, the construction of the maturity scales must be based on microscopic criteria that allow an objective identification of the different states of maturation. The fact that microscopy techniques are becoming faster and easier to apply, allowing a large number of individuals to be analysed with relatively little effort, results in the increasing use of scales based on microscopic criteria such as with sardines and horse mackerel (Perez and Figueiredo, 1992; Costa and Borges, 1996; Costa, 2001). Several international fisheries commissions (i.e. ICES - International Council for the Exploration of the Sea, PICES - North Pacific Marine Science Organization, GFCM - General Fisheries

Commission for the Mediterranean) strongly recommend standardising of methodologies across countries and researchers. Although each fishery institute often uses its own maturity scale, reporting to international databases should be done with the same scale. With this purpose, and since 2007, ICES has promoted several workshops for calibration of maturity stages assignment, adopting internationally agreed macroscopic maturity scales and the corresponding conversion scales for reporting maturation to international databases (ICES, 2018).

The blue jack mackerel, *Trachurus picturatus* (Bowdich, 1825), can be found from the southern Bay of Biscay to southern Morocco (https://www.fishbase.se/summary/1279). Around the islands of Madeira and Azores, it is one of the most common fish, used fresh for human consumption and as live bait for fishing of tuna and black scabbard fish (Jesus, 1992). In mainland Portugal, it is used for human consumption and it is increasingly common to see its presence in markets. In Portuguese landing ports such as Peniche, Portimão and/or Matosinhos, the blue jack mackerel has a distribution in length between 14 to 45 cm, with a spawning season that starts in December and extends until April–May, with a peak in March (Costa, 2019). Since the end of the 1960's, official landings statistics exist for mainland Portugal, Azores and Madeira Islands (https://www.dgrm.mm.gov.pt/esta), which show strong fluctuations, not just due to changes in fishing effort, but also due to the observation of fluctuations in the abundance indices obtained from research surveys carried out along the continental Portuguese coast (Chaves, 2018). An annual TAC (Total Allowable Catch) is set for *Trachurus* spp. in ICES Division 27.9.a (thus applied to the combined catches of the three species (*Trachurus trachurus, T. picturatus, and T. mediterraneus)* around the Iberian Peninsula as well as to *Trachurus picturatus* in area 27.10 (Azores). Regardless, the ICES advice for Portuguese waters pertains to *T. trachurus* (ICES, 2021), since blue jack mackerel has no assessment in Portuguese continental waters. For Division 27.10 (ICES, 2017) and since 2018, assessment and advice were included in the Working Group on Southern Horse Mackerel Anchovy and Sardine (WGHANSA) report (ICES, 2021).

Due to this growing presence, it is thus important to know the life cycle of blue jack mackerel, for which correct identification of the maturity stages has to be validated by histology. Although there is no operational assessment model, knowledge of biological information can be relevant to improve the assessment of this species.

In this study, we observed histological sections of gonads of blue jack mackerel of the Portuguese mainland with the aim of validating, for the first time, a microscopic maturity scale, using the information of macroscopic maturity scale to better identify the maturity stages of this species. According to the recommendation from the Workshop for Advancing Sexual Maturity Staging in Fish (WKASMSF) (ICES, 2018), the 6-stages maturity scale applied in this work was also converted into the scale proposed during that Workshop for all teleosts.

MATERIAL AND METHODS

Since 2007, on a fortnightly basis, samples have been obtained from the commercial fleet of bottom trawlers operating in the northern continental coast of Portugal and purchased at the auction market in Matosinhos. Opportunistically, samples were collected during research campaigns (2010-2011). Each specimen was measured (TL ± 0.1 mm), total weight (TW ± 0.01 g) and gutted weight (GW ± 0.01 g) were registered for all sampled individuals. The sex was determined and the gonads were observed and macroscopically classified in six stages, according to the maturity scale of Walsh et al. (1990) (Table 1).

Table 1 – Blue jack mackerel macroscopic maturity stages (according to Walsh et al., 1990).

Tabela 1 – Estados de maturação macroscópicos de carapau negrão (segundo Walsh et al., 1990).

Stage	State	Macroscopic appearance – Females	Macroscopic appearance – Males
1	Immature	Gonads are small. Ovaries wine red and clear, torpedo-shaped	Gonads are pale, flattened, and transparent
2	Early ripening	Gonads occupy ¼ to ¾ of the body cavity. Opaque eggs visible in ovaries give pale pink to yellowish coloration, largest eggs without oil globule	Testes off-white, milt not running
3	Late ripening/partly spent (early)	Gonads occupy ¾ to almost filling the body cavity. Ovaries are yellow to orange with the presence of opaque eggs. Largest eggs may have oil globules	Testes creamy white
4	Ripe	Ovaries are characterised by externally visible hyaline eggs no matter how few or how early the stage of hydration is. Ovaries with hyaline eggs only in the lumen are not included. Ovary size variable from full to ¼	Testes filling body cavity, milt freely running
5	Partly spent (late)	Gonads occupy ¾ to ¼ of body cavities. Ovaries are slacker than in stage 3 and often bloodshot. It may have residual eggs (opaque and a few hyaline) in the gonad	Testes with sperm remain and shrivelled at the anus end
6	Spent / Recovering spent	Gonads occupy ¼ or less of body cavity. Ovaries reddish and often murky in appearance, sometimes with a scattering or patch of opaque eggs	Testes opaque with a brownish tint and no trace of milt

To validate the macroscopic observations and create a microscopic maturity scale for *T. picturatus*, some gonads were stored in formalin for processing, to cover all maturity stages between the period 2010 and 2013.

Table 2 shows the monthly distribution of the 384 gonads collected and identified by the macroscopic maturity stage.

Table 2 – Number of gonads collected monthly, by sex (Females – F and Males – M) and by macroscopic (MACRO) states (1 to 6) (according to Walsh et al., 1990).

Tabela 2 – Número de gónadas recolhidas mensalmente, por cada sexo (Fêmeas – F e Machos – M) e por cada estádio de maturação macroscópica (MACRO) (1 a 6) (segundo Walsh et al., 1990).

MACRO	Number of gonads																									
	Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		Oct		Nov		Dec		Total	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
1	5		2				4					1			8	3			7						26	4
2	4	6	11	8	6	4	6	4	5	2	4	4	5	4	8	9	6	5	5	4				1	60	51
3	1	3	6	2	3	4	1			1		1	1	1		7									12	19
4					3	11	1	1				1			1											5	13
5	6	8	4	5	11	12	14	9	1	6	9	6	4	5	6	2	1		4	2					60	55
6	5	5	2		5	1	9	2	4		7	3	3	3	8	6	6	4	5	1					54	25
Total	21	22	25	15	28	32	35	16	10	9	20	16	13	13	31	27	13	9	21	7	0	0	0	1	217	167

The gonads were preserved in a solution of 4% buffered formalin and subsequently, a piece of tissue from each one was subjected to histological analysis. Tissues were dehydrated, cleared in xylol, and embedded in paraffin wax, and from these, sections 3–5µm thick were cut, then stained with eosin and hematoxylin (Gonçalves et al*.,* 2004). Histological classification of the gonads was also defined according to the six maturity stages from the macroscopic scale, based on the developmental stage of the most advanced gametes (oocytes in females and sperm in males).

RESULTS

Due to the variability and subjectivity of the sampler, the attribution of macroscopic states is difficult. However, direct observation of the gonads is the most used method. Macroscopic classification is used to determine various breeding parameters. The correspondence or disagreement between the macroscopic and microscopic classifications allows the macroscopic scale to be checked and certain macroscopic aspects to be determined that must be taken into account to obtain the most objective classification possible.

In species with indeterminate fecundity, like *T. picturatus* (Vasconcelos et al., 2016, 2017) only the observation of histological sections allows the identification of the evolution of the development of the gonads ensuring the correct assignment of each state of maturation. In females, during the oogenesis changes occur in the structure of the cytoplasm, oocyte nucleus, and follicle (oocyte growth phase); afterward occurs vitellogenesis, which consists of the synthesis and accumulation of cell components

outside the oocyte. These reserve substances form the yolk and are stored as nutrients, the main source of energy during embryonic development. In males, during spermatogenesis germ cell development occurs within cysts formed by Sertoli cells. Successive mitosis produces smaller and smaller daughter cells, which undergo transformations consisting of the reorganisation of the cytoplasm and nucleus, until the final stage of differentiation is reached, with the appearance of an elongated cell and a flagellum.

1. Description of microscopic characteristics of each maturity stage

The microscopic description of the reproductive cycle of blue jack mackerel is based on the characteristics of the female and male gametes in the different stages of development (Figures 1 and 2).

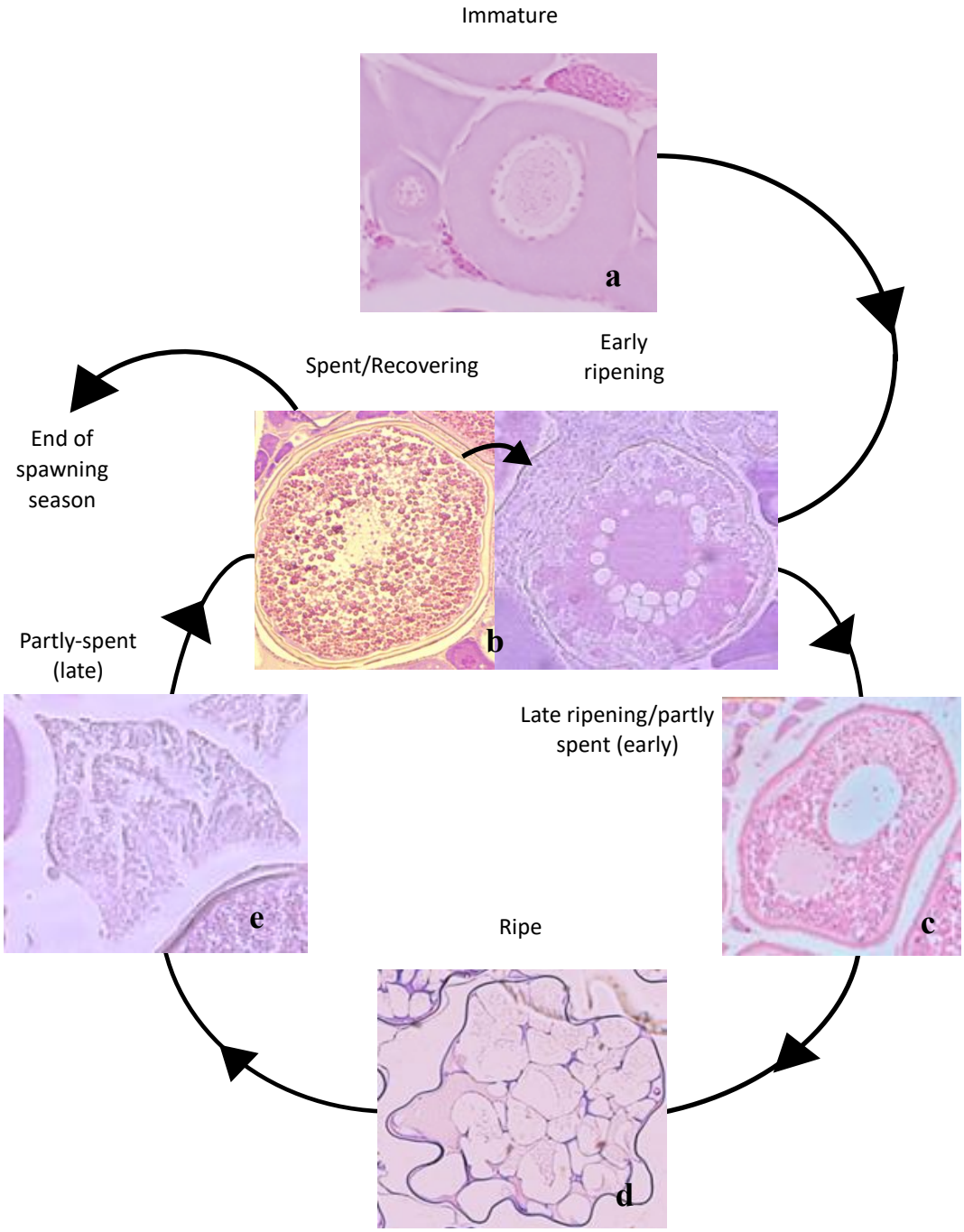


Figure 1 – Representative diagram of the stages of oocyte maturation in female blue jack mackerel. Legend: a – non-vitellogenic oocyte; b – partially yolked oocyte; c – migrated nucleus oocyte; d – hydrated oocyte; e – post-ovulatory follicle.

Figura 1 – Diagrama representativo dos estados de maturação dos oócitos das fêmeas de carapau negrão. Legenda: a – oócito não-vitelado; b – oócito parcialmente vitelado; c – oócito com núcleo migrado; d – oócito hidratado; e – folículo pós-ovulatório.

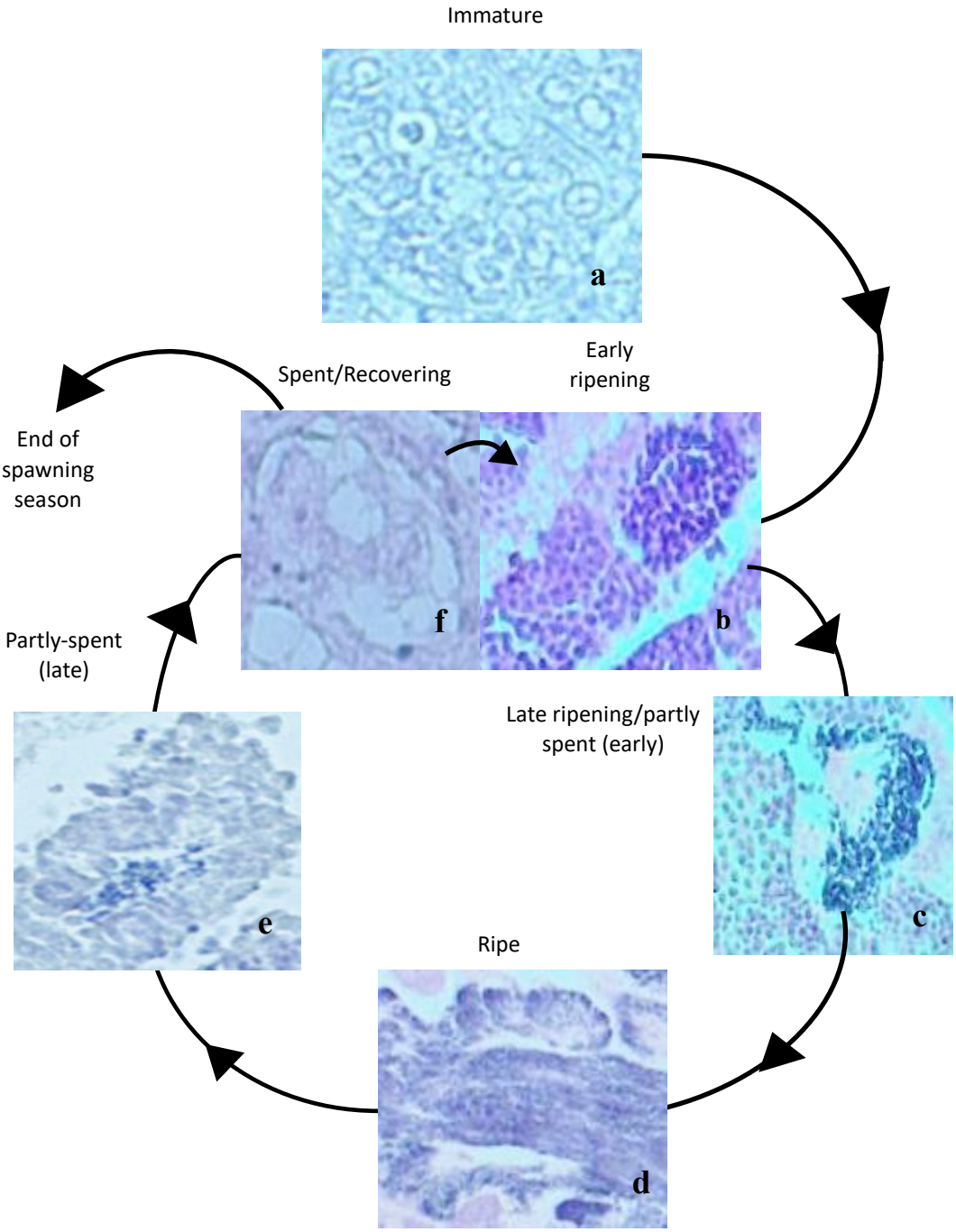


Figure 2 – Representative diagram of the stages of spermatozoa maturation in male blue jack mackerel. Legend: a – spermatogonia; b – spermatocytes in cists; c – spermatids in cists; d – abundant spermatozoids in cists and tubules; e – residual spermatozoids in tubules; f – empty tubules with thick interstitial tissue.

Figura 2 – Diagrama representativo dos estados de maturação do esperma dos machos de carapau negrão. Legenda: a – espermatogónias; b – espermatócitos em cistos; c – espermatídeos em cistos; d – espermatozoides abundantes em cistos e túbulos; e – espermatozoides residuais em túbulos; f – túbulos vazios com tecido intersticial espesso.

1.1. Female gonads

1.1.1. Non-vitellogenic oocytes (also called primary oocytes)

This class comprises the spherical oogonia with a large central round nucleus and little cytoplasm and

oocytes in the first growth stage, where the nucleoli are already identified forming a crown inside the nucleus. These oocytes are covered by a membrane, the follicle, with an inner layer consisting of cubic cells (the granulosa) and an outer layer of large elongated cells (the theca), which are difficult to distinguish (Figure 3).

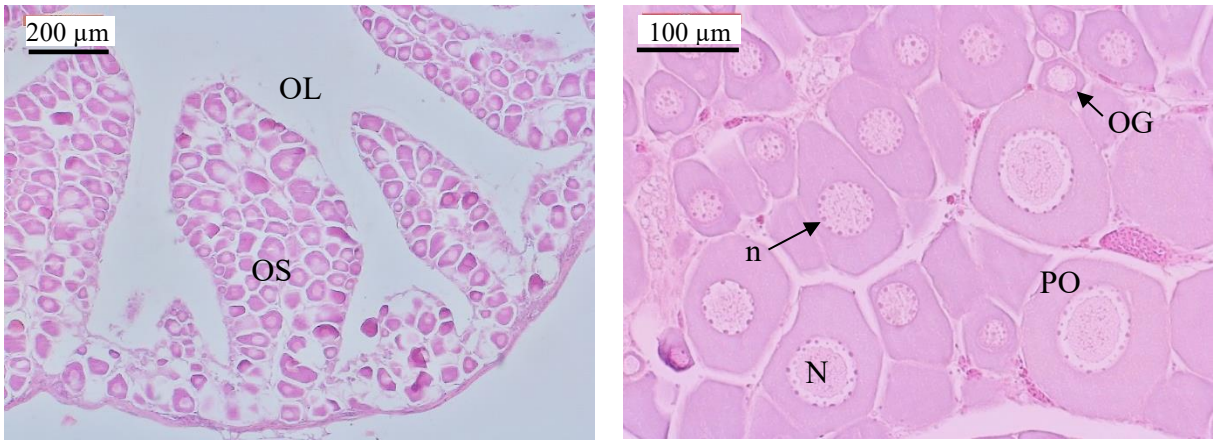


Figure 3 – Microscopic aspect of an immature ovary. OL – ovary lumen; OS – ovary septa; OG – oogonia; PO – primary oocyte; N – nucleus; n – nucleoli.

Figura 3 – Aspeto microscópico de um ovário imaturo. OL – lúmen do ovário; OS – septos do ovário; OG – oogónias; PO – oócito primário; N – núcleo; n – nucléolos.

1.1.2. Partially yolked oocytes (or at the beginning of vitellogenesis)

In this stage, the oocytes are already larger, due to the beginning of vitellogenesis and two protective layers of the oocyte develop inside the follicle: the zona pellucida, non-cellular, and the zona radiata, which is a cellular layer. Cortical alveoli, cytoplasmic vacuoles (lipid droplets), and small yolk granules can be identified first in the cytoplasmic periphery while subsequently spreading internally to the perinuclear zone. As the maturation proceeds, the follicle becomes larger because of its increased thickness and the proliferation of the granulosa cells. Theca cells do not increase in length but show an elongated appearance (Figure 4).

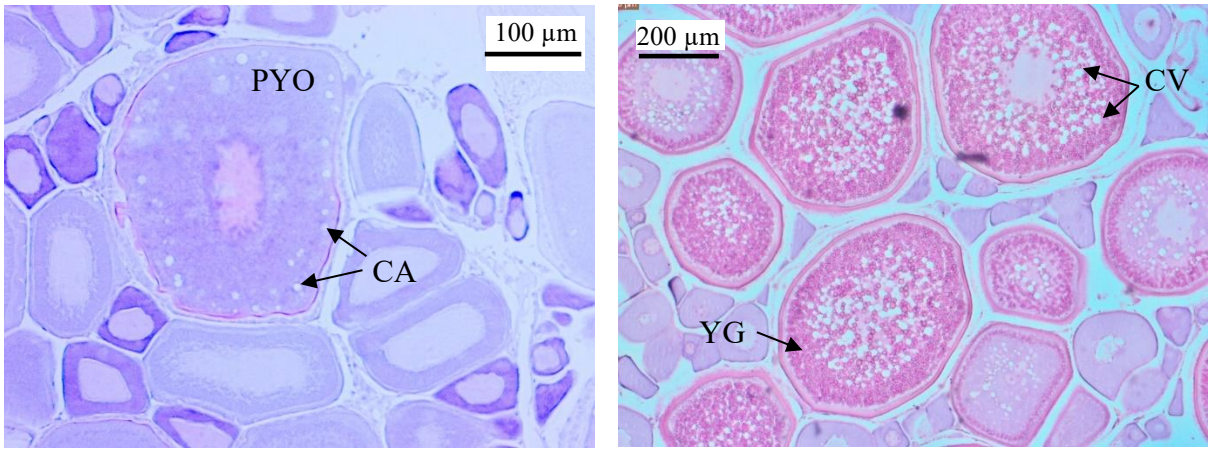


Figure 4 – Microscopic aspect of partially yolked oocytes (PYO). CA – cortical alveoli; CV - cytoplasmic vacuoles; YG – yolk granules.

Figura 4 – Aspeto microscópico de oócitos parcialmente vitelados (PYO). CA – alvéolos corticais; CV – vacúolos citoplasmáticos; YG – grânulos de vitelo.

1.1.3. Yolked oocytes

In this stage, these oocytes are bigger and in late vitellogenesis. Yolk granules become larger; oil droplets spread throughout the cytoplasm and accumulate around the nucleus; at the end of the stage, they coalesce to form yolk plates. The nucleus begins its migration to the animal pole (MN) and the oil droplets merge into plates and accumulate on one side of the nucleus. In the follicle, granulosa cells have a long rectangular shape (Figure 5).

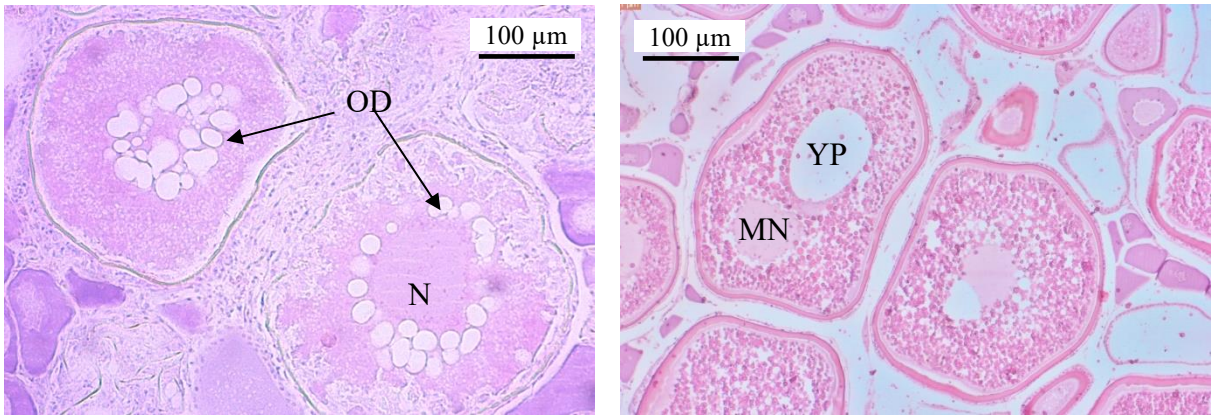


Figure 5 – Microscopic aspect of yolked oocytes. OD – oil droplets; N – nucleus; YP – yolk plates; MN – migrated nucleus.

Figura 5 – Aspeto microscópico de oócitos vitelados. OD – gotas lipídicas; N – núcleo; YP – placas de vitelo; MN – núcleo migrado.

1.1.4. Hydrated oocytes

Hydration begins when the nucleus has completed its migration to the animal pole and is no longer

visible, since at this stage the nuclear membrane disintegrates, the nucleus releases its contents into the cytoplasm and the oocytes become hyaline. Due to the rapid uptake of fluids through the microvilli of the plasma membrane, the oocytes lose their spherical shape at the end of hydration. The granulosa and the theca extend, presenting as a very thin layer (Figure 6).

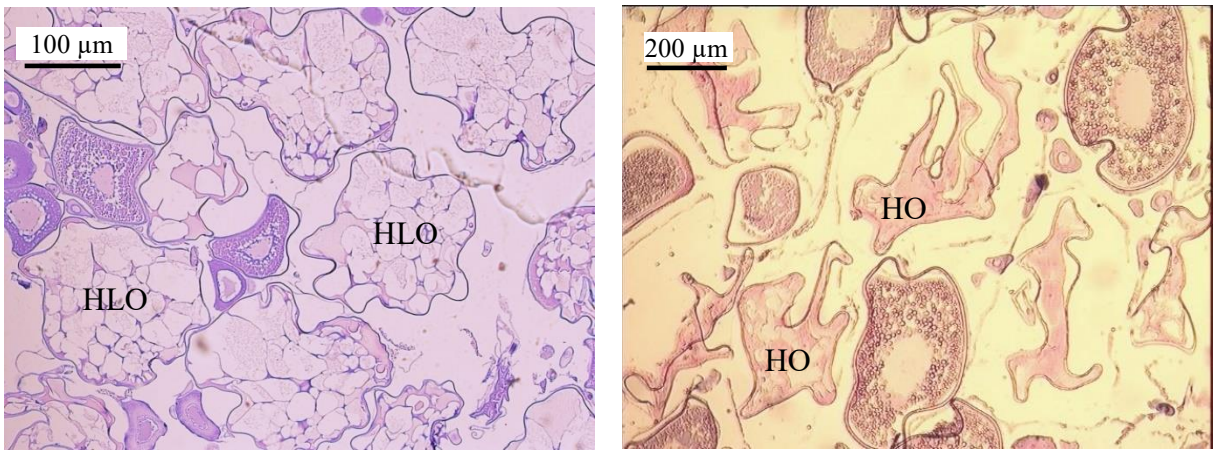


Figure 6 – Microscopic aspect of hyaline oocytes (HLO) and hydrated oocytes (HO).

Figura 6 – Aspecto microscópico de oócitos hialinos (HLO) e oócitos hidratados (HO).

1.1.5. Post-ovulatory follicles

The follicles are structures formed during vitellogenesis that wrap the oocytes until ovulation. They consist of an internal epithelial cell layer (granulosa) and an outer layer of connective tissue (the theca) with some blood capillaries. In ovulation, each hydrated oocyte is released to the gonad lumen from the follicle that surrounds it, which is not released along with the oocyte, thus maintaining its integrity and remaining in the gonad as a post-ovulatory follicle (POF). Its degeneration goes through several phases, described in Figure 7, in which they become smaller and smaller until they are completely reabsorbed.

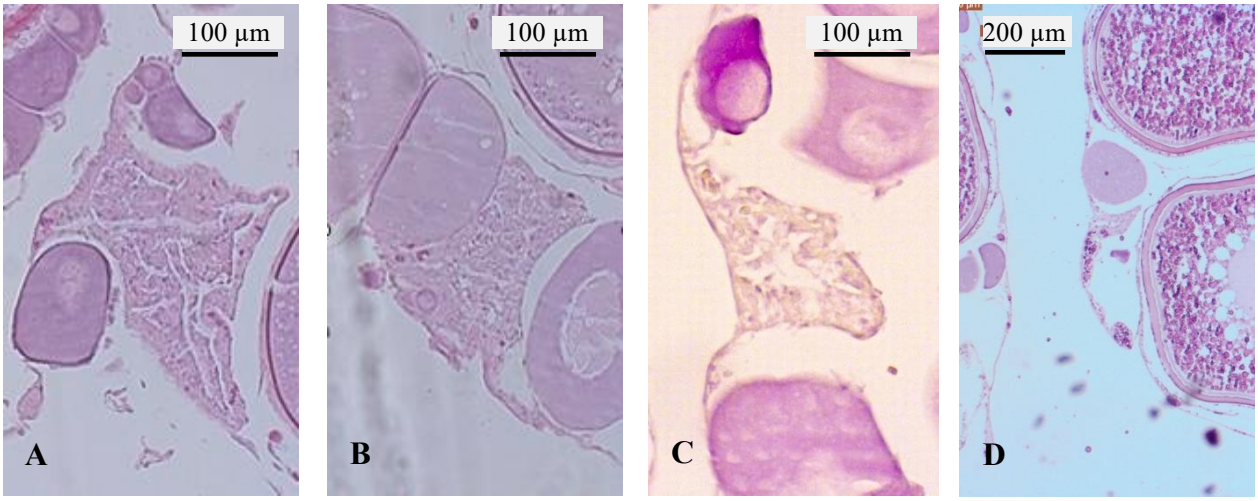


Figure 7 – Microscopic aspect of the degeneration stages of postovulatory follicles. (A) Stage 1: Large, irregularly shaped; thick granulosa and with revolutions, without signs of degeneration; very fine theca with central or basal nuclei; great lumen; (B) Stage 2: Rectangular; barely visible lumen; finer granulosa with pyknotic nuclei (round, small and black) and vacuoles; thicker theca; (C) Stage 3: Smaller and roughly triangular; reduced granulosa and no healthy nuclei; thick theca attached to the granulosa; (D) Stage 4: Very small and triangular; granulosa is almost non-existent; there is almost only theca.

Figura 7 – Aspeto microscópico dos estados de degeneração dos folículos pós-ovulatórios. (A) Estado 1: Grandes, de forma irregular; granulosa espessa, com revoluções, sem sinais de degeneração; teca muito fina, com núcleos centrais ou basais; grande lumen; (B) Estado 2: Retangulares; lumen quase ausente; granulosa fina com núcleos picnóticos (redondos, pequenos e pretos) e vacúolos; teca mais espessa; (C) Estado 3: Mais pequenos e aproximadamente triangulares; granulosa reduzida e sem núcleos saudáveis; granulosa espessa e ligada à granulosa; (D) Estado 4: Muito pequenos e triangulares; granulosa quase inexistente; praticamente só teca.

1.1.6. Atretic oocytes

Atresia is a degenerative process that occurs in the ovaries, corresponding to the absorption of non-emitted oocytes. Oocytes pass through several phases, characterised by thickening and fragmentation of the oocyte wall (zona pellucida), contraction and reabsorption of the cytoplasm, and some granulosa cells with a pyknotic nucleus (nucleus heavily stained), and a large intracellular vacuole. The final stage of atresia presents vacuoles easily confused with POFs. However, while in the latter the cavities are small and all the same size, in the atretic follicles they are of various sizes (Figure 8). This process occurs with a low incidence during the spawning season but becomes more marked towards its end. Some oocytes are also observed at the beginning of vitellogenesis, with small lipid droplets present in the cytoplasm Many blood vessels and some old degenerating post-ovulatory follicles can be present.

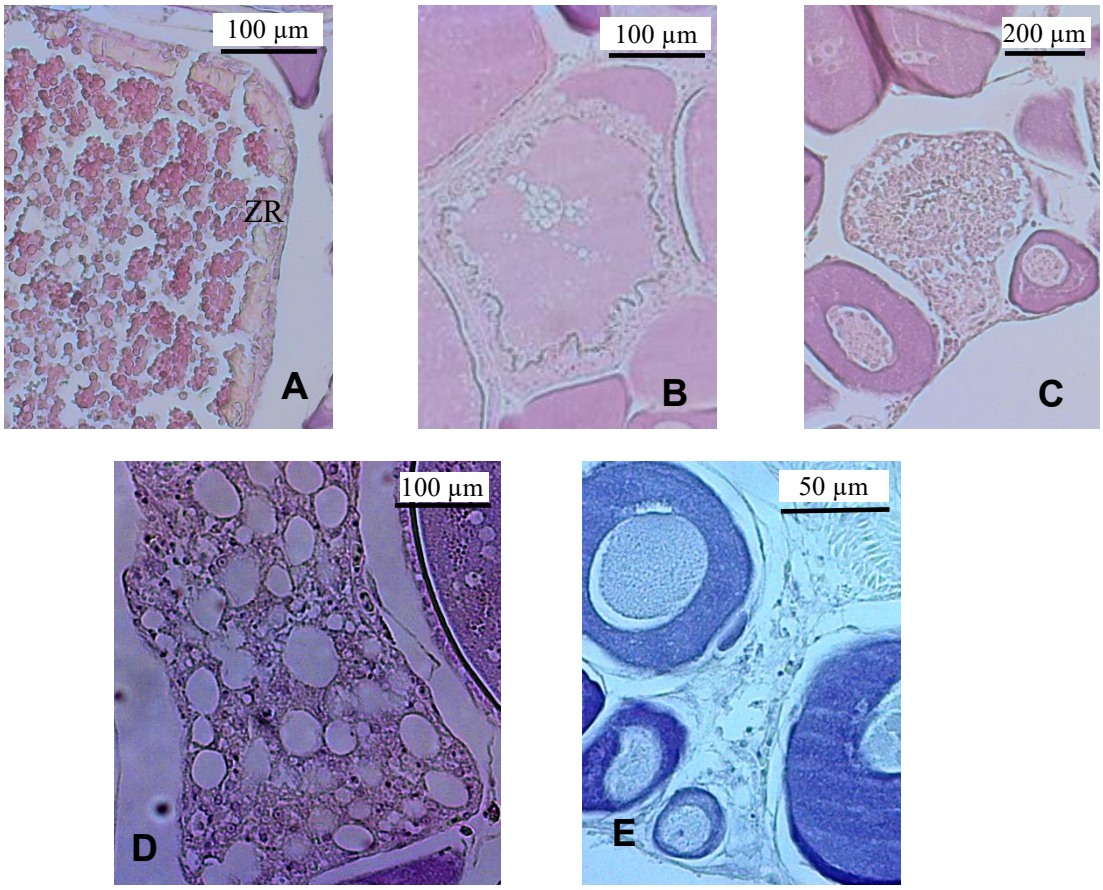


Figure 8 – Microscopic aspect of the oocytes atretic stages. (A) Alfa (α) atresia: the disintegration of the nucleus; enlargement of the granulosa cells and rupture of the zona radiata (ZR), ; (B) At the end of this stage yolk adjacent to the granulosa cells liquifies and appears as a homogeneous area; (C) Beta (β) atresia: numerous disorganised granulosa cells, with some pyknotic nuclei; thin theca; (D) Gama (γ) atresia: almost the same relative size as in β-stage, but less stained; theca with some pyknotic nuclei; large intercellular cavities; (E) Delta (δ) atresia: net of filamentous material with dark basophilic structures; very small granulosa cells and nuclei; without intercellular cavities.

Figura 8 – Aspecto microscópico dos estados de atrésia dos oócitos. (A) Alfa atrésia (α): desintegração do núcleo; aumento de tamanho das células da granulosa e ruptura da zona radiata(ZR) ; (B) No fim deste estado o vitelo adjacente às células da granulosa liquefaz-se e apresenta-se como uma área homogénea; (C) Beta atrésia (β): muitas células da granulosa desorganizadas, com alguns núcleos picnóticos; teca fina; (D) Gama atrésia (γ): quase do mesmo tamanho relativo da beta atrésia mas menos corada; teca com alguns núcleos picnóticos; grandes cavidades intercelulares; (E) Delta atrésia (δ): rede de material filamentoso com estruturas basofílicas escuras; células e núcleos da granulosa muito pequenos; sem cavidades intercelulares.

1.2. Male gonads

1.2.1. Spermatogonia

Spermatogonia are male germ cells located along the basal membrane of the seminiferous tubules of the testis but not attached to it (Figure 9). These are spherical cells of about 7µm diameter.

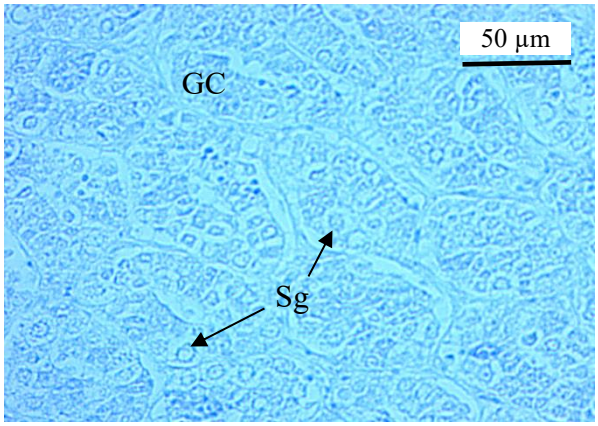


Figure 9 – Microscopic aspect of the seminiferous tubules with spermatogonia (Sg) within the germinal cysts (GC).

Figura 9 – Aspecto microscópico dos túbulos seminíferos com espermatogónias (Sg) dentro de cistos germinativos (GC).

1.2.2. Spermatocytes and spermatids

The growth phase of spermatogonia originates from the spermatocytes, smaller cells (diameter ≈ 3 µm) present in the seminiferous tubules. This is followed by the maturation phase, resulting in even smaller cells, the spermatids (diameter ≈ 1.5 µm) (Figure 10).

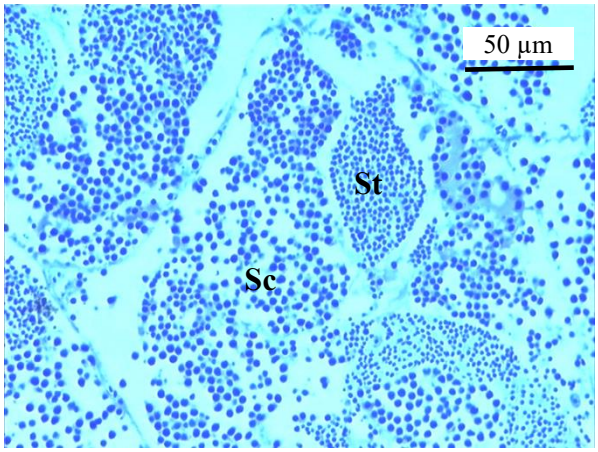


Figure 10 – Microscopic aspect of spermatocytes (Sc) and spermatids (St) in the seminiferous tubules.

Figura 10 – Aspecto microscópico de espermatócitos (Sc) e espermatídeos (St) nos túbulos seminíferos.

1.2.3. Spermatozoids

The spermatozoids develop from spermatids. They are cells with active mobility, capable of swimming freely, consisting of an oval head (about 1 µm diameter) and a tail or flagellum. They are usually grouped in the form of parachutes (Figure 11).

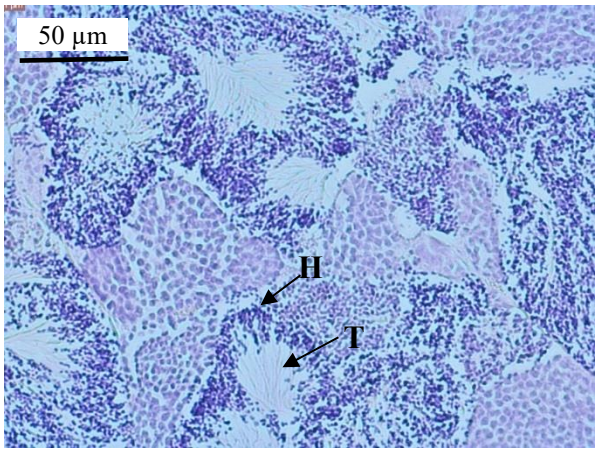


Figure 11 – Microscopic aspect of seminiferous tubules with spermatozoids in parachute form, showing heads (H) and tails (T).

Figura 11 – Aspecto microscópico dos túbulos seminíferos com espermatozoides em forma de paraquedas, onde se identificam as cabeças (H) e as caudas (T).

1.2.4. Atretic Spermatozoids

After spawning, the testis structure changes and seminiferous tubules presents strongly thickened walls and hollow tubes are left by the resorption of spermatozoa and little or no spermatocyte development observed. Little or no residual sperm in lobules (Figure 12).

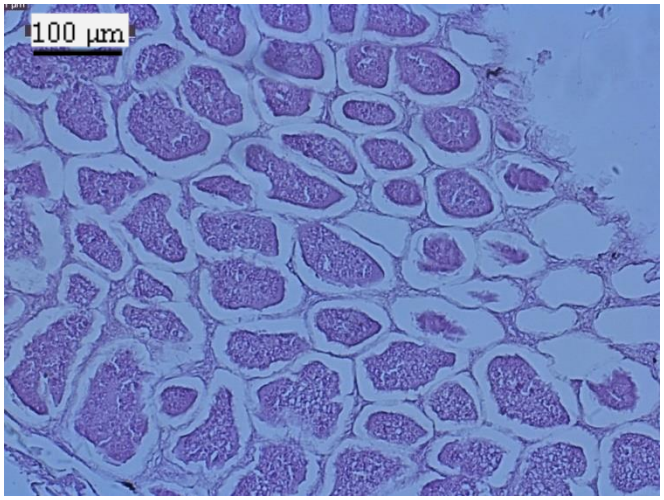


Figure 12 – Microscopic aspect of seminiferous tubules presents thickened walls and presence of blank spaces by resorption of spermatozoa.

Figura 12 – Aspecto microscópico dos túbulos seminíferos com sinais de reabsorção de espermatozoides. As paredes celulares são finas e observam-se os espaços livres dentro dos túbulos.

2. Description of macroscopic characteristics of each maturity stage with linkage to the microscopic description

2.1 Female Gonads or Ovaries

Stage 1 – Immature:

In this stage, all oocytes in gonads are in the primary growth stage, well packaged in folds oriented towards the center of the ovary.

These are small rounded ovaries, wine red and without visible oocytes (Annex I – Table I – Id.1).

Stage 2 - Early ripening:

In this stage, ovaries are characterised by oocytes bigger than in stage 1 due to the beginning of vitellogenesis, with cortical alveoli but without cytoplasmic lipid droplets. Non-vitellogenic oocytes may also be present, as well as some atresia.

Macroscopically, they are elongated, pink, and firm, still without visible oocytes (Annex I – Table I – Id.2).

Stage 3 - Late ripening/partly spent: (early):

In this stage, the oocytes are bigger and in late vitellogenesis (LVO). Yolk granules become larger and are located around the nucleus and at the end of the stage, they coalesce to form yolk plates; oil droplets spread throughout the cytoplasm and accumulate around the nucleus; there are no post-ovulatory follicles.

The gonads are thick and yellowish, already showing opaque, white, oocytes, without empty spaces (Annex I – Table I – Id.3).

Stage 4 – Ripe:

This stage is characterised by the presence of hydrated (or hyaline) oocytes (HO). Without post-ovulatory follicles but other oocytes in the earlier stages of development are present.

At this stage, the gonads are big and bulky, with externally visible hyaline oocytes, that are easily released with a slight pressure on the abdomen (Annex I – Table I – Id.4).

Stage 5 - Partly spent (late):

In this stage, ovaries show many empty spaces and few yolked oocytes. Post-ovulatory follicles (POF) are present in variable number which indicates previous spawning activity. Oocytes in several stages of atresia (AO) can also be present.

In this stage of development, the gonads have a dark pink colour, with many haemorrhagic zones. Sometimes, they have a rubbery texture. More flaccid than in stage 3, still with some hyaline oocytes mixed with opaque ones (Annex I – Table I – Id.5).

Stage 6 - Spent / Recovering spent:

In this stage, most of the vitellogenic oocytes are atretic. Atresia is a degenerative process that occurs in the ovaries, corresponding to the absorption of non-emitted oocytes. This process occurs with a low incidence during the spawning season but becomes more marked towards its end. Many blood vessels and some old degenerating post-ovulatory follicles can be present.

The gonads are flaccid, shrunken, and dark red, with many empty spaces but with some small residual opaque oocytes visible, and sometimes present hard and dark spots (Annex I – Table I – Id.6).

2.2. Male Gonads or Testes

Stage 1 – Immature:

There is no lumen in the lobules and only spermatogonia (male germ cells) are present in the seminiferous tubules.

The testes are small, very thin and flat, pale and transparent (Annex I – Table II – Id.1).

Stage 2 - Early ripening:

The seminiferous tubules contain primary spermatocytes in cysts.

Testes are a little more coloured and already with some consistency, making them a little firmer. Sometimes, *T. picturatus* testes present some hard and dark spots, the opposite of *T. trachurus*. Without sperm (Annex I – Table II – Id.2).

Stage 3 - Late ripening/partly spent (early):

The seminiferous tubules with spermatocytes and spermatids.

At this stage, spermatozoids are already present in the cysts. White testes are larger than those of the previous phase, becoming opaque (Annex I – Table II – Id.3).

Stage 4 – Ripe:

This stage is characterised by the presence of large numbers of spermatozoids in the lumen. The previous stages (spermatocytes and spermatids) may also be present in the gonad.

Large, thick, and whitish testes. Milt freely runs even without any pressure on the abdomen (Annex I – Table II – Id.4).

Stage 5 - Partly spent (late):

The walls of the seminiferous tubules begin to thicken and some amount of sperm is still observed. Gonads are flaccid, with grey spots and some sperm remains (Annex I – Table II – Id.5).

Stage 6 - Spent / Recovering spent:

Seminiferous tubules have strongly thickened walls and blank spaces left by the resorption of spermatozoa.

Small testes are dark brown, with many empty spaces, and with no sperm (Annex I – Table II – Id.6).

3. Comparison of results

From 384 gonads collected and identified by macroscopic maturity stage (Table 2), 143 were observed microscopically to validate the macroscopic stages (85 females and 58 males) and results presented in Table 3.

Table 3 – Correspondence between macroscopic (MACRO) and microscopic (MICRO) identification of the gonads. N - total gonads in each macroscopic maturity stage and n - total gonads in each microscopic maturity stage. Correctly matched macro and microscopic states - bold numbers and percentage of agreement - %.

Tabela 3 – Correspondência entre a identificação do estado macroscópico (MACRO) e microscópico (MICRO) das gónadas amostradas no estudo. N - número total de gónadas para cada estado macroscópico e n - número total de gónadas para cada estado microscópico. A correspondência correta dos estados macro e microscópicos - números destacados a negrito e percentagem de concordância em %.

	Females							Males						
	MICRO							MICRO						
MACRO	1	2	3	4	5	6	N	1	2	3	4	5	6	N
1	**6**	2					8	**1**		1			2	4
2	3	**11**	2	1	1	2	20	1	**0**	3	1	5	1	11
3		4	**3**	1		1	9			**1**	1	1		3
4				**2**	2		4			2	**6**			8
5		10	4		**2**	10	26	2		3	7	**6**	1	19
6	2	6	1			**9**	18	3		2	2	4	**2**	13
n	11	33	10	4	5	22	85	7	0	12	17	16	6	58
%	55	33	33	50	40	41		14		8	35	38	33	

As showed in Table 3, the concordance is set at 39% globally for females (33 in 85) and 48% for males (16 in 33). The stages that are the most misclassified are stages 2 and 3 for females and 1 and 3 for males, although for males there's a low agreement.

DISCUSSION

In certain species, it is not possible to distinguish between immature and resting individuals (Morgan and Trippel, 1996; Saborido-Rey and Junquera, 1998; Domínguez-Petit, 2006). In both stages, no oocytes are macroscopically visible in females, whereas in males the outer appearance of the gonads is similar. The misclassification of these two stages has an impact on the estimate of the mature proportion of the stock since the latter have already contributed to this year's spawning biomass. For this reason, the validation of the macroscopic scale through microscopy is of great importance.

Trachurus species have undetermined fecundity (Gordo et al., 2008), in which germ cells do not mature at the same time. Particularly in the ovaries, where this aspect is more evident, cells in different degrees of development being visible in the same gonad (Figure 13). Like the vast majority of commercially important marine fish species have partial spawning, a reproductive strategy characterised by spawning more than once during their lifetime (Murua and Saborido-Rey, 2003).

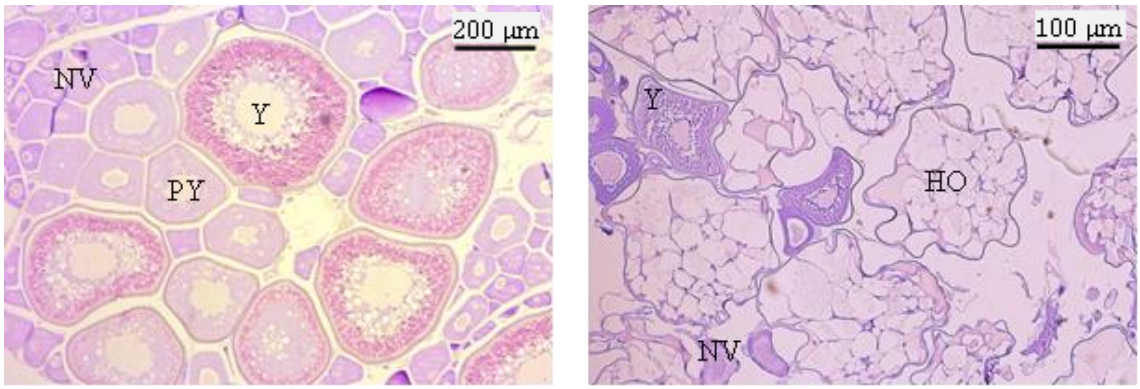


Figure 13 – Examples of ovaries with oocytes in different stages of development: NV – Non-vitellogenic oocytes; PY – Partly-yolked oocytes; Y – Yolked oocytes; HO – hydrated oocytes.

Figura 13 – Exemplos de ovários com oócitos em diferentes estádios de desenvolvimento: NV – Oócitos não-vitelogénicos; PY – Oócitos Parcialmente vitelados; Y – Oócitos vitelados; HO – Oócitos hidratados.

In these species, the major problems in macroscopic observation arise from the identification of stages 2 (Beginning of development) and 6 (Recovering/Spent), as this is a continuous process during the spawning season until the last batch is released, and in the distinction between stages 3 (Late ripening/Partly spent (early)) and 5 (Partly spent (late)). Only microscopic observation allows us to distinguish the various stages, based on the type of structures present in higher percentages.

In this work, as shown in Table 3, macroscopic maturity stages 2 and stages 6 (from males and females) were misidentified. In 20 macroscopic maturity stages 2 (females), 45% were misidentified. There's no male macroscopic identity for stage 2. In 31 macroscopic maturity stages 6, 58% were females, and 85% males were misidentified. This methodology also allows the identification of females in stage 4

(Ripe) with greater precision. As previously described, in this stage the gonads have hyaline oocytes, which are spawning indicators. However, microscopic observation may reveal the presence of post-ovulatory follicles, not externally identifiable, but indicating that this female is already in post-spawning.

For the correct attribution of the state of macroscopic maturation, the external appearance of the gonads is also very important. The use of fixatives such as alcohol and formaldehyde produces changes in the colour and consistency of the gonads, and when tissues are cooled slowly, water migrates out of cells, and ice forms in the extracellular space. Too much extracellular ice can cause mechanical damage to the cell membrane due to crushing.

The ovaries of species with indeterminate fecundity, such as those of *Trachurus* (Gordo et al., 2008), present oocytes in different stages of development, and only when hydration occurs it is possible to distinguish a stock of oocytes of greater diameter, which will be released during spawning, while oocytes in the less advanced stages start to develop. This strategy allows these species to spawn several times in each spawning season (Murua and Saborido-Rey, 2003). However, in these species, it is not possible to distinguish with the naked eye between immature and resting individuals (Morgan and Trippel, 1996; Saborido-Rey and Junquera, 1998; Domínguez-Petit, 2006). In both stages, no oocytes are macroscopically visible in females, whereas in males the outer appearance of the gonads is similar. The misclassification of these two stages has an impact on the estimate of the mature proportion of the stock since the latter have already contributed to this year's spawning biomass.

In females, during the oogenesis, changes occur in the structure of the cytoplasm, oocyte nucleus, and follicle (oocyte growth phase); afterward occurs vitellogenesis, which consists of the synthesis and accumulation of cell components outside the oocyte. These reserve substances form the yolk and are stored as nutrients, the main source of energy during embryonic development. In males, during spermatogenesis germ cell development occurs within cysts formed by Sertoli cells. Successive mitosis produces smaller and smaller daughter cells, which undergo transformations consisting of the reorganisation of the cytoplasm and nucleus, until the final stage of differentiation is reached, with the appearance of an elongated cell and a flagellum.

Macroscopic and microscopic identification of maturity stages can be applied in several studies, such as the estimation of fecundity and length of the first maturity (L_{50}), for stock assessment purposes. In these cases, the macroscopic and microscopic scales should be validated to reduce errors and build a comparison table of the two observations, where the matched percentage of each maturity stage can be used as a correction factor for all macroscopic observations.

With the results obtained during the study period, we were able to prepare the aforementioned table comparing the percentages of macroscopic and microscopic observations (Table 4), which can be

applied as a correction factor for the blue jack mackerel macroscopic maturity stages.

Despite what was mentioned before about the identification of stage 2 *vs* stage 6 and stage 3 *vs* stage 5, in this work, it was found that the higher percentage of staging mistakes of females in macroscopic stage 2, microscopically corresponded to stages 4 and 5, and of males in stage 5 that were microscopic stage 6 (Table 4).

Table 4 – Percentage of correspondence between macroscopic (MACRO) and histological examinations (MICRO) for each maturity stage.

Tabela 4 – Percentagem de correspondência entre as observações macroscópicas (MACRO) e observações histológicas (MICRO) para cada estado de maturação.

	Females							Males						
	MICRO							MICRO						
MACRO	1	2	3	4	5	6	%	1	2	3	4	5	6	%
1	75	25					100	25		25			50	100
2	15	55	10	5	5	10	100	9		27	9	46	9	100
3		45	33	11		11	100			34	33	33		100
4				40	40	20	100			25	75			100
5		40	16		8	36	100	10		16	37	32	5	100
6	11	33	6			50	100	23		15	15	31	15	100

The reasons for the discrepancies between macroscopic and histological observations are various: the state of the fish after its capture; the time between capture and the observation of the gonads; the accuracy of the description of the maturity stages in the tables used; the subjectivity of the observer. To overcome these problems, the analysis of the gonads should be done directly on board and applying a very concise maturity scale. As reported in Lasker (1985), the gonad tissues have to be sampled within 2 h of the catch, before degradation becomes significant. When assigning the macroscopic classification, it is necessary to bear in mind that the interior of the gonad can have a different appearance from what is observed (not visible to the sampler) on the surface, so one should also try to investigate the interior to have a more comprehensive view.

Also very important is the need for exchanges or standardization meetings among the various observers not only of the same laboratory but also among the countries that study the fecundity of the same resource, which have been applying different maturity scales over the years. This means that comparing datasets from different countries shows variability that may be higher than the one due to misclassifications.

To overcome this issue, the Workshop for Advancing Sexual Maturity Staging in Fish (WKASMSF), held in Copenhagen in 2018, proposed a unique maturity scale for pelagic fish, which is mandatory when reporting maturity data to ICES databases since the 1st. January 2020 (ICES, 2018).

As a conclusion to this work, Table 5 shows the correspondence between the Walsh et al. (1990) scale applied and the scale proposed during the WKASMSF (ICES 2018). For a better understanding, this new correspondence should be used in the post-processing of data after assigning macroscopic states (with eventual microscopic validation), during responses to ICES datacalls and working groups while the new maturation states are not automated during sampling biological.

Table 5 – Correspondence between the two macroscopic maturity scales – Walsh 1990 versus WKASMSF 2018.

Tabela 5 – Correspondência entre as duas escalas de maturação macroscópica – Walsh 1990 versus WKASMSF 2018.

Walsh et al., 1990		ICES, 2018	
Stage	State	Stage	Sub-stage
1	Immature	A	Immature
2	Early ripening	Ba	Developing but functionally immature
3	Late ripening/Partly spent (early)	Bb	Developing but functionally mature
4	Ripe	Ca	Actively spawning
5	Partly spent (late)	Cb	Spawning capable
6	Spent/Recovering spent	Da	Regressing
		Db	Regenerating

Nevertheless, histology seems to be the only way to obtain an accurate classification of maturity stages for reproduction studies in multiple-spawning fishes, like the blue jack mackerel (Vasconcelos, 2016).

FINAL REMARKS

The necessity for exchanges or standardisation meetings among the many observers for the same or comparable species is crucial to reducing misclassifications, especially when assigning the macroscopic categorization during surveys at sea (with very fresh fish) or landing samples (with hours or days passed after fishing), which could be extremely different. Furthermore, it must be remembered that even in well-studied species, changes may occur throughout the life cycle, necessitating occasionally a more in-depth examination of fish reproduction utilising histological methods to confirm macroscopic stages. Given that the attribution of macroscopic maturation states is prone to some subjectivity, it is preferable to obtain as much information as possible, even when utilising the new scales proposed during ICES' reproduction workshops.

ACKNOWLEDGMENTS

This work was supported by the PNAB/EU DCF - European Commission's Data Collection Framework (Council Regulation EC no. 199/2008). We would like to thank colleagues Hugo Mendes, Andreia V. Silva and Alberto Rocha for their knowledge and comments. We would also like to thank the two anonymous reviewers and Corina Chaves as editor who, with their contributions and comments, helped to improve this work.

CONTRIBUTIONS

AMC and DF coordinated the work and sample collection. GC and DF performed the biological and macroscopic classification of the gonads. DF has collected images of the macroscopic aspects. CNS, MI and PA performed the histology. AMC performed the microscopic classification of the gonads, reading the slides, classifying the gonads at the microscopic level and collecting images of the microscopic aspects. AMC carried out the statistical analysis of the results. AMC coordinated the writing of the report, elaborated the first version of the report and elaborated the description of the microscopic scale. DF and CNS wrote and revised the final version of the report. All authors reviewed the text and document before being submitted.

REFERENCES

Chaves, C., 2018. Relatório da Campanha Demersal 2018 - 02041018 IBTS PT-PGFS-Q4-2018. Relatórios de Campanha, 45p.

Claramunt, G., Cubillos, L., Herrera, G., Díaz, E., 2019. Spawning marker patterns of *Engraulis ringens* of northern Chile. *Fisheries Research*, 219. https://doi.org/10.1016/j.fishres.2019.06.004

Costa, A.M. 2001. Contribuição para o estudo da fecundidade temporal e espacial do carapau (*Trachurus trachurus* L.) na costa portuguesa. Dissertação original apresentada para acesso à Categoria de Investigador Auxiliar. IPIMAR, 2001, 180 pp.

Costa A.M. 2019. Reproductive cycle of the blue jack mackerel, *Trachurus picturatus* (Bowdich, 1825), off the Portuguese continental coast. Aquat. Living Resour. 32: 14

Costa, A.M., Borges, M.F. 1996. Total fecundity and atresia of horse mackerel (*Trachurus trachurus*) in Division IXa. ICES CM 1996/H:26.

Dominguez-Petit, R. 2006. Study of Reproductive Potential of *Merluccius merluccius* in the Galician Shelf. Doctoral Thesis. University of Vigo (Spain). 253 pp.

Gonçalves, P., Cunha, E., Costa, A. 2004. Escala de maturação microscópica e macroscópica das gónadas femininas de pescada (*Merluccius merluccius*). *Relat. Cient. Téc. IPIMAR, Série digital* (http://ipimariniap.ipimar.pt) nº 21, 27 p.

Gordo, L.S., Costa, A.M., Abaunza, P., Lucio, P., Eltink, A.T.G.W. 2008. Determinate versus indeterminate fecundity in horse mackerel. *Fisheries Research*, 89, 181-185.

ICES. 2017. Blue jack mackerel (*Trachurus picturatus*) in Subdivision 10.a.2 (Azores grounds). Stock Annex. 8 pp. https://www.ices.dk/sites/pub/Publication%20Reports/Stock%20Annexes/2017/jaa.27.10a2_SA.pdf

ICES. 2018. Report of the Workshop for Advancing Sexual Maturity Staging in Fish (WKASMSF), 30 April - 4 May 2018, ICES Headquarters, Copenhagen, Denmark. ICES CM/EOSG: 38. 75 pp.

ICES. 2021. Working Group on Southern Horse Mackerel Anchovy and Sardine (WGHANSA). ICES Scientific Reports. 3:55. 689 pp. https://doi.org/10.17895/ices.pub.8138.

ICES. 2022. Advice on fishing opportunities. In Report of the ICES Advisory Committee, 2022. ICES Advice 2022, section 1.1.1. https://doi.org/10.17895/ices.advice.19928060

Jesus GT. 1992. Study of the growth and reproduction of *Trachurus picturatus* (Bowdich, 1825) in Madeira. Doc. No XIV/C/1-1991/03 (DG XIV/CE), 1–66.

Lasker, R. 1985. An Egg Production Method for Estimation Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*. *NOAA Technical Report NMFS*, 36, 99 pp.

Methot, R.D. and Wetzel, C.R. 2013. Stock Synthesis: A biological and statistical framework for fish stock assessment and fishery management. *Fisheries Research*, 142: 86-99. https://doi.org/10.1016/j.fishres.2012.10.012

Morgan, M.J., Trippel, E. A. 1996. Skewed sex ratios in spawning shoals of Atlantic cod (*Gadus morhua*). *ICES Journal of Marine Science*, 53, 820–826.

Murua, H., Saborido-Rey, F. 2003. Female reproductive strategies of marine fish species of the North Atlantic. *Journal of Northwest Atlantic Fishery Science*, 33, 23-31.

Pérez, N., Figueiredo, I., 1992. First approach to the study of atresia in the ovary of sardine Sardina pilchardus (Walb.). Boletin Instituto Español de Oceanografía, 8, 191-199.

Rothschild, B.J., Osborn, T.R., Dickey, T.D., Farmer, D.M. 1987. The physical basis for recruitment variability in fish populations. ICES C.M. 1987/ Mini Nº 8, 19 pp.

Saborido-Rey, F., Junquera, S. 1998. Histological assessment of the variations in sexual maturity of cod (*Gadus morhua*) in Flemish Cap (Northwest Atlantic). *ICES Journal of Marine Science*, 55, 515–521.

Scott, F.; Jardim, E.; Millar, C.P.; Cerviño, S. 2016. An Applied Framework for Incorporating Multiple Sources of Uncertainty in Fisheries Stock Assessments. PLoS ONE 11(5): e0154922. https://doi.org/10.1371/journal.pone.0154922

Shepherd, J.G. 1993. “Why fisheries need to be managed” and “Why technical conservation measures on their own are not enough”. *Laboratory Leaflet, MAFF Directorate of Fisheries Research*, Lowestoft, 71, 15 pp.

Sparre, P., Ursin, E., Venema, S.C. 1989. Introduction to tropical fish stock assessment. Part I. Manual. *FAO Fisheries Technical Paper* No. 306.

Vasconcelos, J. 2016. Estrutura Populacional do Chicharro, *Trachurus picturatus* do Atlântico Nordeste. Tese de Doutoramento em Ciências Biológicas. Universidade da Madeira, 277 pp.

Vasconcelos, J.; Faria, G.; Freitas, R.; Gordo, L. 2017. Fecundity regulation strategy of the blue jack mackerel, *Trachurus picturatus* (Bowdich, 1825), off Madeira Island (NE Atlantic). *Fisheries Research*, 190(), 150–156. doi:10.1016/j.fishres.2017.02.009

Walsh, M., Hopkins, S. P., Witthames, P., Greer-Walker, P., Watson, J. 1990. Estimation of total potential fecundity and atresia in the western mackerel stock, 1989. ICES CM 1990/H:31.

ANNEXES/ANEXOS

Table I – Diagnostic characters and some aspects of the macroscopic and microscopic maturity stages of female gonads based on the applied scale (Walsh et al., 1990).

Tabela I – Caracteres diagnosticantes e alguns aspectos dos estados de maturação macroscópicos e microscópicos das gónadas femininas com base na escala aplicada (Walsh et al., 1990).

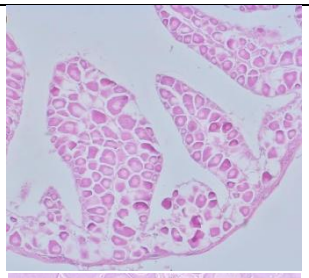
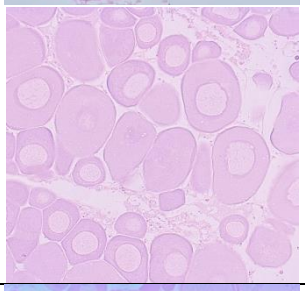
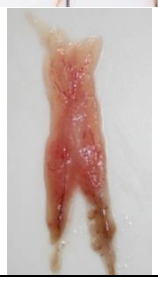
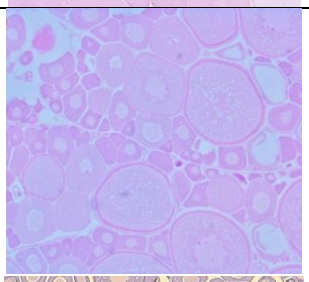
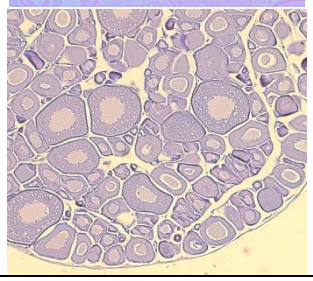
Id.	Diagnostic characters	Macroscopic aspect	Microscopic aspect	Mat. stage
1	Immature **Macroscopic:** No visible oocytes **Microscopic:** All oocytes in the primary growth stage	 	 	1
2	Early ripening **Macroscopic:** Still without visible oocytes **Microscopic:** Cortical alveoli	 	 	2

Table I – Continued

Tabela I – Continuação

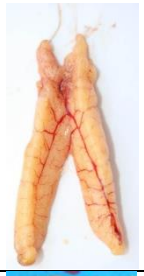
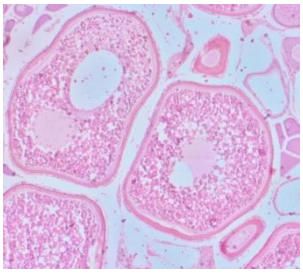
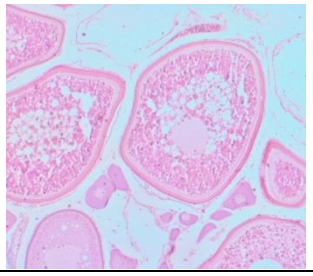
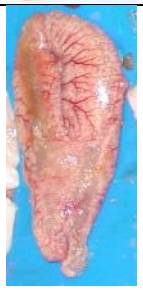
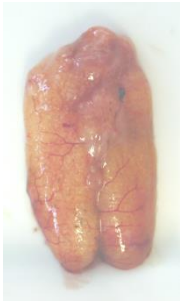
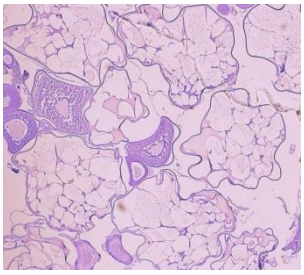
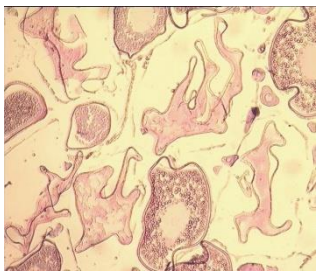
Id.	Diagnostic characters	Macroscopic aspect	Microscopic aspect	Mat. Stage
3	Late ripening/ partly spent **Macroscopic:** Already showing opaque white oocytes **Microscopic:** Migrated nucleus and no post-ovulatory follicles	 	 	3
4	Ripe **Macroscopic:** With externally visible hyaline oocytes **Microscopic:** Hydrated and/or hyaline oocytes	 	 	4

Table I – Continued

Tabela I – Continuação

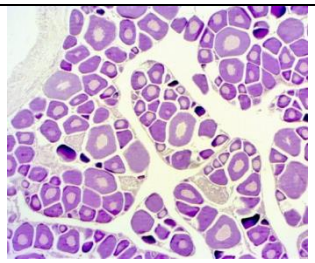
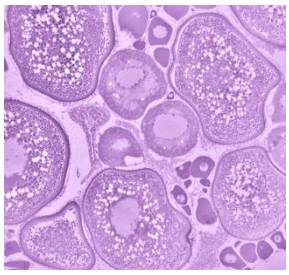
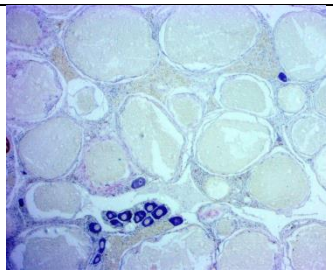
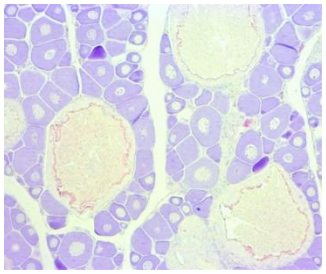
Id.	Diagnostic characters	Macroscopic aspect	Microscopic aspect	Mat. Stage
5	Partly spent **Macroscopic:** Flaccid, with many haemorrhagic zones **Microscopic:** Presence of POFs	 	 	5
6	Spent/ Recovering spent **Macroscopic:** Flaccid and shrunken with many empty spaces **Microscopic:** Most of the vitellogenic oocytes are atretic	 	 	6

Table II – Diagnostic characters and some aspects of the macroscopic and microscopic maturity stages of male gonads based on the applied scale (according to Walsh et al., 1990).

Tabela II – Caracteres diagnosticantes e alguns aspectos dos estados de maturação macroscópicos e microscópicos das gónadas masculinas com base na escala aplicada (segundo Walsh et al., 1990).

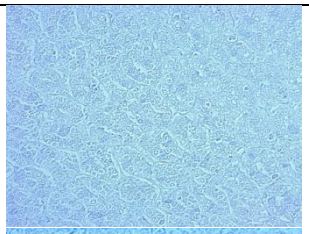
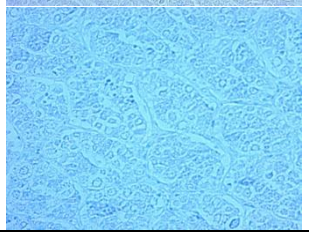
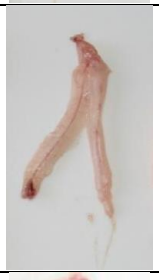
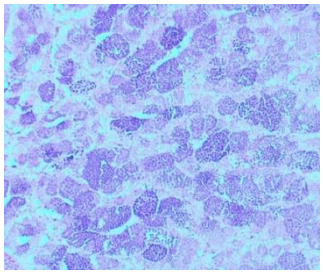
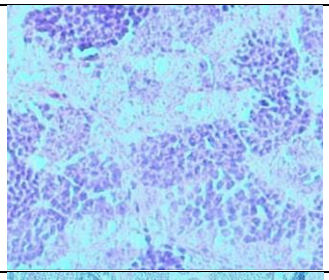
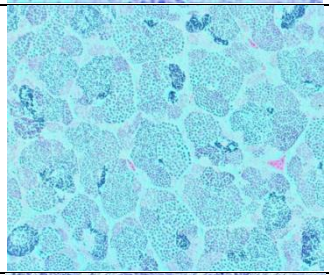
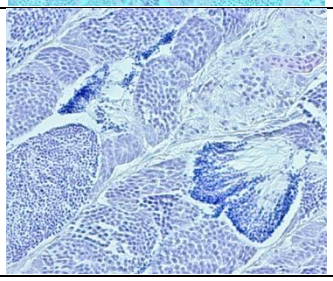
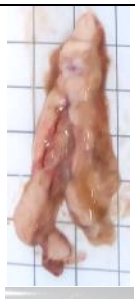
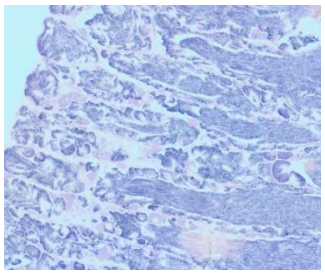
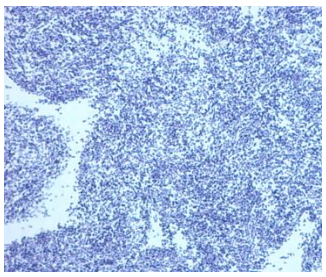
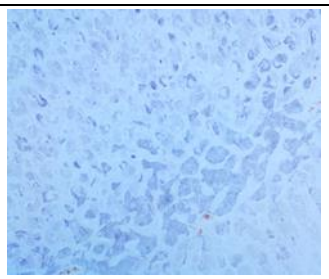
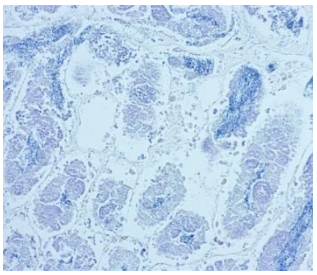
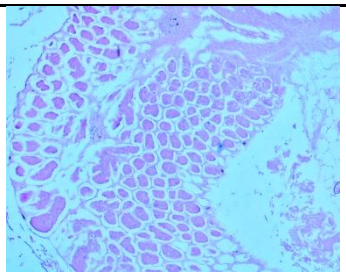
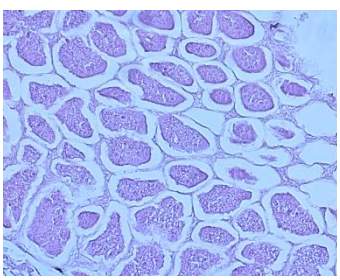
Id.	Diagnostic characters	Macroscopic aspect	Microscopic aspect	Mat. stage
1	Immature **Macroscopic:** Small, thin and transparent gonads **Microscopic:** Only spermatogonia		 	1
2	Early ripening **Macroscopic:** With some consistency. Without sperm **Microscopic:** Spermatocytes and spermatids in cysts	 	 	2
3	Late ripening/ partly spent **Macroscopic:** Testes larger and opaque **Microscopic:** Spermatocytes and spermatids in tubules; spermatozoids in cysts	 	 	3

Table II – Continued

Tabela II – Continuação

Id.	Diagnostic characters	Macroscopic aspect	Microscopic aspect	Mat. Stage
4	Ripe **Macroscopic:** Milt freely running **Microscopic:** Spermotozoids in the lumen	 	 	4
5	Partly spent **Macroscopic:** Flaccid, with some sperm remains **Microscopic:** Some amount of sperm is still observed	 	 	5
6	Spent/ Recovering spent **Macroscopic:** Empty spaces and no sperm **Microscopic:** Thick walls and blank spaces	 	 	6


MINISTÉRIO DO MAR
IPMA
Instituto Português
do Mar e da Atmosfera