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WHY DO WE NEED HISTOLOGY OF HAKE (*Merluccius merluccius*) GONADS? THE PORTUGUESE CASE

Ana Maria Costa e Maria do Carmo Silva

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WHY DO WE NEED HISTOLOGY OF HAKE (*MERLUCCIUS MERLUCCIUS*) GONADS? THE PORTUGUESE CASE

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ABSTRACT

With a monthly sampling frequency between 2010 and 2013, 4238 fish were collected and their gonads were macroscopically and microscopically staged. The percentages of error encountered with the macroscopic assignments were analysed by sex, length class and month, both for all stages together and for stage 1 separately, where the highest percentages of error are found on the identification of the immature stages that in fact were already mature. When considering all the individuals the highest errors occurred in the females (11%), in the length classes of 28 and 40 cm (26% and 25%, respectively) and in June (17%). The greatest misidentifications of maturity stage 1 were verified in males in length classes under 32 cm (92%) and over 57 cm (100%) and in the months of June, July and October (100%). The L_{50} decreased 8.1 cm (from 34.4 to 26.3 cm) with the microscopic identification, considering combined sexes and 2.3 cm (from 40.6 to 38.3 cm) only in females.

Key words: Hake, histology, length-at-first maturity.

RESUMO

Título: Porque fazer histologia das gónadas de pescada (*Merluccius merluccius*)? O caso português

Com uma amostragem mensal entre 2010 e 2013 foram recolhidos 4238 exemplares e foi atribuído o seu estado de maturação macroscópico e microscópico. As percentagens de erro encontradas na identificação macroscópica foram analisadas por sexo, classe de comprimento e mês, para todos os estados de maturação em conjunto e para o estado 1 em separado, tendo as maiores percentagens de erro sido encontradas na identificação dos estados imaturos em gónadas que na realidade já estavam maturas. Considerando todos os indivíduos, os maiores erros ocorreram nas fêmeas (11%), nas classes de comprimento dos 28 e 40 cm (26% e 25%, respectivamente) e em Junho (17%). O maior erro na atribuição do estado 1 verificou-se nos machos (92%), nas classes de comprimento abaixo dos 32 cm (92%) e acima dos 57 cm (100%) e nos meses de Junho, Julho e Outubro (100%). O L_{50} decresceu 8.1 cm com a identificação microscópica, de 34,4 para 26,3 cm, considerando os sexos combinados e 2,3 cm nas fêmeas, de 40,6 para 38,3 cm.

Palavras-chave: Pescada, histologia, comprimento de primeira maturação.

REFERÊNCIA BIBLIOGRÁFICA

COSTA, A.M. and SILVA, M.C., 2016. Why do we need histology of hake (*Merluccius merluccius*) gonads? The Portuguese case. ***Relat. Cient. Téc. do IPMA* (http://ipma.pt) *nº 11 14 p.***

INTRODUCTION

Knowledge of the dimension of the reproductive stock is essential for the assessment of the state of exploitation of fish resources (Bromley, 2003). This evaluation is based on the combined modelling of biological information (reproduction, growth, feeding habits, among others) and fisheries (fishing effort, catches, landings and discards) (Rothschild *et al.*, 1987; Sparre *et al.*, 1989; Shepherd, 1993). However, the biological characteristics must be the fundamental basis for the conservation and management of marine living resources (Cadima, 2000). In any reproductive study the first step is the identification of the sex of the individual, and the identification of the level of maturation is the subsequent one (Vazzoler, 1996). West (1990) points out that the identification of the reproductive cycle of a species is made by classifying the development of the gonads and Guerra and Sánchez (1998) state that the study of the maturation of the gonads is an essential item for the identification of reproductive seasons and areas, of the achievement of the gonads' first maturation and of fecundity. One of the main criteria for assessing the state of exploitation of a fishery relies on the knowledge of spawning stock biomass (SSB) (Bromley, 2003), forwhich the maturation stage is its principal component. To know the spawning season and the number of mature and immature fish of a certain species, information about the maturity stages of those individuals is necessary. To obtain the maturity ogives it is necessary to correctly identify maturity stages which present specific characteristics of oocyte developmental status in the case of the female gonads and spermatozoa in the male gonads. The correct assignment of the maturity stage is extremely important since errors in its determination may lead to an incorrect estimation of the spawning biomass and therefore of the exploitation status of the stock. Gonad maturity stages are usually assigned by macroscopic observation of their external characteristics. This methodology allows the analysis of a great number of individuals with a reduced effort and in a very short time period. However, doubts raised by some experienced observers justified a review of the maturity scale by contrast to histological observation. In particular, doubts were raised regarding the occurrence of immature individuals much larger than expected for first maturity, which therefore may be resting, and externally present gonads of characteristically immature individuals (Morgado and Gonçalves, 2007). These issues led to the development of a microscopic maturity scale formerly with five stages (Gonçalves *et al.*, 2004) and finally converted to four stages (Morgado and Gonçalves, 2007), which must always be applied in order to correctly distinguish between mature and immature individuals.

The aim of this paper is to demonstrate the need of histology to assign maturity stages to hake gonads, and the implications of its application in the correct definition of the spawning season

and in the determination of length at first maturity.

MATERIAL AND METHODS

This study is based on the observation of 4238 individuals (1233 males, 2870 females and 135 indeterminate specimens) caught from 2010 to 2013 by the fishing fleets that land on the Portuguese coast in Póvoa do Varzim, Matosinhos, Peniche and Olhão. The characteristics of the samples studied each month are indicated in Table 1. All fish were measured (total length in cm), weighted (total weight in g), sexed and the maturity stage was assigned according with the four stage maturity scale (ICES, 2007). Gonads were removed and fixed in 10% formaldehyde neutralized with phosphate salts for subsequent histological processing. Sections of 3-5 μm were stained with hematoxylin-eosin and analysed under a light microscope to determine microscopic maturity stage according to Morgado and Gonçalves (2007). The macroscopic and microscopic characteristics of each maturity stage are given in Table 2. The error in the macroscopic identification was evaluated by sex, length class and month, not only taking into account all fish sampled but also considering only macroscopic stage 1, which correct identification is crucial to get the real percentage of mature and immature specimens. Due to the importance of knowing the error in the length at first maturity induced by incorrect determination of maturity, macroscopic and microscopic maturity ogives were designed considering only the females and both sexes combined (the current method used in hake assessment).

Table 1 - Number of fish sampled per month
Tabela 1 - Número de peixes amostrados por mês

Year	Month												Total	Length range (cm)
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
2010	51	98	119	178	131	24	52	128	178	41	239		1239	20.7-76.7
2011	191	49	89	120	187	52	103	38	93	5	163	44	1134	25.0-76.5
2012	167	101	62	36	76	41	95	34	69	80	119	70	950	23.0-92.0
2013	172	91	75	32	179	15	46	57	23	50	60	115	915	23.0-86.1

Table 2 - Macroscopic and microscopic characteristics of each maturity stage
Tabela 2 - Características macroscópicas e microscópicas de cada estado de maturação

Maturity stage	Macroscopic appearance - Females	Microscopic appearance - Females
1. Immature / Resting	Small ovaries, with firm consistence and minimal vascularization, transparent or pinkgrey, without opaque or hyaline oocytes	This stage includes two stages that are not macroscopically distinguishable - immature and resting. In the immature females all the oocytes in the ovary are in primary growth stage and the oocytes are well packaged. The resting ovary doesn't present mature oocytes, has a wide ovary wall, lamellae are not as compact as in immature ovaries and blood vessels may be more abundant
2. Developing / Maturing	Medium or large ovaries, pink or yellow to orange, with vascularization variable, present and obvious. Opaque oocytes present but without hyaline oocytes	This stage is characterized by the occurrence of cortical alveoli and/or vitellogenic oocytes, but POFs are not present and no signs of advanced spawning processes, such as a thick ovary wall, high vascularization of gonad and/or disorganization of lamellae
3. Spawning	**A - Hydrated -** Large ovaries, with firm consistence and vascularization, pink or redish orange. Opaque and hyaline oocytes present **B - Partial spawning -** Large ovaries, flaccid, with vascularization, pink or redish orange. With opaque oocytes present but without hyaline oocytes	Presence of high percentage of hydrated oocytes or at the beginning of the hydration process POFs are observed throughout the ovary together with vitellogenic oocytes in different stages. There are no signs of advanced spawning process, such as high number of blood vessels, swelling ovary wall, atresia, disorganization of ovary structures, etc.
4. Post-spawning	Small or medium ovaries, flaccid, dark pink, orange or purple. Opaque and hyaline oocytes absent or residual	Females at this stage will no longer produce more oocytes to be released during the current breeding season. They are characterized by a high level of atresia, old POFs, disorganization of ovary structures, numerous blood vessels, thick ovary wall and absence of yolked oocytes groups (excepting some atretic yolked oocytes)

Maturity stage	Macroscopic appearance - Males	Microscopic appearance - Males
1. Immature / Resting	Small testis, transparent or white, with the shape of a thin ribbon, with no signs of development. Without sperm	There are only spermatogonia and primary spermatocytes present
2. Developing / Maturing	Medium testis, with the shape of develop bands. Sperm flows when testis are cut	The presence of secondary spermatocytes and spermatids is the main characteristic
3. Spawning	Large white testis, with the shape of large bands. Sperm flows with pressure on the abdomen	Seminiferous tubules are thick and full of spermatozoa
4. Post-spawning	Large testis, white or light pink, empty and deformed. Sperm absent or residual	Seminiferous tubules are empty, with some residuals spermatozoa. Spermatogonia are present in the testis cortex

RESULTS

The results of the comparison between macroscopic and microscopic assignment highlight differences in the macroscopic identification of immature fish which have been found to be mature when analysed microscopically. The highest error occurred in females, 11%, against 6% in males, which results in a 10% error when considering all individuals sampled (Figure 1).

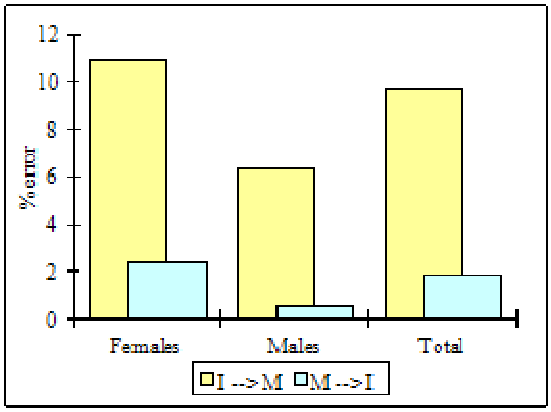

Figure 1 - Percentage of error of macroscopic identification by sex (all stages considered)
Figura 1 - Percentagem de erro na identificação macroscópica por sexo (considerados todos os estados)

Since, as explained above, the macroscopic distinction of mature and immature stage 1 is difficult, the analysis was repeated taking into account only the macroscopic stage 1 assignements, which lead to different results, as shown in Figure 2. In this case the percentage of error was extremely high 84% with sexes combined, 82% for females and 92% for males.

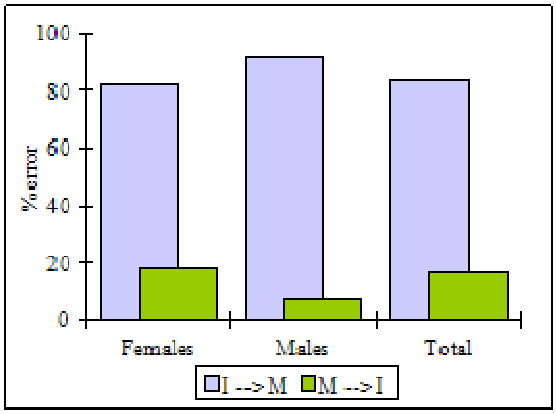

Figure 2 - Percentage of error of macroscopic identification by sex (stage 1)
Figura 2 - Percentagem de erro na identificação macroscópica por sexo (estado 1)

The analysis by length class show highest percentage error between 26 and 45 cm, with a maximum of 26% and 25% in the 28 and 40 cm length classes, respectively. Individuals under 26 cm and over 63 cm were all correctly identified (Figure 3).

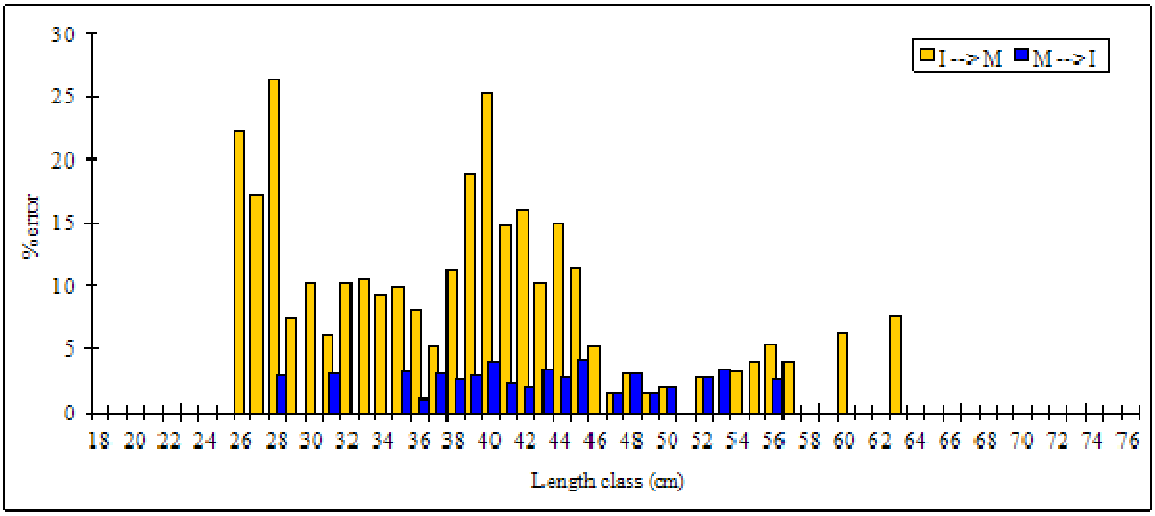

Figure 3 - Percentage error in macroscopic identification by length class (all stages)
Figura 3 - Percentagem de erro na identificação macroscópica por classe de comprimento (todos os estados)

Considering once again only macroscopic stage 1, the distribution of errors is very different (Figure 4): all fish bellow 32 cm and above 57 cm were identified as immature but in fact they were all mature. On the other hand, 47, 53 and 54 cm length classes contained no mature individuals although identified as such macroscopically.

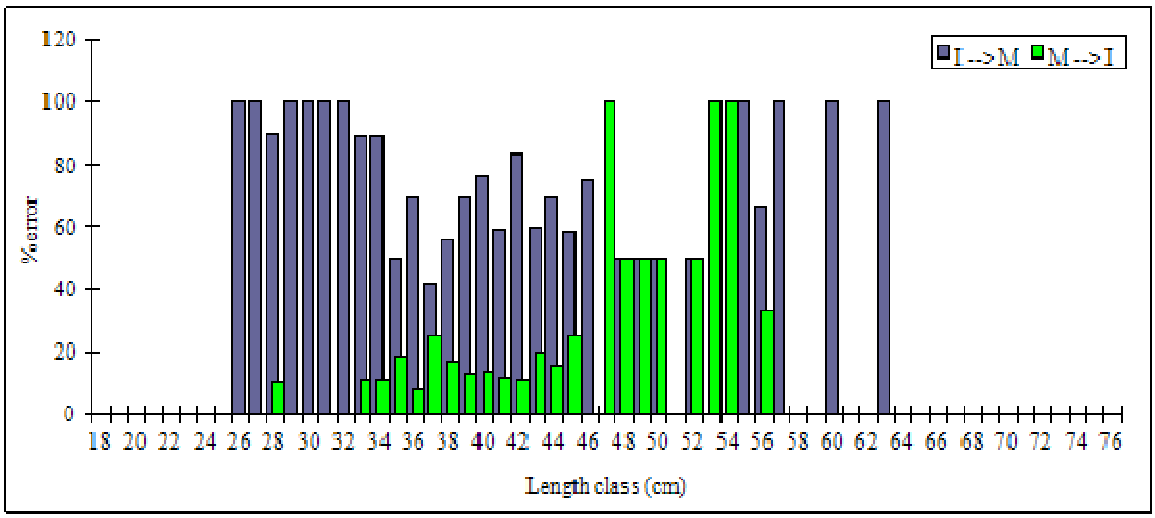

Figure 4 - Percentage of error in macroscopic identification by length class (stage 1)
Figura 4 - Percentagem de erro na identificação macroscópica por classe de comprimento (estado 1)

About the monthly distribution of all maturity stages there is a great similarity between macroscopic and microscopic identifications (Figure 5), although the latter showed smaller percentages of immature fish in the samples.

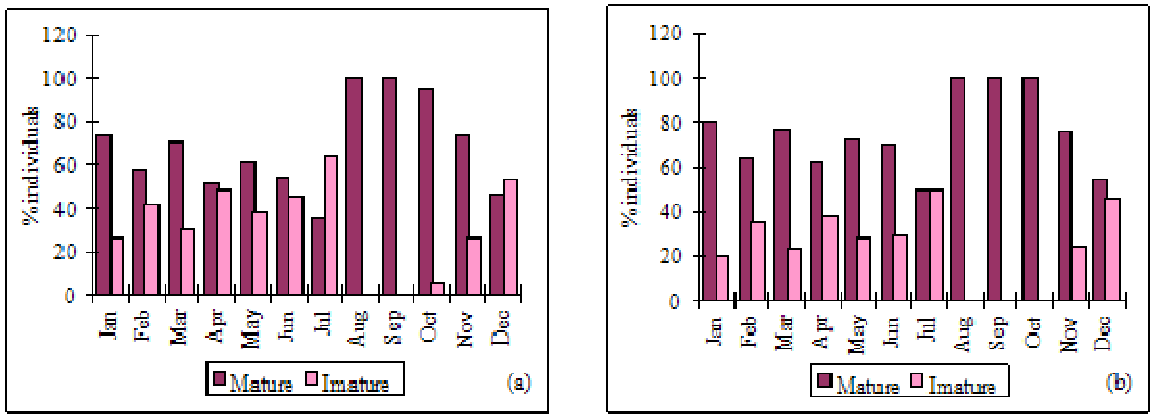

Figure 5 - Percentages of mature and imature individuals (macroscopic (a) and microscopic (b) identification)
Figura 5 - Percentagem de indivíduos maturos e imaturos (identificação macroscópica (a) e microscópica (b))

To understand the error introduced in these identifications the analysis of Figure 6 shows that in both cases the highest errors occurred out of the spawning season (17% in June and 14% in July for all stages), reaching 100% in June, July and October when considering only macroscopic stage 1.

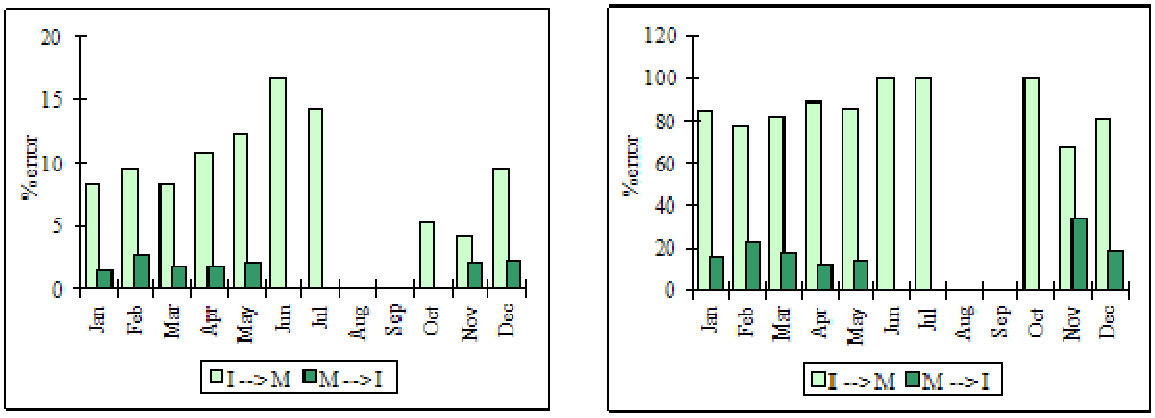

Figure 6 - Percentage of error of macroscopic identification by month of all stages (left) and of stage 1 alone (right)
Figura 6 - Percentagem de erro na identificação macroscópica por mês de todos os estados (esquerda) e apenas do estado 1 (direita)

The analysis of the distribution of the maturity stages by month (Figure 7) shows that there is no great discrepancy between macroscopic and microscopic identifications for males. On the

other hand, regarding the females the microscopic identification reveals some data not displayed with the macroscopic staging: the macroscopic maturity stage 1 (immature) actually includes not only immature individuals but also resting ones (mature); in August and September there were fish spawning (Stage 3A), which were not identified macroscopically and the post-spawning fish (Stage 3B) occurred all year round and not only during the spawning season; the high percentages of resting fish (Stage 4) which were identified just prior to the spawning season were not found by microscopic analysis.

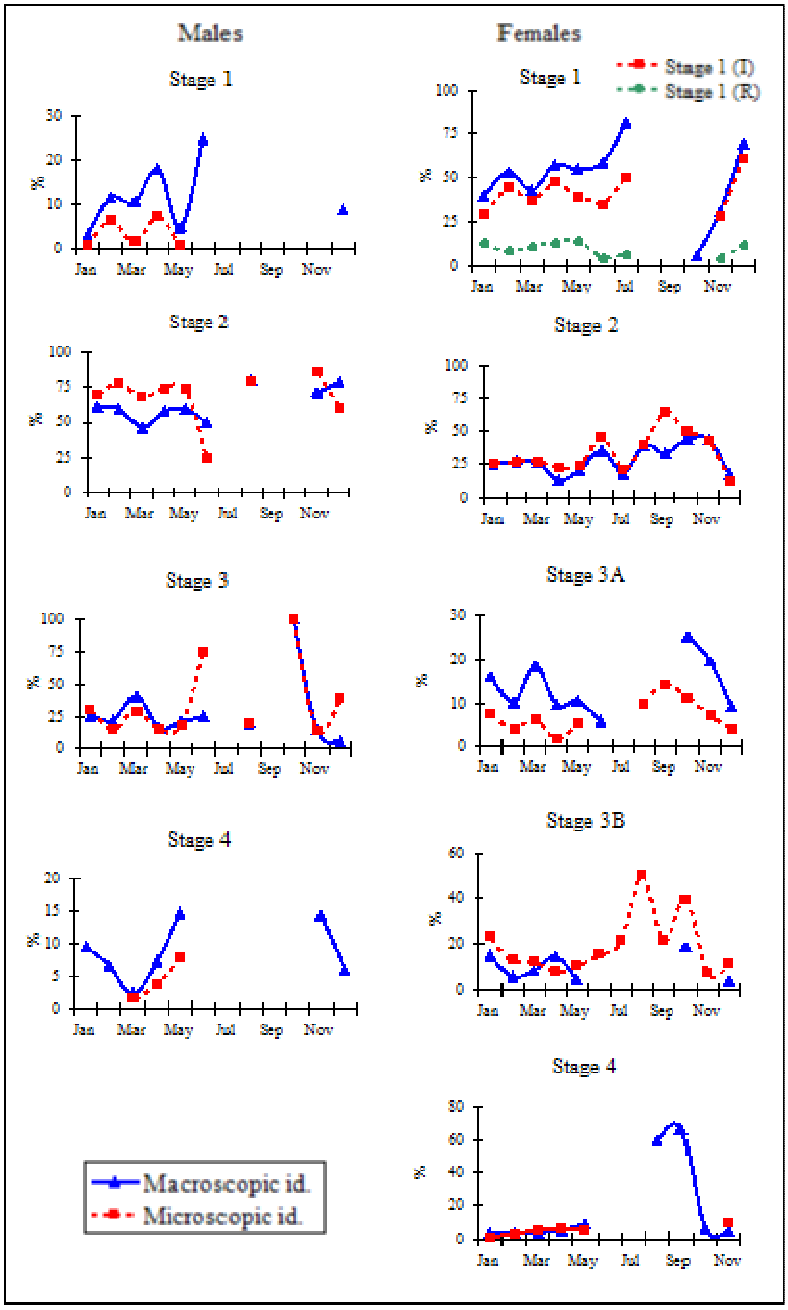

Figure 7 - Monthly distribution of macroscopic and microscopic maturity stages
Figura 7 - Distribuição mensal dos estados de maturação macroscópicos e microscópicos

Length at first maturity also showed differences, not only between determinations made from all individuals (males and females) versus only females, but also between the ones obtained under, macroscopic and microscopic observations, as shown in Figure 8 and Table 3.

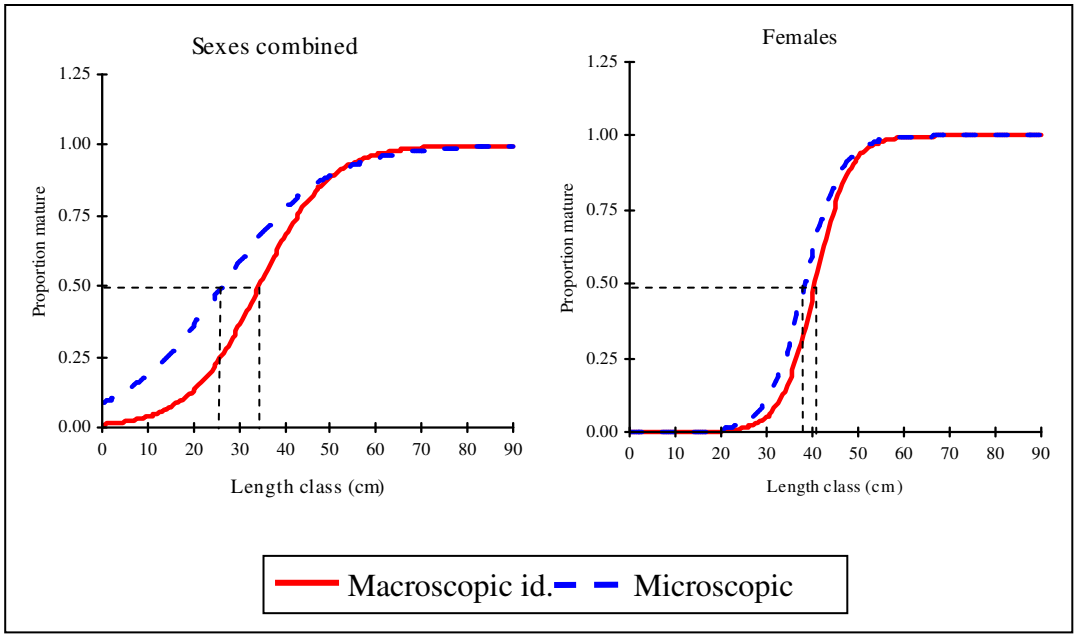

Figure 8 - Macroscopic and microscopic maturity ogives of combined sexes (N = 4103) and only females (N = 2870)
Figura 8 - Ogivas de maturação macroscópicas e microscópicas de sexos combinados (N = 4103) e só fêmeas (N = 2870)

Table 3 - Parameters of the maturity ogives of combined sexes and only females
Tabela 3 - Parâmetros das ogivas de maturação de sexos combinados e só de fêmeas

	Macroscopic identification		Microscopic identification	
	Sexes combined	Females	Sexes combined	Females
a	4.5156	11.1032	2.3670	9.6926
b	0.131	0.273	0.090	0.253
R	0.952	0.994	0.921	0.994
L_{50}	34.4	40.6	26.3	38.3

The correct identification of mature and immature fish through the microscopic observation leads to an L_{50} lower than the one obtained under macroscopic identification - 8 cm for sexes combined and 2 cm for females only.

DISCUSSION

The maturity scales proposed by Lucio *et al.* (2000) and Gonçalves *et al.* (2004) do not separate immature from resting individuals since they are not distinguishable macroscopically, which may lead to an underestimation of the spawning biomass. Adult hake which at the end of the spawning season are resting (macroscopically similar to the immature stage), will not be considered in the determination of SSB, although they have contributed to the reproductive potential of that year. Similarly to other species, such as *Gadus morhua* (Vitale *et al.*, 2006), this is a common issue also to other species of the genus *Merluccius*, for which the use of histology is essential for the correct identification of the immature and resting maturity stages, as referred by Honji *et al.* (2006), Louge and Christiansen (1993), Louge (1996) and Vaz-dos-Santos *et al.* (2005) for the argentine hake (*Merluccius hubbsi*). The application of histology to the gonads of adult individuals of different geographic areas and periods combined with the distribution of eggs and larvae of the species can at the same time provide some important information about the migratory behaviour of that species (Christiansen *et al.*, 1986). At the same time, histology also allows a verification of the synchrony or asynchrony of ovarian development, which is valuable information to characterise a multispawner species, as reported by Balboutin and Fischer (1981) and Goldberg (1985) for *Merluccius gayi gayi*. Regarding stock management, the error introduced by the incorrect assignment of the mature and immature stages may have a strong impact on stock size progression estimates.

This work shows that the monthly distribution of maturation states and consequent identification of spawning season is not much different considering macroscopic and microscopic identifications. The value obtained macroscopically for the length at first maturity of females, 40.6 cm, is slightly lower than the ones indicated in other references (41.1 cm in Martos *et al.*, 1996, for the Northwest African waters; 44.1 cm in Cardador, unpublished, for the Portuguese waters; 45.4 cm in Piñeiro and Saínza, 2003, for the Northwest Iberian Peninsula; 47.1 cm (in 2003) and 45.7 cm (in 2004) in Domínguez-Petit, 2006, on the Galician shelf; 46.5 cm in Domínguez-petit *et al*., 2008, for Galicia and the Bay of Biscay; 32.5 cm in Al-Absawy, 2010, for Egyptian Mediterranean waters; 45-50 cm in Murua, 2010, for Galicia and the Bay of Biscay; 41.3 cm in Costa, 2014, for the Portuguese coast and 21.5 cm in Soykan *et al.*, 2015, for the Central Aegean Sea, Turkey), as well as the 34.4 cm for sexes combined (36.0 cm in Cardador, unpublished; 37.9 cm in Piñeiro and Saínza, 2003 and 34.5 cm in Costa, 2014). However, the microscopic analysis corrects these figures allowing a more accurate assessment of the Iberian hake stock.

So, the results obtained with this study seem to indicate that in order to get a good assessment

of the stock of hake all states considered immature macroscopically should be examined histologically.

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REFERENCES

AL-ABSAWY, M., 2010. The reproductive biology and the histological and ultrastructural characteristics in ovaries of the female gadidae fish *Merluccius merluccius* from the Egyptian Mediterranean water. *African Journal of Biotechnology*, 9 (17): 2544-2559.

BALBONTIN, F.; FISCHER, W., 1981. Sexual cycle and fecundity of the hake, *Merluccius gayi gayi*, from the coasts of Chile. *Revista de Biología Marina y Oceanografía de Valparaíso*, 17 (3): 285-334.

BROMLEY, P.J., 2003. The used of market sampling to generate maturity ogives and to investigate growth, sexual dimorphism and reproductive strategy in central and south-western North Sea sole (*Solea solea* L.), *ICES Journal of Marine Science*, 60 (1): 52-65

CADIMA, E., 2000. Manual de avaliação de recursos pesqueiros. *FAO Documento Técnico sobre as pescas*. No. 393. Roma, FAO. 162 pp.

CHRISTIANSEN, H.E.; GLORIOSO, P.D.; OLIVIERI, C.E., 1986. Application of the histological determination over hake stocks (*Merluccius hubbsi*), typical tissues fecundity evaluation and their relation with environmental conditions. *Publicación de la Comisión Técnica Mixta del Frente Marítimo (Argentina/Uruguai)*, 1 (2): 567-574.

COSTA, A.M., 2014. Variação do comprimento da pescada na primeira maturação entre 2000 e 2010 na costa portuguesa. *Relatórios Científicos e Técnicos do IPMA* (http://ipma.pt) *nº 1. 16p.*

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

DOMÍNGUEZ-PETIT, R.; KORTA, M.; SABORIDO-REY, F.; MURUA, H.; SAINZA, M.; PIÑEIRO, C., 2008. Changes in size at maturity of European hake Atlantic populations in relation with stock structure and environmental regimes. *Journal of Marine Systems*, **71** (3-4):

260–278.

GOLDBERG, S.R., 1985. Seasonal spawning cycle of merluza *Merluccius gayi* (Merluccidae) from Chile. *Bulletin of the Southern California Academy of Sciences*, 84 (3): 172-174.

GONÇALVES, P.; CUNHA, M.E.; COSTA, A.M., 2004. Escala de maturação microscópica e macroscópica das gónadas femininas de pescada (*Merluccius merluccius*). *Relatórios Científicos e Técnicos, IPIMAR, Série digital*, nº 21, 35 pp.

GUERRA, A.; SÁNCHEZ, J.L., 1998. Fundamentos de explotación de recursos vivos marinos. Zaragoza, Editorial Acribia, 249 p.

HONJI, R.M.; VAZ-DOS-SANTOS, A.M.; ROSSI-WONGTSCHOWSKI, C., 2006. Identification of the stages of ovarian maturation of the Argentine hake *Merluccius hubbsi* Marini, 1933 (Teleostei: Merlucciidae): advantages and disadvantages of the use of the macroscopic and microscopic scales. *Neotropical Ichthyology*, 4 (3):329-337.

ICES, 2007. Report of the Workshop on Sexual Maturity Staging of Mackerel and Horse Mackerel (WKMSMAC) ICES CM 2007/ACFM:26.

LOUGE, E.B., 1996. Time and space reproductive differences of hake *Merluccius hubbsi* Marini, 1933 during its winter concentration in the Argentinean-Uruguayan common fishing zone. *Boletín del Instituto Español de Oceanografía,* 11 (2): 123-139.

LOUGE, E.B.; CHRISTIANSEN, H.E., 1993. Fecundidad de la merluza *Merluccius hubbsi*, durante la temporada estival de 1988. *Boletín del Instituto Español de Oceanografía*, 9 (2): 343-346.

LUCIO, P.; MURUA, H.; SANTURTÚN, M. 2000. Growth and reproduction of hake (*Merluccius merluccius*) in the Bay of Biscay during 1996-1997. *Ozeanografika*, 3: 325-354.

MARTOS, A.R.; YRAOLA, I.S.; PERALTA, L.F., 1996. Biologia de la merluza europea *Merluccius merluccius* (Linnaeus, 1758), de las costas de Africa Noroccidental. *Oceanografía y Recursos Marinos en el Atlántico Centro-Oriental*, *Instituto Canario de Ciencias Marinas*,: 225-239.

MORGADO, C.; GONÇALVES, P., 2007. Maturação de pescada (*Merluccius merluccius* Linnaeus, 1758): exercício de calibração e proposta de uma nova escala de maturação. *Relatórios Científicos e Técnicos, IPIMAR, Série digital* nº 39, 13 p + anexo.

MURUA, H., 2010. The Biology and Fisheries of European Hake, *Merluccius merluccius*, in the North-East Atlantic. *Advances in Marine Biology*, 58: 97-154.

PIÑEIRO, C.; SAÍNZA, M.A., 2003. Age estimation, growth and maturity of the European hake (*Merluccius merluccius* (Linnaeus, 1758)) from Iberian Atlantic waters. *ICES Journal of Marine Science*, 60 (5): 1086-1102.

ROTHSCHILD, B.J.; OSBORN, T.R.; DICKEY, T.D.; FARMER, D.M., 1987. The physical basis for recruitment variability in fish populations. I.C.E.S. – Recruitment Processes in Marine Ecosystems, C.M. 1987/ Mini Nº 8, 19 p.

SHEPHERD, J.G., 1993. "Why fisheries need to be managed" and "Why technical conservation measures on their own are not enough". *Laboratory Leaflet, Ministry Agriculture, Fisheries and Food, Directorate of Fisheries Research*, Lowestoft, 71, 15 pp.

SOYKAN, O.; ÍLKYAZ, A.T.; METÍN, G.; KINACIGÍL, H.T., 2015. Age, growth and reproduction of European hake (*Merluccius merluccius* (Linn., 1758)) in the Central Aegean Sea, Turkey. *Journal of Marine Biological Association of U.K.*, 95 (4): 829-837.

SPARRE, P.; URSIN, E.; VENEMA, S.C., 1989. Introduction to tropical fish stock assessment. Part I. Manual. *FAO Fisheries Technical Paper* No. 306.

VAZ-DOS-SANTOS, A.; HONJI, R.M.; ROSSI-WONGTSCHOWSKY, C.L.D.B., 2005. Comprimento e idade da primeira maturação gonadal de fêmeas de merluza *Merluccius hubbsi* Marini, 1933 na região sudeste-sul do Brasil. *Boletim do Instituto de Pesca*, São Paulo, 31 (2): 109-117.

VAZZOLER, E., A., E. de M., 1996. Biologia da Reprodução de Peixes Teleósteos: teoria e prática. Maringá, EDUEM, 169 pp.

VITALE, F.; SVEDÄNG, H.; CARDINALE, M., 2006. Histological analysis invalidates macroscopically determined maturity ogives of the Kattegat cod (*Gadus morhua*) and suggests new proxies for estimating maturity status of individual fish. *ICES Journal of Marine Science*, 63 (3): 485-492.

WEST, G., 1990. Methods of assessing ovarian development in fishes: a review. *Australian Journal of Marine and Freshwater Research*, 41 (2): 199-222

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