Updated maturity parameters based on histology of megrim (*Lepidorhombus whiffiagonis*) and four spot megrim (*L. boscii*) stocks in Atlantic Iberian waters (Div. 8.c, 9.a) and in Celtic Seas (Div. 7.b-k)

Domínguez-Petit, R.[1], Landa, J.[2], Fernández, J.C.[3,4], Abad, E.[1]

1. Instituto Español de Oceanografía (IEO, CSIC). Centro Oceanográfico de Vigo. Subida a Radio Faro, 50. 36390 Vigo. Spain.
2. Instituto Español de Oceanografía (IEO, CSIC). Centro Oceanográfico de Santander. Promontorio San Martín, s/n. 39004 Santander. Spain.
3. Cooperativa de Armadores de Pesca del Puerto de Vigo (ARVI). Puerto Pesquero, Edificio Ramiro Gordejuela. 36202 Vigo. Spain,
4. Organización de Productores de Pesca Fresca del Puerto de Vigo (OPPF-4). Puerto Pesquero, Edificio Ramiro Gordejuela. 36202 Vigo. Spain.

Abstract

The maturity ogives of megrim (*L whiffiagonis*) and four spot megrim (*L. boscii*) stocks in Atlantic Iberian waters (ICES Div. 8.c, 9.a) and Celtic Seas and northern Bay of Biscay (ICES Div. 7.b-k, 8.abd) currently used in the stock assessment are based on macroscopic observations and were estimated in 1998. This study presents updated maturity ogives and parameters by length and age in Div. 8.c,9.a2 (Galician waters and Cantabrian Sea), and in Div. 7.b-k (Celtic Sea, West and South of Ireland) by specie for both sexes combined based on a more robust microscopic methodology.

A total of 1708 individuals were sampled between 2017 and 2020 on board the commercial vessels and research surveys, 876 *L. whiffiagonis* of which 538 in Div. 7.b-k and 338 in 8.c, 9.a2, and a total of 832 *L. boscii*, 435 in Div. 7.b-k and 397 in 8.c, 9.a2.

L. whiffiagonis in 8.c, 9.a2 showed a L_{50} for both sexes combined of 18.9 cm and an A_{50} of 1.6 years, while in 7.b-k were 17.8 cm and 2.4 years, respectively. On the other hand, *L. boscii* in 8.c, 9.a2 showed a L_{50} and an A_{50} for both sexes combined of 14.7 cm and 1.6 years, respectively, while in 7.b-k were 16.7 cm and 2.3 years respectively.

Keywords: reproduction; maturity ogive; histology; L50; A50; flatfish, Northeast Atlantic.

1. Introduction

The state of the megrim (*Lepidorhombus whiffiagonis*) and the four spot megrim (*L. boscii*) stocks in Atlantic Iberian waters (ICES Div. 8.c, 9.a) and that of the megrim stock in Celtic Seas and northern Bay of Biscay (ICES Div. 7.b-k, 8.abd) is annually assessed in ICES since more than two decades ago. The catches of the Spanish fleet, mainly Galician, represent a very significant percentage of the total landings of these stocks. Analytical models (XSA-VPA) have always been used in the assessment of the three stocks, but 2016 was the first year in which a Bayesian (analytical) assessment model was used for the *L. whiffiagonis* stock in 7.b-k, 8.abd. The *L. boscii* stock in ICES Div. 7.b-k, 8.abd has now also been included in the assessment process in 2017 but still without analytical model (ICES, 2017). The Spawning Stock Biomass (SSB) is one of the parameters estimated in the

assessment process, basic information needed for the calculation of recruitment projections and the biological reference points (Blim, Bpa, Bloss, etc).

SSB is estimated based on the percentage of mature individuals by age or size (maturity ogive). The maturity ogives used by the ICES working group for the assessment of the *L. whiffiagonis* stock in 7.b-k, 8.abd, and for both Atlantic Iberian stocks, were estimated in 1998 based on macroscopic maturity data (BIOSDEF project, Pereda et al., 1998). The maturity parameters of *L. whiffiagonis* in 7.b-k, also estimated based on macroscopic information, were updated in the Benchmark Workshop on Flatfish (WKFLAT) (Landa et al., 2012) although they were not incorporated into the stock assessment process. Maturity parameters need to be updated periodically and, as far as possible, validated by microscopic (histological) methods.

Maturity and growth are two of the most plastic features of the life history of exploited fish. The maturity ogive can vary over time, and with it, the SSB. These shifts can be phenotypic, in response to environmental variations and, therefore, reversible, or they can be genotypic, due to genetic changes in the population and hardly reversible. Gerritsen (2016) observed some variability in the length at first maturity (L_{50}) of the *L. whiffiagonis* stock in 6.a and 7.abgj between 2004 and 2015, in addition to significant differences with respect to the maturity ogive used in the assessment process. However, these differences and variations could be due to sampling limitations (insufficient spatial coverage) and methodological errors, because the maturity stages were assigned based on macroscopic observations that have a higher error than the microscopic ones.

Taking into account the need to obtain updated information on the main biological parameters of these two species for a better assessment of the state of these stocks, the objective of the present study is to estimate, in a robust way (histological), the maturity ogive of the four stocks of interest to the Spanish fleet: *L. whiffiagonis* and *L. boscii* in Div. 7.b-k, 8.abd, and in Div. 8.c, 9.a.

2. Materials and methods

A total of 1708 individuals were sampled between 2017 and 2020, 973 in ICES Div. 7.b-k (Celtic Sea, West and South of Ireland) and 735 in Div. 8.c, 9.a2 (Galician waters and Cantabrian Sea). A total of 876 *L. whiffiagonis* were sampled, 538 in Div. 7.b-k and 338 in 8.c, 9.a2, and a total of 832 *L. boscii*, 435 in Div. 7.b-k and 397 in 8.c, 9.a2. The composition of the sample according to species and sex is shown in Table 1.

The sampling of individuals larger than 20 cm in total length was performed in March and April 2017 on board the commercial vessels "Manuel Laura", "Pescasar" and "Skellig Light II". Because the minimum landing size for both *Lepidorhombus* species is 20 cm, fish between 5 and 20 cm were obtained from the Instituto Español de Oceanografía (IEO, CSIC) research surveys, “Demersales” (in Div. 8c-9a2) and “Porcupine” (in Div. 7b,c,k) that took place in September-October 2017 on board of the research vessels “Miguel Oliver” and “Vizconde de Eza”, respectively. In all cases, bottom trawling was the fishing gear used.

The total length (mm), weight (g), sex and macroscopic maturity stage from each individual were taken on board and the sagitta otoliths were removed for age estimation in the lab. In addition, gonads were collected and immediately fixed in 4% buffered formaldehyde. In the laboratory, the gonads were weighed (to the nearest 0.01 g) and a histological study was performed. For this, a sub-sample was extracted from the central zone of each gonad. The histological processing consisted in the inclusion in paraffin of the sub-samples, the cutting using a microtome to sections of 3.5 microns and the staining with hematoxylin-eosine. The observation methodology and the age estimation criterion

followed the protocol of Anon (1997) described for *L. whiffiagonis*, and that is similar for both *Lepidorhombus* species. The age interpretation in these species is based on the count of the translucent rings (hyaline) that are considered as annual in whole otoliths.

The histological sections of the gonads were examined to determine the maturity stage of each individual. In males, individuals were classified as mature or immature, while a scale of 6 maturity stages was used for females (Table 2). The percentage of mature individuals was estimated by length and age for each species, sex and study area. The maturity ogives and the length and age at first maturity (L_{50} and A_{50}) were estimated, fitting the data to the logistic model of Ashton (1972).

The maturity ogives at age were performed fitting the data of each individual, using its "absolute age". This "absolute age" was estimated as a function of the capture season, adding to the age estimated the proportional part of the year (month) in which it was caught. Thus, in the specimens collected in the period of February-March, which coincides with the spawning season and, therefore, close to the theoretical month of birth, their "absolute ages" were the same as the estimated ages (i.e. ages 1, 2, 3, etc.). However, in the specimens caught in the research surveys (performed in September-October) the aforementioned fit was incorporated, and the "absolute age" for those specimens was approximately half a year older (i.e. ages 1.5, 2.5, 3.5, etc.) than those captured in March-April.

The maturity ogives at age and length was estimated by using the sizeMat R package (Torrejón-Magallanes, 2020).

3. Results and Discussion

Table 3 summarizes the parameters of the maturity ogives for each stock. Results showed that the length and age at maturity was larger in the northern stock than in the southern one for both species of *Lepidorhombus*.

L. whiffiagonis in **7.b-k** showed a length at first maturity (L_{50}) for both sexes combined of **17.8** cm, which corresponded to an age at first maturity (A_{50}) of **2.4** years; while in **8.c, 9.a2** showed a L_{50} of **18.9** cm and a A_{50} of **1.6** years. The maturity ogive by length and age is shown in Table 4 and 5 respectively.

L. boscii in **7.b-k** showed a length at first maturity (L_{50}) for both sexes combined of **16.7** cm which corresponded to an age at first maturity (A_{50}) of **2.3** years; while in **8.c, 9.a2** showed a L_{50} of **14.7** cm and a A_{50} of **1.6** years. The maturity ogive by length and age is shown in Table 4 and 5 respectively.

The observed differences between the maturity ogives currently used in the assessment and those estimated in the present study could be due to wrong macroscopic assignation of maturity stages (Pereda et al., 1998) (Table 4). As previously mentioned, and especially in the case of males, maturation occurs very early in these species and it is not possible to macroscopically identify the maturity stage of the small gonads of the youngest mature individuals. On the other hand, the maturity ogives estimated by Pereda et al. (1998) were obtained from individuals larger than 16 cm, while in the present study individuals larger than 4 cm were sampled, thus covering a wider length range of small individuals. It is also relevant to consider the difference of 20 years between the study of Pereda et al. (1998) and the current study. It is known that the fish stocks maturity in parameters are very plastic features that can present high interannual variability since they are influenced not only by environmental factors, but also by density-dependent factors (Cardinale and Modin, 1999, Domínguez-Petit et al. al., 2007; Pérez-Rodríguez et al., 2013). Considering this plasticity and our results, it is recommended a regular update of the maturity ogives to be taken into account by the relevant stock assessment group (WGBIE), on which the estimation of the spawning stock biomass

(SSB) depends and, therefore the perception of the state of the stock. In that sense, it is advisable doing a special effort to obtain samples of individuals smaller than 15 cm and estimating the maturity ogives based on microscopic observations, since it is impossible to distinguish visually the true maturity stage in the smallest gonads.

Acknowledgements

We thank Andrés Villaverde and Marisol Pérez for their help in the laboratory analyses and Lorena Rodríguez for the age estimation of the samples, as well as the scientist and crew on board the IEO research surveys “Demersales” and “Porcupine”, for their collaboration in the sampling of young specimens and the crews from the fishing vessels: "Manuel Laura", "Pescasar" and "Skellig Light II", for their collaboration in the sampling of mature individuals.

This study was funded by the Fisheries Producer Organization, OPPF-4 (*Organización de Productores de Pesca Fresca del Puerto Vigo*) and by the EU through the European Maritime and Fisheries Fund (EMFF) as an action included in a Production and Marketing Plan. It also has received funds from the National Program of collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy.

References

Anon, 1997. Report of workshop on megrim otolith and fin rays age reading. IEO, Vigo, Spain, 47 pp.

Ashton, W.D. 1972. The logit transformation with special reference to its uses in bioassay. Haffner Publishing Co., Inc. New York. 88pp.

Cardinale, M., Modin, J. 1999. Changes in size-at-maturity of Baltic cod (*Gadus morhua*) during a period of large variations in stock size and environmental conditions. Fisheries Research, 41(3): 285-295.

Domínguez-Petit, R. 2007. Study of reproductive potential of *Merluccius merluccius* in the Galician s helf. Tesis Doctoral. Universidad de Vigo. 253 + xxii pp.

Gerritsen, H. 2016. Maturity-at-age estimates for Irish Demersal Stocks in VIa and VIIabgj 2004-15. I CES Working Group for the Celtic Seas Ecoregion 4–13 May 2016. Copenhagen, Denmark.

Gibson, R.N., 2005. Flatfishes: Biology and Exploitation. Ed. R.N. Gibson. Blackwell Publishing, Oxford, 391 pp.

ICES. 2017. Report of the Working Group for the Bay of Biscay and the Iberian waters Ecoregion (WGBIE), 4-11 May 2017, Cadiz, Spain. ICES CM 2017/ACOM: 12. 522 pp.

Landa, J., Fontenla, J., Gómez, A. 2012. Sexual maturity of megrim (*Lepidorhombus whiffiagonis*) caught by the Spanish fleet in ICES Subarea VII. Working document to Benchmark Workshop on Flatfish (WKFLAT), Bilbao, Spain, 1-7 March 2012.

Pereda, P., Afonso, M.H., Azevedo, M., Dawson, W., Duarte, R., Dupouy, H., Franco, J., Godinho, M.L., Landa, J., Loureiro, I., Lucio, P., Macara, H., Mahé, J.C., Pérez,

N., Piñeiro, C., Saínza, M., Santurtún, M., Trujillo, V., 1998. Final Report of the project “Biological st udies of demersal fish (BIOSDEF)”. UE DG XIV 95/038.

Pérez-Rodríguez, A., Morgan, J., Koen-Alonso, M., Saborido-Rey, F. 2013. Disentangling genetic cha nge from phenotypic response in reproductive parameters of Flemish Cap cod Gadus morhua. Fisherie s research, 138: 62-70.

Tables and Figures

Table 1. *Descriptive statistics of analyzed variables by specie, area and sex. Values corresponds to mean (S.D.), saving age that is presented as a range (min-max). N: nº of sampled specimens. North: ICES division 27.7.b-k-8.abd, South: ICES division 27.8.c, 9.a. n.s.: not sampled.*

Sex	Variable	*L. boscii*		*L. whiffiagonis*	
		South	North	South	North
Females	Length (mm)	223.9 (69.2)	239.3 (71.4)	238.7 (69.3)	314.1 (120.7)
	Weight (g)	71.6 (72.7)	144.7 (136.3)	78.2 (72.0)	353.5 (370.5)
	Age	1-9	1-12	1-7	1-11
	N	209	220	192	296
Males	Length (mm)	204.4 (53.5)	219.9 (57.5)	204.0 (47.5)	227.9 (64.8)
	Weight (g)	32.5 (19.4)	58.7 (49.4)	33.3 (13.3)	77.8 (70.4)
	Age	1-8	1-12	1-5	1-9
	N	158	191	146	217
Indetermined	Length (mm)	59.3 (13.9)	98.6 (7.6)	n.s.	93.0 (13.7)
	Weight (g)	1.3 (1.1)	6.1 (2.4)	n.s.	8.8 (6.8)
	Age	0-1	1	n.s.	0-1
	N	30	24	n.s.	25

Table 2. *Histological description of maturity stages used in the present study to classify gonads. Adapted from Brown-Peterson et al. (2011).*

STAGE	DESCRIPTION	
	Females	**Males**
0		Immature. Only primary spermatogonias are observed in testes.
1	Immature. Ovary only presents oogonias and/or oocytes in primary growth stage, well packaged, without atresia or postovulatory follicles. Ovary tunica is thin.	Mature. Testes present secondary spermatogonia, spermatocytes, spermatids and/or sperm.
1b	Regenerating. Ovary presents oogonias and/or oocytes in primary growth stage. Ovary tunica is swollen. Blood vessels are numerous; rest of atresia and postovulatory follicles in advanced degeneration stage can exist.	
2	Developing. Ovary presents oogonias and oocytes in primary growth and cortical alveoli stage. Oocytes in vitelogenic and migratory nucleous stage can be also present. No hydrated oocytes or postovulatory follicles exist. Some atretic oocytes can be present but are not abundant.	
2b	Spawning capable-between batches. Equal to stage 2 but with postovulatory follicles (recents or not) and/or residuals hydrated oocytes. Atretic oocytes can be more or less abundant.	
3	Actively spawning. Equal to stage 2 but with numerous hydrated oocytes. No recent postovulatory follicles.	
4	Regressing. Rest of oocytes at different developing stage can be present. Atretic oocytes and postovulatory follicles are abundant. Numerous and prominent blodd vessels are observed. Ovary tunica is more or less swollen.	

Table 3*. Parameters of the maturity ogives at length (mm) and age (years) estimated in the present study for L. whiffiagonis and L.boscii.in each study areas,* 7.b-k (Celtic Sea) and 8.c, 9.a2 (Galician waters and Cantabrian Sea)

Species	Stock	Studied ICES Division	Length			Age		
			Intercept	Slope	L_{50}	Intercept	Slope	A_{50}
L. whiffiagonis	7.b-k, 8.abd	7b-k	-6.9178	0.0389	178	-4.5405	1.8766	2.4
	8.c, 9.a	8.c, 9.a2	-6.1998	0.0327	188.5	-3.3067	2.1229	1.6
L. boscii	7.b-k, 8.abd	7b-k	-7.7134	0.0459	167.3	-3.8478	1.6764	2.3
	8.c, 9.a	8.c, 9.a2	-6.4671	0.044	147	-2.775	1.7315	1.6

Table 4*. Maturity ogives at length (mm) estimated in the present study for L. whiffiagonis and L.boscii.in each study areas, 7.b-k (Celtic Sea) and 8.c, 9.a2 (Galician waters and Cantabrian Sea)*

Length (mm)	8.c, 9.a		7.b-k, 8.abd	
	L. whiffiagonis	*L. boscii*	*L. whiffiagonis*	*L. boscii*
70	2%	3%	1%	1%
80	3%	5%	2%	2%
90	4%	8%	3%	3%
100	5%	11%	5%	4%
110	7%	16%	7%	7%
120	9%	23%	10%	10%
130	12%	32%	13%	15%
140	16%	42%	19%	22%
150	22%	53%	25%	30%
160	28%	64%	33%	41%
170	35%	73%	42%	52%
180	42%	81%	52%	63%
190	50%	87%	62%	73%
200	58%	91%	70%	81%
210	66%	94%	78%	87%
220	73%	96%	84%	92%
230	79%	97%	88%	94%
240	84%	98%	92%	96%
250	88%	99%	94%	98%
260	91%	99%	96%	99%
270	93%	100%	97%	99%
280	95%	100%	98%	99%
290	96%	100%	99%	100%
300	97%	100%	99%	100%
310	98%	100%	99%	100%
320	99%	100%	100%	100%
330	99%	100%	100%	100%
340	99%	100%	100%	100%
350	99%	100%	100%	100%

Table 5. *Maturity ogives at age (years) estimated in the present study for L. whiffiagonis and L.boscii.in each study areas, 7.b-k (Celtic Sea) and 8.c, 9.a2 (Galician waters and Cantabrian Sea)*

Age	8.c, 9.a		7.b-k, 8.abd	
	L. whiffiagonis	***L. boscii***	***L. whiffiagonis***	***L. boscii***
0	4%	6%	1%	2%
1	23%	26%	7%	10%
2	72%	67%	31%	38%
3	96%	92%	75%	77%
4	99%	98%	95%	95%
5	100%	100%	99%	99%
6	100%	100%	100%	100%
7	100%	100%	100%	100%