

Explorative analysis on red mullet (*Mullus barbatus*) ageing data variability in the Mediterranean

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Summary: The uncertainty in age estimation by otolith reading may be at the root of the large variability in red mullet (*Mullus barbatus*) growth models in the Mediterranean. In the MEDITS survey, red mullet age data are produced following the same sampling protocol and otolith reading methodology. However, ageing is assigned using different interpretation schemes, including variations in theoretical birthdate and number of false rings considered, in addition to differences in the experience level of readers. The present work analysed the influence of these variations and the geographical location of sampling on red mullet ageing using a multivariate approach (principal component analysis). Reader experience was the most important parameter correlated with the variability. The number of rings considered false showed a significant effect on the variability in the first age groups but had less influence on the older ones. The effect of the theoretical birthdate was low in all age groups. Geographical location had a significant influence, with longitude showing greater effects than latitude. In light of these results, workshops, exchanges and the adoption of a common ageing protocol based on age validation studies are considered fundamental tools for improving precision in red mullet ageing.

Keywords: *Mullus barbatus*; age variability; MEDITS; Mediterranean, reader effect, false rings, date of birth.

Análisis exploratorio de los datos de determinación de edad de *Mullus barbatus* en el Mediterráneo

Resumen: La incertidumbre en la estimación de la edad mediante la lectura de otolitos puede ser la principal causa detrás de la gran variabilidad existente en los modelos de crecimiento del salmónete (*Mullus barbatus*) en el Mediterráneo. En la campaña MEDITS, los datos de edad del salmónete se generan siguiendo el mismo protocolo de muestreo y metodología de lectura de otolitos, aunque la asignación de la edad se lleva a cabo usando distintos esquemas para la interpretación de las lecturas, incluyendo variaciones en la fecha teórica de nacimiento y en el número de anillos considerados falsos, además de las diferencias existentes en cuanto al nivel de experiencia de los lectores. En este trabajo, la influencia de las variaciones en los esquemas de interpretación, el nivel de experiencia de los lectores, y la localización geográfica de las muestras, sobre la determinación de la edad en el salmónete se analiza mediante una aproximación multivariante (Análisis de Componentes Principales). La experiencia de los lectores fue el parámetro más correlacionado con la variabilidad. El número de anillos considerados falsos mostró un efecto significativo sobre la variabilidad de los primeros grupos de edad, mientras que su influencia sobre los más viejos fue menor. El efecto de la fecha teórica de nacimiento tuvo poca importancia en todos los grupos de edad. La localización geográfica tuvo una influencia significativa, con la longitud mostrando mayores efectos que la latitud. Teniendo en cuenta estos resultados, los grupos de trabajo e intercambios de otolitos así como la adopción de un protocolo de asignación de edad común basado en estudios de validación de edad, se consideran herramientas fundamentales para mejorar la precisión en la determinación de la edad del salmónete.

Palabras clave: *Mullus barbatus*; variabilidad en determinación de edad; MEDITS; mar Mediterráneo; efecto del lector; anillos falsos; fecha de nacimiento.

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INTRODUCTION

Age and growth data are among the most important data input in stock assessment analytical models (Reeves 2003). However, bias in these data can lead to stock diagnosis failures (Eero et al. 2015). Poor quality ageing data have also contributed in certain cases to misleading evaluation of the population status, sometimes resulting in stock collapse (Beamish and McFarlane 1995, Liao et al. 2013). For these reasons, an increasing effort has been devoted during the last few decades to improving the quality of age data (ICES 2011a, 2013), especially in the context of the European Union Data Collection Framework, which is implementing otolith exchange exercises, workshops and meetings concerning the ageing of the most important species in the European fisheries (ICES 2018).

Several of these workshops (ICES 2009, 2012, 2017a) were dedicated to the ageing analysis of red mullet (*Mullus barbatus*), one of the most important species in terms of landings for the Mediterranean fisheries (FishStat 2016). Despite the number of workshops and exchange exercises done (Mahé et al. 2012, 2016), the precision in *M. barbatus* ageing, in terms of

percentage of agreement and coefficient of variation, is still outside acceptable limits (ICES 2011b). Several sources of disagreement have been recognized during these ageing workshops: i) the identification of the first growth ring with an annual periodicity; ii) the number (one or two) of false increments; iii) the presence/absence of reproductive ring(s) after the first growth ring; iv) disagreement considering the age assignment (theoretical birthdate 1 January versus 1 July); and v) the overlapping of the annulus in the older specimens (ICES 2009, 2012, 2017a). All these issues can result in high red mullet ageing differences (ICES 2017a). While reviewing age data on this species published during the last decade, Carbonara et al. (2018) observed an impressively varying average length at the first year, ranging between 7.54 and 18.93 cm. This high variability is difficult to justify through only the geographical and/or genetic differences (Matić-Skoko et al. 2018). Another source of difference in the age analysis can be the different calcified structures used in age reading (scales or otoliths). In particular, scale reading may cause the underestimation of age in the larger specimens of the species (ICES 2012, Mahé et al. 2012). Consequently, otoliths are considered the most suit-

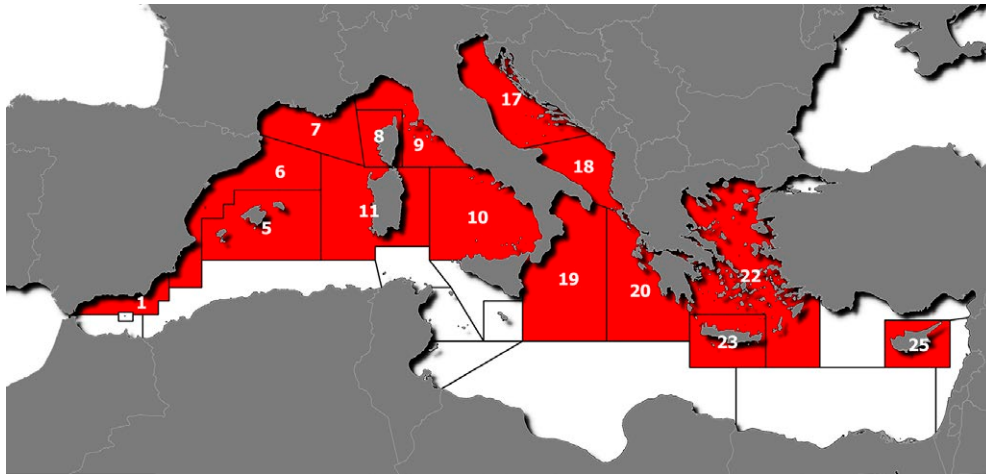


Fig. 1. – Map of the study area showing the 15 geographical sub-areas (GSAs) established by the General Fisheries Commission for the Mediterranean <http://www.gfcm.org> where otoliths of red mullet (*Mullus barbatus*) were sampled.

able calcified structures for red mullet ageing (ICES 2012). Moreover, the growth studies based on indirect methods (length frequency distribution analysis) provided a faster growth pattern than the analysis based on direct age analysis from otolith and scale reading (Carbonara et al. 2018). The uncertainties linked to age analysis are also hampered by the lack of direct (e.g. mark-recapture, radiochemical dating), indirect (e.g. length frequency distribution analysis) and semi-direct (e.g. marginal analysis; marginal increment analysis) validation studies (Bianchini and Ragonese 2011, Sieli et al. 2011). Indeed, direct validation is challenging because of the difficulty of catching specimens alive (Düzbastılar et al. 2015) and the relative short life span of red mullet (Vrantzas et al. 1992, Sieli et al. 2011). Only two validation studies have been performed so far and are available in the literature: one in the southern Tyrrhenian Sea (Sieli et al. 2011) focusing only on the periodicity of the growth increment deposition (marginal analysis) and one in the southern Adriatic (Carbonara et al. 2018) focusing on the periodicity of the growth increment deposition (marginal analysis and marginal increment analysis) and the indirect validation method (length frequency distribution analysis).

The impact of age analysis uncertainties on the stock assessment of red mullet can be significant, since the identification of the first growth ring (ICES 2012) can lead to the over-/underestimation of one or more year in age (Vrantzas et al. 1992, Sieli et al. 2011). In general, the growth of this species follows a biphasic pattern: it is high in the first year, reaching about a third of the maximum size, and once it has reached the size at the first maturity, there is a significant decrease in the growth rate (Fiorentino et al. 1998, 2013, Carbonara et al. 2018).

Analysing the percentage of agreement (PA) and coefficient of variation (CV) among the readers obtained by the workshops/exchanges on age reading of *M. barbatus* in the last decade (ICES 2009, 2012, 2017a), an improvement in precision was obtained (PA, from 51.6% in Exchange 2008 to 67% in Exchange 2016; CV, from 68.5% in Exchange 2008 to 64.6% in Exchange 2016), but not sufficient to reach the accept-

able threshold limit of precision (PA 80%, CV 20%) (ICES 2011b). Most of the readers who participated in the exchanges contributed their age readings to the stock assessment analysis. In this context, a common ageing protocol is an important tool for decreasing the relative/absolute bias, improving precision (reducing the CV and increasing the PA) (ICES 2017a) in age determination, and increasing reproducibility among the age readers of different laboratories (ICES 2011b). Therefore, in order to reach this goal, it is useful to assess the effect of the specific factors influencing the age reading variability (i.e. theoretical birthdate, ageing criteria, age scheme, reader experience).

Red mullet otoliths have been collected since 2012 throughout the European Mediterranean waters during the international MEDITS bottom trawl survey. However, individual age determination is affected by sources of variation, including different ageing schemes, different reader experience and geographical differences in growth (Carbonara et al. 2018, Sonin et al. 2007). The objective of this work is to investigate the potential influence of these factors on red mullet ageing in the Mediterranean using a multivariate approach. Results could be useful for defining a standardized reading protocol aimed at obtaining unbiased age-length keys for red mullet stocks in the Mediterranean.

MATERIALS AND METHODS

Data

The red mullet otoliths used in this study were collected during the MEDITS surveys, which are carried out in spring and early summer (usually from April to July) following a standardized sampling protocol (Anonymous 2017). In our analysis, we used the otolith reading (length/age) data collected in 2014 surveys at geographical sub-areas (GSAs) established by the General Fisheries Commission for the Mediterranean (Fig. 1).

For each GSA age data set, we considered the following meta-data: sex, theoretical birth date applied for ageing (1 January or 1 July), reader experience

Table 1. – Number of otoliths (n) of red mullet analysed by geographical sub-area (GSA), with information on total length (TL, cm), age range (years), birthdate (Jul, 1 July; Jan, 1 January), number of false rings (FR) and level of experience of readers (Exp; L, low: <2000 otolith readings; M, medium: 2000-6000 otolith readings; H, high: >6000 otolith readings).

GSA	n	TL	Age	Birthdate	FR	Exp
1	151	10.9-24.1	0-3	Jul	1	M
5	49	11.1-22.2	0-3	Jul	1-2	M
6	113	11.2-25.7	0-5	Jul	1-2	M
7	681	10-30	1-5	Jul	1	H
8	432	9.5-21	1-6	Jan	1	H
9	242	5-26.5	0-5	Jan	1	M
10	469	5.5-24.5	0-6	Jul	1	M
11	413	10.2-23.1	0-4	Jul	1	M
17	354	10-25	0-7	Jul	2	M
18	679	4-24.5	0-5	Jul	1	H
19	525	8.7-22.9	0-5	Jul	1	M
20	59	11.3-18.9	1-3	Jan	1-3	H
22	402	4.7-23.5	0-5	Jul	0-3	L
23	294	6.1-21.6	0-4	Jul	0-2	L
25	192	8.6-19.9	1-4	Jul	1	M

(low, <2000 otolith readings; medium, 2000-6000 otolith readings; high, >6000 otolith readings), number of false rings considered before the first winter ring (0, 1 or 2) and geographic location as an average between the latitude and longitude of each GSA. The readers analysed the otoliths from their own area, providing information on the theoretical birth date applied, reader experience and number of false rings considered. In total, the scientific staff from 13 laboratories (GSAs 7 and 8 and GSAs 10 and 18 were analysed by the same scientific staff) analysed 5055 pairs of otoliths (2815 female and 2240 male) collected in 15 GSAs (Fig. 2).

Multivariate analysis

Principal component analysis (PCA) was applied to identify the most informative variables influencing the differences in the ageing data of red mullet. PCA is a multivariate statistical technique (Jolliffe 2002, Abdi and Williams 2010) used to extract the important information from a multivariate data set and express this information as a set of a few new variables called principal components (PCs). The PCs are calculated as linear combinations of the original variables aimed at identifying directions (or PCs), along which the variation in the data is maximized. Hence the number of selected PCs is less than or equal to the number of original variables. The information in a given data set corresponds to the total variation it contains. The PCA aims to identify the directions (or PCs) along which the variation in the data is largest. To measure the effects of each variable in the system, the correlation level with PCs is used. In other words, PCA reduces the dimensionality of a multivariate data set to a lower number of principal components with minimal loss of information (Kassambara 2017). PCA was performed using the FactoMineR library (Lê et al. 2008) available in R (R Development Core Team 2018). The main feature of the FactoMineR library is the ability to perform the analysis using different types of variables (quantitative or categorical).

The first analysis considered all the age groups together, using total length (TL), age of the specimens and GSA coordinates (latitude and longitude) as quantitative variables and number of false rings,

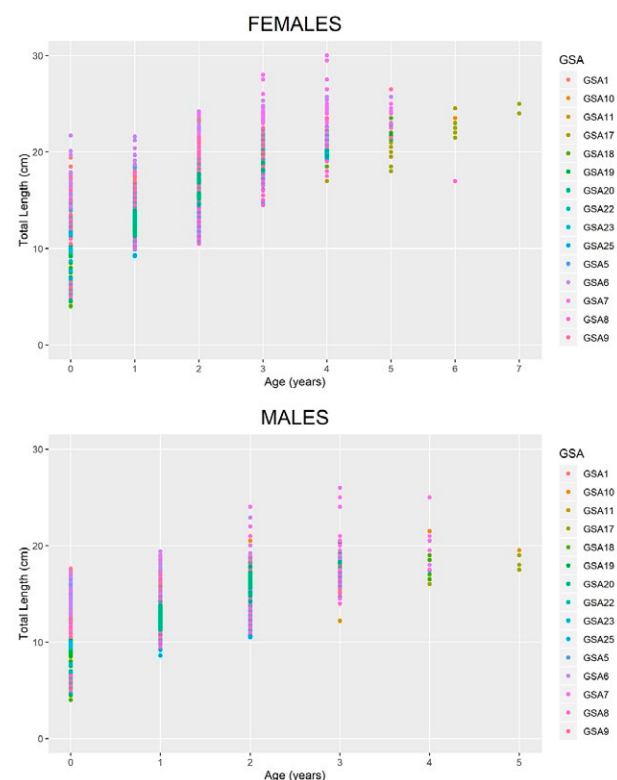


Fig. 2. – Length-at-age data by geographical sub-area (GSA) and sex, estimated from otolith readings of red mullet (*Mullus barbatus*) and used in the present study.

sex, theoretical birthdate and reader experience as qualitative variables. The age groups from 0 to 4 were the most represented in the data set, while the age groups of more than 4 years were present only in 9 GSAs (Table 1). Thus, ten PCAs were performed for each age group from 0 to 4 years and for both sexes using TL and GSAs coordinates as quantitative variables and theoretical birthdate, reader experience and number of false rings as qualitative variables. The temporal factor was not included in the analysis, because all data were collected in 2014 during a short period (April-July) as foreseen by the MEDITS protocol (Anonymous 2017).

The number of PCs to be considered for each PCA was determined using the Kaiser rule (Kaiser 1960),

Table 2. – Values of variance (Var), percentage of variance (%Var) and cumulative percentage of variance ($\Sigma\%$ Var) that accounted for each dimension (Dim) in the PCAs.

Sex and age	Variables	Dim 1	Dim 2	Dim 3	Dim 4
Both 0-4	Var	2.11	1.27	0.50	0.12
	%Var	52.86	31.63	12.52	3.00
	$\Sigma\%$ Var	52.86	84.49	97.00	100.00
Females 0	Var	1.66	1.17	0.18	-
	%Var	55.18	38.92	5.91	-
	$\Sigma\%$ Var	55.18	94.10	100.00	-
Females 1	Var	1.82	0.96	0.22	-
	%Var	60.67	32.03	7.30	-
	$\Sigma\%$ Var	60.67	92.70	100.00	-
Females 2	Var	2.05	0.76	0.19	-
	%Var	68.29	25.30	6.41	-
	$\Sigma\%$ Var	68.29	93.59	100.00	-
Females 3	Var	1.65	1.14	0.22	-
	%Var	54.95	37.89	7.16	-
	$\Sigma\%$ Var	54.95	92.84	100.00	-
Females 4	Var	1.51	1.32	0.18	-
	%Var	50.34	43.84	5.82	-
	$\Sigma\%$ Var	50.34	94.18	100.00	-
Males 0	Var	1.67	1.13	0.20	-
	%Var	55.61	37.78	6.61	-
	$\Sigma\%$ Var	55.61	93.39	100.00	-
Males 1	Var	1.79	1.02	0.19	-
	%Var	59.71	33.92	6.37	-
	$\Sigma\%$ Var	59.71	93.63	100.00	-
Males 2	Var	1.96	0.90	0.14	-
	%Var	65.44	29.87	4.69	-
	$\Sigma\%$ Var	65.44	95.31	100.00	-
Males 3	Var	1.67	1.05	0.28	-
	%Var	55.68	34.92	9.40	-
	$\Sigma\%$ Var	55.68	90.60	100.00	-
Males 4	Var	1.48	1.25	0.27	-
	%Var	49.21	41.82	8.98	-
	$\Sigma\%$ Var	49.21	91.02	100.00	-

retaining only those PCs whose variance exceeds 1. PCs with variance lower than 1 are less informative and thus not worth being retained (Jolliffe 2002). Only significant correlations ($p < 0.05$) were considered in the analysis.

RESULTS

In the PCA performed on the whole dataset, the first two PCs were retained, accounting for 84.5% of the total variability (Table 2). The first principal component (PC1) was strongly correlated with all four original variables (TL, age, latitude and longitude), with TL showing the highest correlation (Table 3). Size, age and latitude variables varied together, being positively correlated with PC1. In contrast, longitude was an opposite effect.

Although none of the qualitative variables showed a strong correlation with PC1, the highest contribution was shown by reader experience, followed by number of false rings and birth date. Unlike latitude, longitude showed a higher correlation with the second principal component (PC2). The qualitative variables showed the following order of decreasing correlation with PC2: reader experience, birth date and sex.

The PCAs performed on each age group and sex showed a strong geographical effect mostly driving PC1. Indeed, longitude and latitude were the best-correlated variables in almost all the age groups, at least in the PC1, but with opposite directions. Moreover, TL was mostly correlated with PC2, except for the age group 0, in which latitude had the highest correlation value.

Table 3. – Summary of the correlation coefficients of both continuous (TL, total length; Lat, latitude; Lon, longitude; Age of the specimens) and qualitative (Exp, reader experience; NFR, number of false rings; B, theoretical birthdate; Sex) variables (VAR) for dimensions (Dim) 1 and 2 in the PCAs. Non-significant correlations ($p > 0.05$) are shown in bold.

Both Sexes / Ages 0-4				Females				Males			
VAR	Dim 1	Dim 2	Age	VAR	Dim 1	Dim 2	Age	VAR	Dim 1	Dim 2	
TL	0.82	0.43	0	TL	0.81	-0.53	0	TL	0.88	-0.38	
Lat	0.72	-0.51		Lat	0.32	0.93		Lat	0.15	0.97	
Lon	-0.61	0.69		Lon	-0.95	-0.14		Lon	-0.93	-0.20	
Age	0.75	0.58		Exp	0.69	0.47		Exp	0.66	0.52	
Exp	0.28	0.14		NFR	0.37	0.06		NFR	0.34	0.10	
NFR	0.15	0.01		B	0.02	0.37		B	0.00	0.38	
B	0.12	0.14	1	TL	0.45	0.88	1	TL	0.19	0.98	
				Lat	0.86	-0.42		Lat	0.92	-0.24	
				Lon	-0.94	0.04		Lon	-0.95	-0.04	
				Exp	0.39	0.13		Exp	0.44	0.10	
				NFR	0.04	0.01		NFR	0.04	0.00	
				B	0.23	0.11		B	0.28	0.03	
			2	TL	0.67	0.73	2	TL	0.46	0.88	
				Lat	0.84	-0.47		Lat	0.92	-0.31	
				Lon	-0.94	0.10		Lon	-0.95	0.13	
				Exp	0.19	0.14		Exp	0.28	0.10	
				NFR	0.01	0.04		NFR	0.02	0.06	
				B	0.26	0.07		B	0.29	0.00	
			3	TL	0.33	0.92	3	TL	0.14	0.98	
				Lat	0.80	-0.52		Lat	0.89	-0.28	
				Lon	-0.94	-0.12		Lon	-0.92	-0.11	
				Exp	0.34	0.12		Exp	0.16	0.05	
				NFR	0.02	0.29		NFR	0.06	0.15	
				B	0.31	0.00		B	0.23	0.08	
			4	TL	0.46	0.86	4	TL	0.45	0.85	
				Lat	0.61	-0.75		Lat	0.62	-0.73	
				Lon	-0.96	-0.07		Lon	-0.94	-0.07	
				Exp	0.31	0.24		Exp	0.03	0.47	
				NFR	0.00	0.66		NFR	0.00	0.62	
				B	0.45	0.05		B	0.52	0.09	

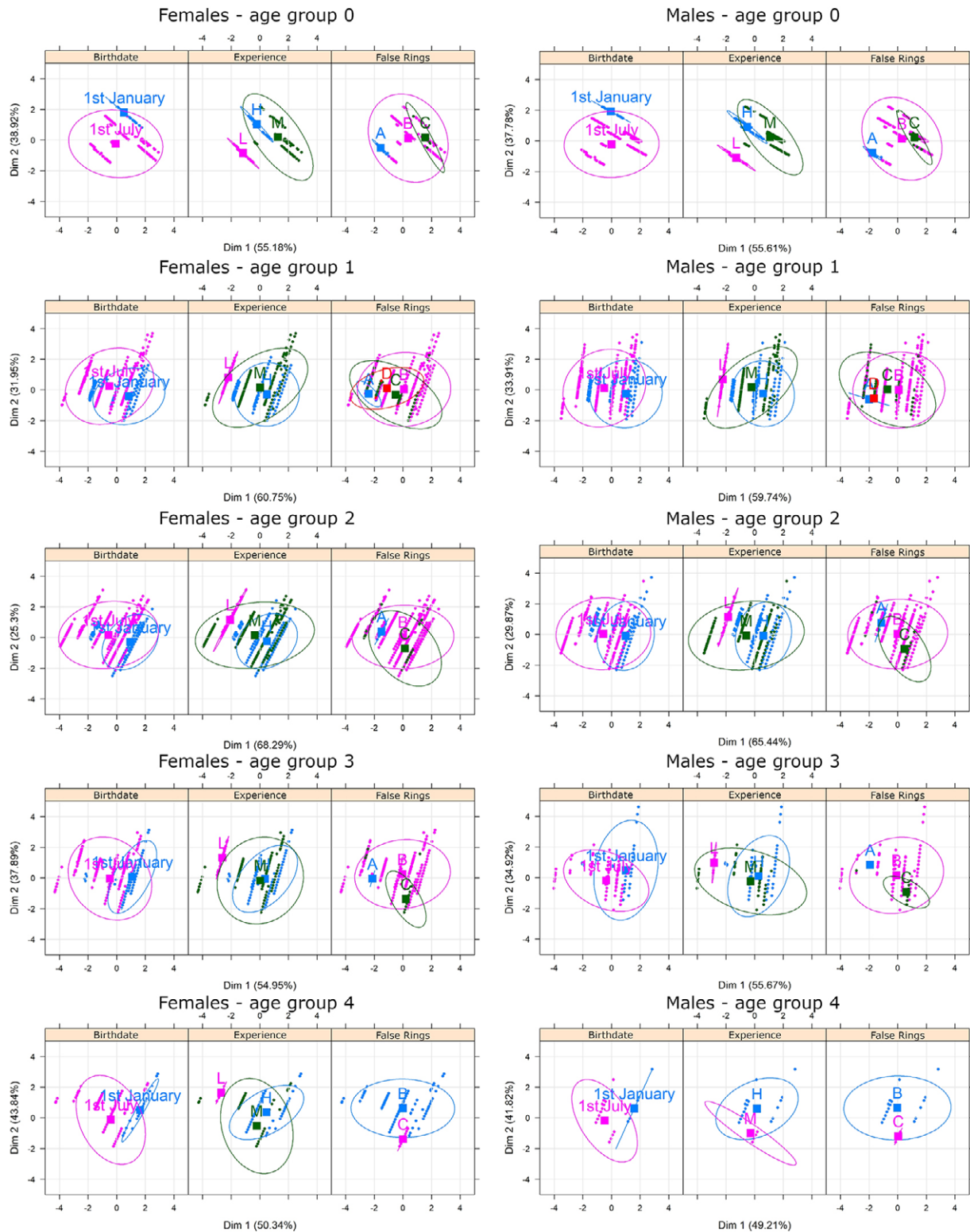


Fig. 3. – Confidence ellipses drawn around the levels of the categorical variables considered in each PCA (confidence level = 0.95) by sex and age group, taking into account the variables theoretical birthdate (1 January, 1 July), reader experience (L, low: <2000 otolith readings; M, medium: 200-5000 otolith readings; H, high: >5000 otolith readings) and number of false rings considered before the first winter growth increment (A, 0; B, 1; C, 2; D, 3).

Among the qualitative variables, the highest correlation with PC1 was shown by reader experience, especially in the lower age classes, and birthdate, mostly

in the oldest age groups (Fig. 3). The contribution of number of false rings was important for the age groups 0 (PC1) and 4 (PC2).

DISCUSSION

The results of the present paper confirm the high variability occurring in the age and growth of the red mullet in the Mediterranean reported in previous studies (Bianchini and Ragonese 2011, Carbonara et al. 2018). The variability in age data can be attributed to several factors, including the use of different sampling methodologies (commercial fishing or scientific surveys: Coggins et al. 2013), age schemes (ICES 2011a), otolith preparation methods (Smith et al. 2016), age criteria (Hüssy et al. 2016), reader experience (Kimura and Lyons 1991) and geographical differences (Carbonara et al. 2018). These factors affect both the accuracy and the precision of the age and growth data, having a negative impact on stock status assessment and the application of management measures aiming to achieve a sustainable exploitation of red mullet in the Mediterranean. Most of the stock assessment models used, especially the analytical ones such as virtual population analysis (e.g. Extended Survivor Analysis) and statistical catch-at-age (e.g. the state-space assessment model and assessment-for-all), require knowledge of the demographic structure of the stocks. One of the first steps for running these models is the conversion of the LFD of catches to age structure, which is performed by means of age slicing procedures using growth parameters from the von Bertalanffy growth function (VBGF) or age-length keys. Inappropriate growth parameters or age-length keys to convert size distribution into age structure can lead to unreliable scientific advice (STECF 2017). If an age overestimation occurs, the stock assessment will provide an erroneous scenario with a population composed of older individuals and, consequently, affected by lower fishing mortality, while in the opposite case, fish would be younger with an overestimation of fishing mortality (Campana 2001). Moreover, age and growth also affect the estimation of natural mortality and maturity-at-age data. As a result, they can affect the estimation of recruitment strength and spawning stock biomass. Ultimately, the most important effect is linked to short-term predictions of the stock status and the related management measures (Punt et al. 2008, Eero et al. 2015, Hüssy et al. 2016).

Our findings revealed that samples geographical location was the most important factor significantly correlated to age variability, in particular the longitudinal (west-east) influenced more than latitudinal (north-south) samples geographical component. Considering the relative genetic homogeneity of this species in the area (Matić-Skoko et al. 2018), the detected ageing variability could be attributed to geographical and environmental differences. A reduction in the growth rate of red mullet from west to east in the Mediterranean has already been described (Sonin et al. 2007, Carbonara et al. 2018). In support of this observation comes the hypothesis by Sonin et al. (2007) for red mullet called “Levantine nanism”, according to which specimens are characterized by smaller body size in the Levantine basin than the conspecifics in the western Mediterranean. These findings can be explained by

the lower productivity of the eastern Mediterranean compared with the western basin, with a higher chlorophyll concentration in the western than the eastern part (Moutin and Raimbault 2002). The higher water temperature in the southeastern Mediterranean may be another explanation for Levantine nanism (Sonin et al. 2007). The higher water temperature may result in a higher metabolic rate in that area for the red mullet, resulting in earlier sexual maturity and a consequent decrease in growth rate (Saborido-Rey and Kjesbu 2005). Other environmental factors such as salinity, food competition and invasive species could also be factors driving to dwarfism (Sonin et al. 2007, Edelstet al. 2014, Corrales et al. 2017).

Reader experience has been identified as an important factor affecting the precision of age data for many species in both marine and freshwater environments (Appelberg et al. 2005, Kimura and Anderl 2005, Rude et al. 2013). In the present study, this factor was also found to be important in ageing variability of red mullet in the Mediterranean, especially when we compared the results of readers with high-medium vs. low experience. This factor emerged as a key issue in estimating the age mostly in the youngest ages (0 and 1 years) and oldest ages (4 years). The identification of the true first growth increment and the overlapping of the growth rings have been mentioned as reasons for disagreement in ageing analysis of this species (ICES 2009, 2012, 2017a). However, the precision of these results was calculated for all readers together, without an assessment of reader experience because of the low number of readers (Mahé et al. 2012, 2016, ICES 2009, 2017a).

The theoretical birthdate has also been reported as another important element in the process of age estimation (Morales-Nin and Panfili 2002). In our analysis, birthdate had a lower influence in the first age group than reader experience and number of false rings. In the rest of the age groups, birthdate had a greater influence on ageing. The specific date of birth at individual and/or population level, as established by studies of reproduction and/or analysis of daily increments, is not always known. Therefore, for convenience during the stock assessment process the conventional birthdate for the entire population was established at 1 January (Morales-Nin and Panfili 2002). The reproduction of red mullet in the Mediterranean takes place from April to September (Carbonara et al. 2015). Thus, an age scheme based on 1 July as the birthdate of the species has been suggested as more appropriate, avoiding overestimation of age in the first year. Considering 1 January as the birthdate, specimens born during the spawning season (April-September) will be aged as 1 year old, even if they are caught after 6 months. During the last workshop on red mullet age validation, the use of a single ageing scheme based on the birthdate of 1 July was endorsed with agreement among all readers in order to overcome this kind of bias (ICES 2017a). Nevertheless, though the mid-year birthdate is consistent with the species, it could generate a mismatch between age groups and year classes for the year time-step on which the assessment is run. For this reason, for other species, such as anchovy (*Engraulis encrasi-*

colus), despite a peak of spawning in early summer, a birthdate of 1 January has been adopted (ICES 2017b). Thus, the annual catches-at-age obtained by slicing the LFDs using VBGF parameters and/or age-length keys correspond directly to the annual catch (ICES 2017b). However, it must be considered that birthdate is also an important factor in the estimation of spawning stock biomass (SSB). In fact, the SSB estimated for the real spawning period (in the case of a birthdate of 1 January, before the spawning period) may lead to an overestimation of this stock variable because of the underestimation of natural and fishing mortality before the spawning period.

The interpretation of the first growth ring has been mentioned as another source of discrepancy among readers (ICES 2009, 2012, 2017a). In particular, two different hypotheses have been proposed: i) only one false ring can be detected before the first growth ring (winter area), reflecting the transition between the pelagic and the demersal phase (the demersal ring; e.g. Tursi et al. 1994, Sonin et al. 2007, Carbonara et al. 2018); and ii) two false growth increments can be identified before the first growth ring, the first laid down during the pelagic phase and the second reflecting the settlement (pelagic and demersal rings, respectively; e.g. Vrantzas et al. 1992, Fiorentino et al. 1998, Sieli et al. 2011). These two hypotheses result in two different growth scenarios: i) the slow-growth hypothesis if only one false ring is detected; and ii) the fast-growth hypothesis if two false rings are detected before the first winter growth increment. A recent study carried out in the southern Adriatic (GSA 18) using marginal analysis, back-calculation and morphological analysis on juveniles of red mullets demonstrated that only one false ring (the demersal ring) is laid down before the first true winter ring (Carbonara et al. 2018), thus supporting the slow-growth hypothesis. Nevertheless, the present results have shown that the number of false rings is less important than reader experience, except for the age group 0. This point, however, may be also linked to the different ageing experience of the readers. Therefore, workshops, age exercises and exchanges are considered fundamental tools for improving precision in red mullet age analysis (ICES 2011b). Internal and external ageing exercises should be implemented before age data are included in stock assessment, and at least a minimum level of agreement with experienced readers should be achieved (90% PA and 10% CV; ICES 2011b).

The results of the present analysis further demonstrate the importance of a handbook to clarify and standardize ageing schemes (e.g. birthdate) and criteria (e.g. number of false rings before the first winter growth increment). The use of a common and standardized protocol among all institutes and experts is fundamental in order to decrease the relative/absolute bias associated with the activities of age determination and to improve the precision (reproducibility and reduction of the CV) of the age readers from the various laboratories involved in the ageing analysis. Furthermore, placing all laboratories under the same standardized protocol can ensure the possibility of applying changes to

datasets horizontally when future breakthroughs and/or ground-breaking discoveries are made. All these actions can make an important contribution to overcome ageing uncertainties, thus providing accurate and robust input data for stock assessments and ensuring a more appropriate fishery management of red mullet in the Mediterranean.

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