MARE/2016/22 "Strengthening regional cooperation in the area of fisheries data collection" Annex III "Biological data collection for fisheries on highly migratory species"

Strengthening Regional cooperation in the area of large pelagic fisheries data collection (Acronym: RECOLAPE)

REPORT

Xiphias gladius age reading exchange

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Index

Exchange exercise

The ageing analysis, the examination of the protocols and literature (Rodríguez-Marín et al., 2007; Williams et al., 2013; ICCAT 2006-2016; Lanteri and Garibaldi, 2019; Quelle et al., 2014) on the large pelagic stocks showed some gaps on:

- Ageing scheme;
- Ageing criteria;
- Ageing validation study;
- Preparation method.

These aspects affect both the precision and the accuracy (Panfili et al., 2002) of the age estimation for the selected stocks. To overcome these gaps and improve the precision, workshop and reading exchange (ICES, 2011; ICES, 2013; ICES, 2015) are useful tools, while validation studies are the means to improve the accuracy (Campana, 2001).

In addition, in the case of swordfish, problems in age estimation using spines can be summarized in the following main sources of errors:

• Presence of multiple bands and false bands;
• Progressive disappearance of the inner bands in larger specimens.

The Exchange approach based on supporting tools (SmartDots, Eltink sheet, full scale exchange) (PGCCDBS 2011; ICES 2016, ICES 2017) was utilized to highlighted the main source of bias and understand the level of precision of Swordfish

1.1 Sampling Collection and Participation

A preliminary step to the exchange was the collection and calibration on a suitable number of HS images (first three ray of the anal fin).The images of prepared spines have been provided by Genoa University and IEO. In total 79 specimens were sampled from 2003 to 2017 in the Mediterranean area (Tab. 1.1.1; Fig. 1.3.1.1).

Table 1.1.1 - Samples distribution of *Xiphias gladius* by the sampling year and area.

Species	Areas	2003	2004	2005	2007	2008	2009	2010	2011	2016	2017	Tot.
X. gladius	Ligurian Sea (1)				2	1	1	1	3	2	54	64
	Alboran Sea (2)	1	5	9								15
Total		1	5	9	2	1	1	1	3	2	54	79

Figure 1.1.1Map of specimens collected: 1Ligurian Sea; 2 Alboran Sea

The length distribution of *X. gladius* (Fig. 1.1.2) there were from 2 different geographical areas. The specimens of Ligurian Sea included the smallest fish below LJFL range included between 69 and 177 cm. Conversely, the fish from Alboran Sea presented the LJFL range from 102to 213 cm (Fig. 1.1.2). In total there were covered a huge range of LJFL that they included juveniles and adult specimens

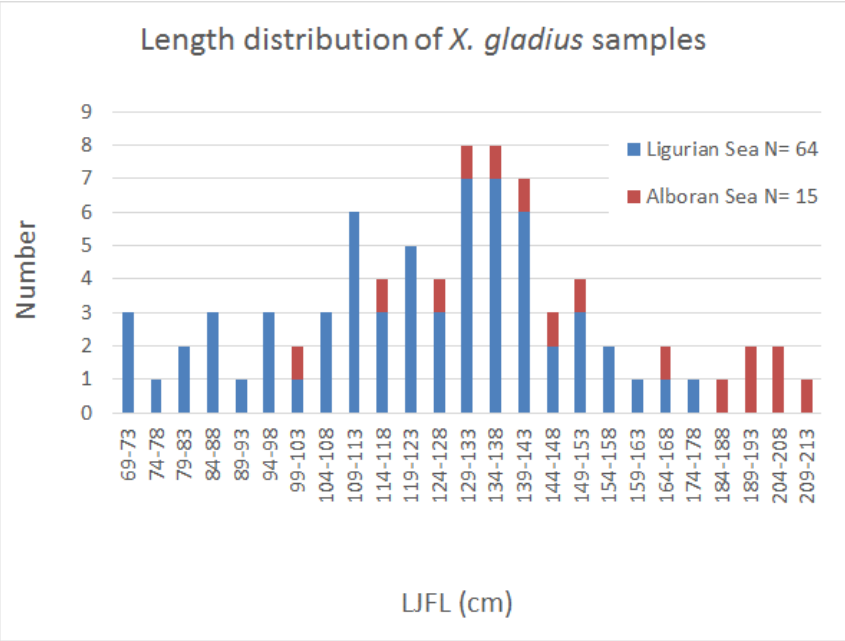

Figure 1.1.2 - Length distribution of *X. gladius* used during the exchange by geographical areas

In total 9 readers participated to the reading exchange exercise from 6 country and 7 laboratories (Tab. 1.1.2). The readers included not only readers from the Institutes involved in the RECOLAPE project but also from others Institution, involved in the DCF.

Table 1.1.2 List of the readers by country and laboratory

Reader	Name	Country	Institution
1	Aurelie Guillou	France	IRD
2	Fulvio Garibaldi	Italy	University of Genoa
3	Sergio Bizzari	Italy	Unimar
4	Daniela Rosa	Portugal	IPMA
5	Luca Lanteri	Italy	University of Genoa
6	Rui Coelho	Portugal	IPMA
7	George Tserpes	Greece	HCMR
8	Ioannis Thasitis	Cyprus	DFMR
9	Pablo Quelle	Spain	IEO

1.2 Reading procedures and data analysis

To all readers were asked to read each digitised images with their own interpretation (positions of the annual rings on a given transect) using the program SmartDOT platform (http://www.ices.dk/marine-data/tools/Pages/smartdots.aspx). SmartDOT is a new set of software tools supports the user in managing all data of ICES age reading

workshops and exchanges. The workshop or exchange manager can manage the meta data related to workshops and exchanges, and the age reader can carry out age readings by annotating HS images. All registered data are available in the connected reporting environment.

The instructions, how to use this software in the context of this exchange, are reported in the Annex 1.

The age was assigned taking into account the number of the transparent rings the date of birthday and the edge type. Moreover the date of capture and the sex were visible by the readers. Then the age for each specimen was assigned following the scheme reported in the Table 3.1.3

Table 1.1.3 - Age scheme used during the exchange

Date of Caprure	Age	Edge
1th semester	N of ring + Internal ring	Trasparent
	N of ring +1 + Internal ring	Opague
2th semester	N of ring + internal ring	Opaque
	N of Ring + internal ring	Trasparent

All data were extracted from SmartDOT and analysed using the GuusEltink spreadsheet (Eltink, 2000).The spreadsheet (Eltink, 2000) was completed according to the instructions contained in Guidelines and Tools for Age Reading Comparisons by Eltink et al. (2000). Modal ages were calculated for each spine red, with percentage agreement (PA), coefficient of variation (CV) and average percent error (APE), as a definition (for each spines):

$$PA = \frac{\sum |n_{diff} \leq 1|}{n}$$

$$CVj(\%) = 100.\frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}}}{x_j}$$

Where R is the number of times each fish is aged, Xij the i(th) age determination of the j(th) fish, Xj is the mean age calculated for the j(th) fish, and ndiff is the difference in age determination between the readings of two readers.

$$APE\text{j}(\%) = 100.\frac{1}{R}\sum_{i=1}^{R}\frac{|\text{Xij} + \text{Xj}|}{\text{Xj}}$$

Where xij is the ith age determination of the jth fish, x j is the average age calculated for the jth fish and R is the number of times each fish was aged.

1.3 Results

In the analysis were utilized the data from all readers and the precision analyse with CV, APE and percent of agreement to modal age for *X. gladius* spines sets was presented in the Table 3.3.1. All data showed the low precision with the percent agreement between 52.7 and 67.2%, the CV from 33.9 to 17.8% and the APE from 22.7 and 24.4%. For the all samples together the CV, APE and percent of agreement to modal age were respectively: 30.8%, 23 and 64.4%.

Table 1.3.1 - Reading's precision for *X. gladius* by sampling area

Species	Geographical area	Otoliths Spine	Length LJFL Range (cm)	Age range (year)	Percentage of Agreement	CV	APE
X. gladius	Ligurian Sea ITA	64	69/177	0/7	67.2%	33.9%	22.7%
	Alboran Sea SPA	15	160/213	1/9	52.7%	17.8%	24.4%
	TOTAL	**79**	**69/213**	**1/9**	**64.4%**	**30.8%**	**23%**

Moreover the precision indices (PA, CV and APE) not showed significant differences (Kruskal–Wallis test; $p>0.05$) if they were stratified by readers' experience (Expert >500 spines read; Basic < 500 spines read) (Tab. 1.3.2).

Table 1.3.2 - Reading's precision for *X. gladius* by sampling area

Species	Expertise	Percentage of Agreement	CV	APE
X. gladius	Expert	65.1%	30.9%	21.6%
	Basic	64.2%	30.5%	24.7%
	TOTAL	**64.4%**	**30.8%**	**23%**

The coefficient of variation (CV), percent agreement and the standard deviation (STDEV) are plotted against MODAL age (Fig. 1.3.1). The results show a decrease trend from the lower age groups to the higher one for PA and STDEV and the opposite trend for the CV. These results could be explained by the position of the first growth increment (Quelle et al., 2014) and the overlapping the growth increments in the older specimens

(Lanteri and Garibaldi, 2019). In general after the first age groups was observed a decrease of the agreement, the increment of STDEV and a constant CV around the 20%.

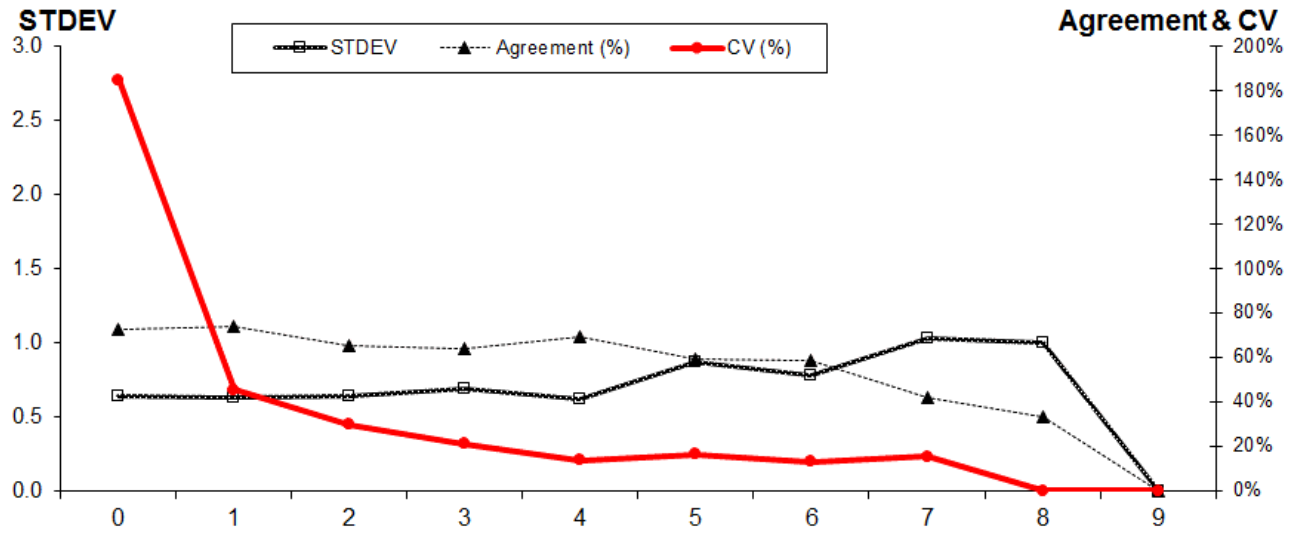

Figure 1.3.1 - The coefficient of variation (CV), percent agreement and the standard deviation (STDEV) are plotted against MODAL age.

The percentage of agreement by readers weighed by the number of samples read are included between 38% to 79.5% (Table 1.3.3). Moreover the PA by age group shows a negative trend passing from 72% for the age 0 to 33% for the age 8.

Table 1.3.3 Percentage of agreement by readers and age group.

	PERCENTAGE AGREEMENT										
	MODAL age	France AG	Italy FG	Italy SB	Portugal DR	Italy LL	Portugal RC	Greece GT	Cyprus IT	Spain PQ	ALL
	0	0%	100%	75%	100%	100%	0%	100%	75%	100%	72%
	1	0%	100%	100%	67%	78%	56%	89%	78%	100%	74%
	2	50%	33%	45%	91%	75%	83%	100%	73%	36%	65%
	3	36%	68%	57%	77%	68%	77%	74%	68%	50%	64%
	4	33%	60%	57%	87%	93%	87%	70%	77%	60%	69%
	5	50%	60%	40%	80%	80%	80%	63%	50%	30%	59%
	6	100%	50%	100%	50%	50%	100%	0%	50%	0%	59%
	7	75%	25%	0%	50%	25%	25%	0%	67%	75%	42%
	8	100%	0%	0%	100%	0%	0%	0%	100%	0%	33%
	9	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-9	38.0%	62.0%	58.1%	79.5%	74.7%	70.9%	75.4%	69.3%	55.1%	64.6%

Relative bias can be defined as a systematic over- or underestimation of age compared to the modal age. In the results of the exchange the bias are higher in the first two age groups (age 0 and age 1) reaching about 0.4 year and in the last age group where the bias reach about 0.6 year (Fig. 1.3.2).

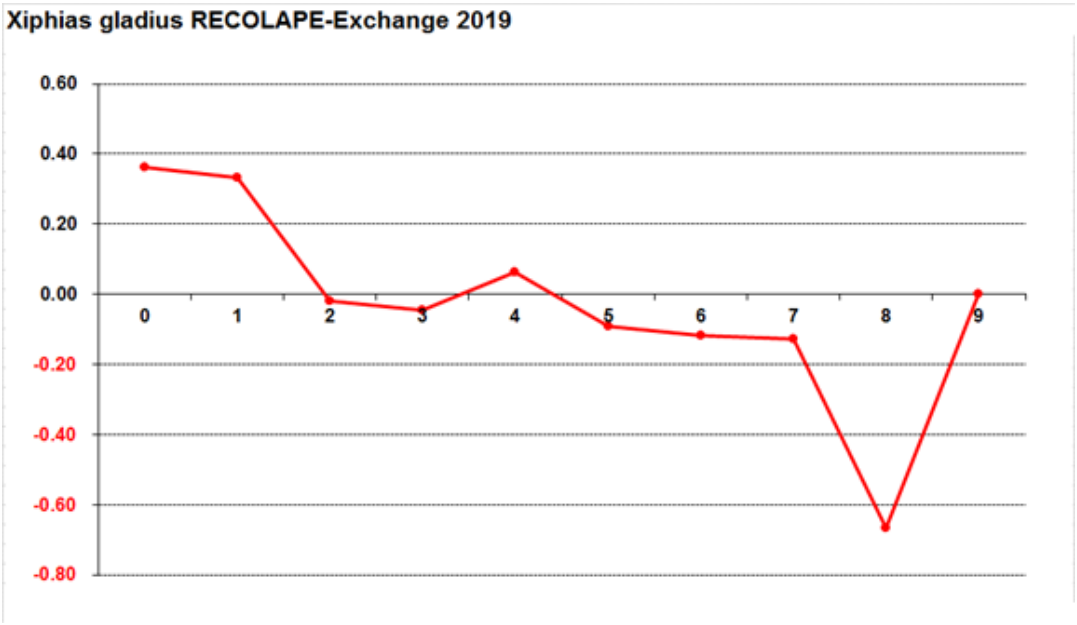

Figure 1.3.2 - The RELATIVE bias by MODAL age as estimated by all age readers combined

The hypothesis of an absence of bias between two readers or between a reader and the modal age estimated was tested non-parametrically with a one-sample Wilcoxon signed rank test. The results of the test (Fig. 1.3.3) highlighted that there is a group of readers that not show significant difference among them and with modal age.

Inter-reader bias test and reader against MODAL age bias test

	France AG	Italy FG	Italy SB	Portugal DR	Italy LL	Portugal RC	Greece GT	Cyprus IT	Spain PQ
Reader AG									
Reader FG	**								
Reader SB	**	–							
Reader DR	**	*	**						
Reader LL	**	**	**	–					
Reader RC	**	*	**	–	–				
Reader GT	**	*	–	–	–	*			
Reader IT	**	**	**	–	–	–	*		
Reader PQ	**	–	–	**	**	**	**	**	
MODAL age	**	*	**	–	*	–	–	–	**

–	= no sign of bias (p>0.05)
*	= possibility of bias (0.01<p<0.05)
**	= certainty of bias (p<0.01)

Figure 1.3.3 - Inter-reader bias test and reader against modal age bias test of *X. gladius* spines.-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)

11 images of the all sample (79 images) presented an agreement ≥ 80% (Tab. 1.3.4). These are from the lower age groups (age group 1, 3 and 4) and they could be represented the base for the age reference collection of the swordfish spines.

Table 1.3.4 – The number of images with an agreement ≥ 80% by modal age.

Criterion 80% agreement	
MODAL AGE	n
0	0
1	4
2	0
3	3
4	4
5	0
6	0
7	0
8	0
9	0
10	0
11	0
12	0
13	0
14	0
15	0
16	0
17	0
18	0
19	0
20	0
	11

Plotting the mean length by age group and readers (Fig. 1.3.4) seems clear that the mean length of the first 6 age groups (from age 0 to age 5 years) are comparable for the mostly of the readers. So this could be explained by the relative easiness to recognize the first growth increments.

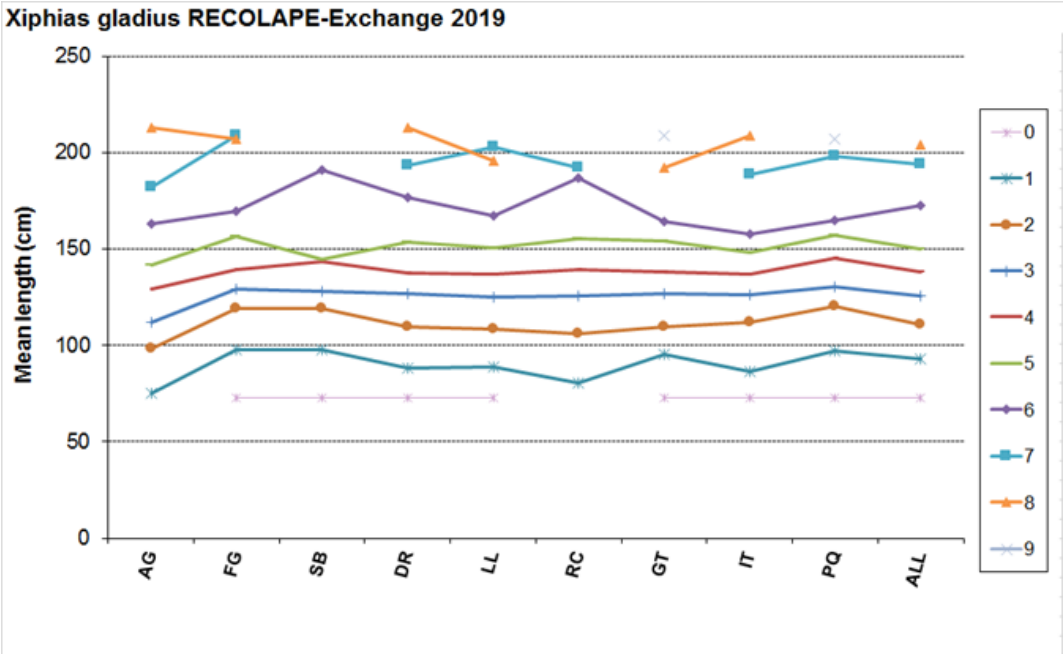

Figure 1.3.4 - The mean length at age as estimated by each age reader.

3.4 Remarks

The exchange exercise was based on a total of 79 fish sampled from 2003 to 2017 in Mediterranean from 2 sites sample: Ligurian sea and Alboran Sea. The pictures of HS (spines of the anal fin) tin section were prepared in the same way (Quelle et al., 2014; Lanteri and Garibaldi, 2019). The overall precision are PA, CV and APE respectively of 64.4%, 30.8% and 23%. These value are respectively lower and higher than those considered acceptable: 80% PA and 20% CV (PGCCDBS 2011). Moreover they were no significantly different if they were stratified by readers' experience, so this factor not explained fully the low PA and high CV reach in this exchange exercise. The analysis of the precision indices by age groups showed a negative trend from the first age group to the older one. In addition, the bias analysis on the all data seems highlight an under-estimation for the older age group, while an overestimation for the first age group (0 and 1 year). These results could be explained by the difficult to recognize the first growth increment and mostly growth increments (overlapping of the rings) in the older fish (age > 5 years).

The comparison of the age readings among the readers and each reader with modal age highlighted that a groups of readers follow a same age criteria. These results are confirmed also of the mean length at age as estimated by each age reader. Indeed in the first 6 age groups (from age 0 to age 5 years) the mean length at age are comparable for the mostly of readers. All these results were discussed during the next workshop.

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