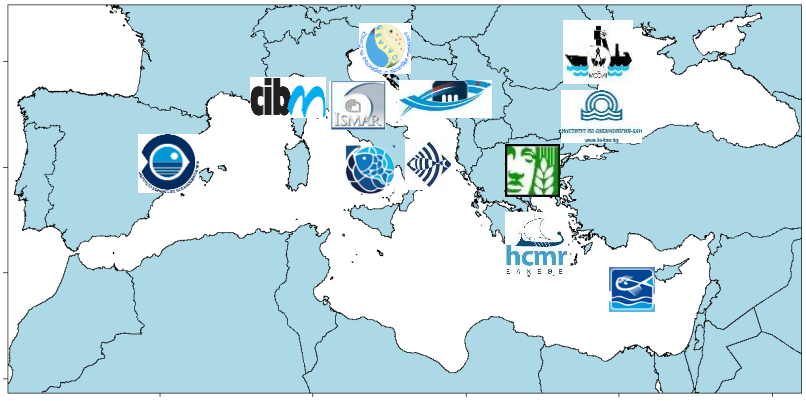

Call for Proposals MARE/2016/22 “Strengthening regional cooperation in the area of fisheries data collection”, Annex 1 “Biological data collection in EU waters” – Agreement Number – MARE/2016/22 – SI2.770115

**STrengthening REgional cooperation in the Area of fisheries biological data collection in the Mediterranean and Black Sea (STREAM)**

# *Pagellus erythrinus* Age Readers exchange

**April 2019**

**Partners involved:** COISPA, CIBM, HCMR, CNR-ISMAR, IEO, DFMR, IOF, IO-BAS, NIMRD

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.

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## Exchange exercise

Workshops and exchanges are regularly implemented by ICES and have recently included Mediterranean and Black Sea countries (ICES, 2016), Hence, the same methodologies and tools (WebGR, exchange exercises, etc.) can be utilized (ICES, 2011). However, some economically important species for the Mediterranean fisheries are not regularly addressed in ICES (e.g. *Pagellus erythrinus*). Under Task 6.2, the aforementioned tools were calibrated on this target species in order to obtain ageing scheme and criteria accepted and shared. The age analysis of the common Pandora still presents some gaps (Carbonara et al., 2019), in particular on the following issues:

- 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 common pandora, *P. erythrinus*, problems in age estimation using otolith can be summarized in the following main sources of errors:

- Presence of multiple bands;
- Presence of the false/s rings before the first winter ring;
- Presence of reproductive rings;
- Overlapping of the growth increment in the older specimens.

The Exchange approach based on supporting tools (SmarDots, Eltink sheet, full scale exchange) (PGCCDBS 2011; ICES 2016, ICES 2017) was utilized to identify the main source of bias and understand the level of precision for *P. erythrinus*

## 1.1 Sampling Collection and Participation

A preliminary step to the exchange was the collection and calibration on a suitable number of otolith images. The images of prepared otoliths have been provided by COISPA and HCMR laboratories to be used by all the participants. In total, 339 images were provided from specimens sampled from 2003 to 2017 in the Mediterranean area (Tab. 1.1.1; Fig. 1.1.1). The images of the otoliths of the specimens from the Tyrrhenian Sea were considered in the exchange with two types of preparation: whole and thin sections.

Table 1.1.1 - Samples distribution of common pandora by year and area.

| Species | Areas | 2016 | 2017 | Total |
|---|---|---|---|---|
| *P. erythrinus* | South Adriatic Italy (1) | 95 | 51 | 146 |
| | Central-Southern Tyrrhenian Italy (2) | 49 | | 49 |
| | Aegean (3) | 137 | 7 | 144 |
| Total | | 281 | 58 | 339 |

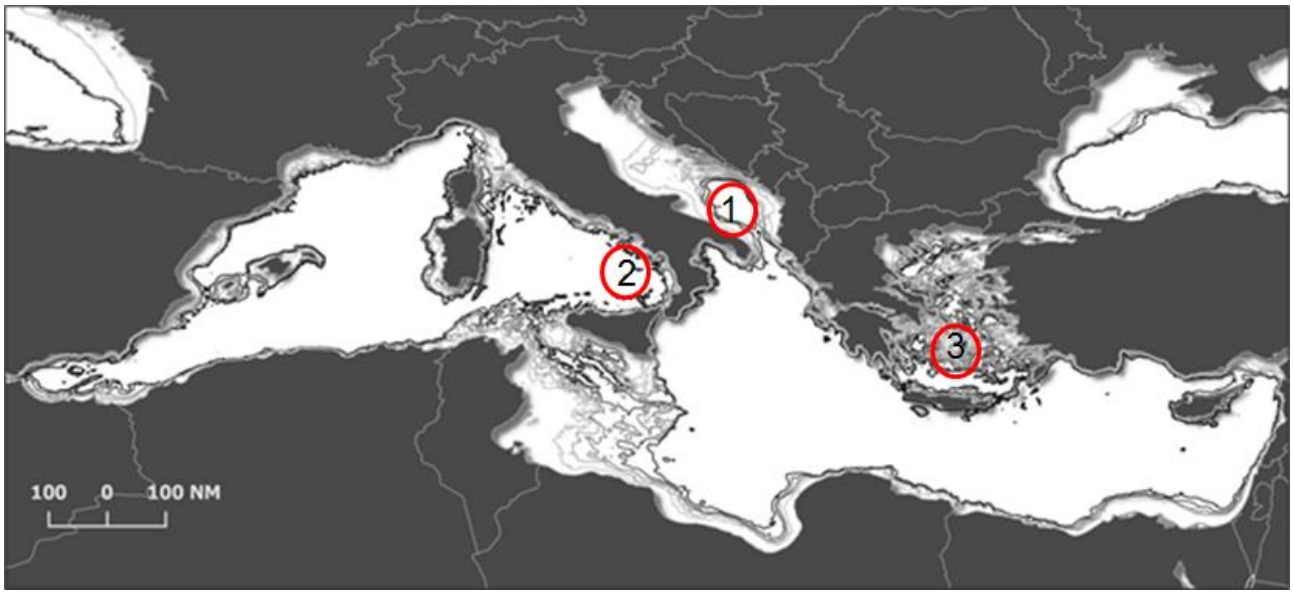



Figure 1.1.1 Map of investigated areas: 1 South Adriatic; 2 Central-Southern Tyrrhenian; 3 Aegean Sea

In total, we covered a wide range of sizes, including juveniles and adult specimens (Fig. 1.1.2).

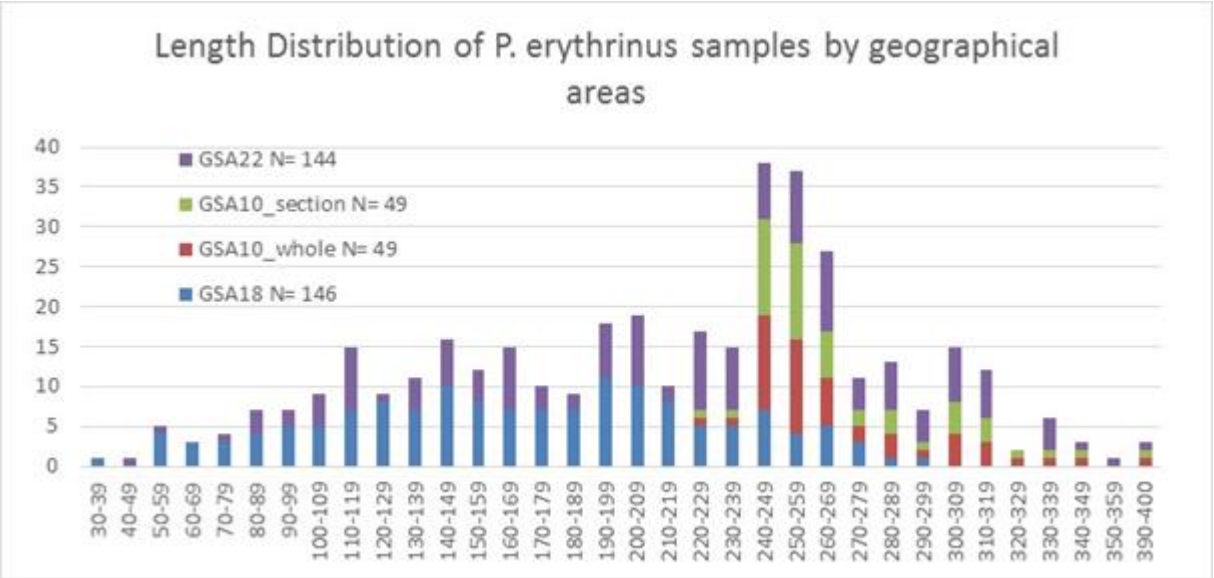



Figure 1.1.2 - Length distribution of X. gladius used during the exchange by geographical areas

In total, 9 readers participated to the reading exchange exercise from 2 countries and 4 laboratories (Tab. 1.1.2). The readers included not only readers from the Institutes involved in the STREAM 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 | Casciaro Loredana | Italy | COISPA |
| 2 | Palmisano Michele | Italy | COISPA |
| 3 | Andrea Massaro | Italy | CIBM |
| 4 | Andrea Bellodi | Italy | UNICA |
| 5 | Paola Pesci | Italy | UNICA |
| 6 | Archontia Chatzispyrou | Greece | HCMR |

## 1.2 Reading procedures and data analysis

All readers were asked to read each digitised image giving their own interpretation (positions of the annual rings on a given transect), and using the program SmartDOT platform. SmartDOT (http://www.ices.dk/marine-data/tools/Pages/smartdots.aspx) 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 otolith images. All registered data are available in the connected reporting environment.

The instructions on 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 transparent rings, the date of birth and the edge type. Moreover the date of capture and the sex were known by the readers. The age of each specimen was assigned following the scheme reported in the Table 1.1.3.

Table 1.1.3 - Age scheme used during the exchange

| DATA OF CAPTURE | EDGE | AGE |
|---|---|---|
| 1st | Translucent | N-1 |
| | Opaque | N |
| 2nd | Translucent | N-1 |
| | Opaque | N-1 |
| 3rd | Translucent | N |
| | Opaque | N |
| 4th | Translucent | N-1 |
| | Opaque | N |

All data were extracted from SmartDOT and analysed using a specific spreadsheet (Eltink, 2000). The spreadsheet (Eltink, 2000) was completed according to the instructions contained in the Guidelines and Tools for Age Reading Comparisons by Eltink et al. (2000). Modal ages were calculated for each otolith, 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

### 1.3.1 Whole otolith

In the analysis the data from all readers were used and the precision was analysed in terms of CV, APE and percent of agreement to modal age for *P. erythrinus*. The results are presented in the Table 1.3.1.1. The results from the whole otoliths showed the low precision with a percent agreement between 42.9 and 61.8%, CV from 25.4 to 47.8% and APE from 32.2 and 36.3%. For the all samples together, the CV, APE and percent of agreement to modal age were respectively: 37.6%, 33.5 and 57.2%.

Table 1.3.1.1 - Reading's precision for *P. erythrinus* by sampling area

| Species | Geographical area | Otoliths number | Length Range (cm) | Age range (year) | Percentage of Agreement | CV | APE |
|---|---|---|---|---|---|---|---|
| *P. erythrinus* | South Adriatic ITA | 146 | 3.5/29.5 | 0/13 | 61.8% | 47.8% | 32.2% |
| | Central-Southern Tyrrhenian ITA | 49 | 22/39.5 | 1/14 | 42.9% | 25.4% | 36.3% |
| | Aegean GR | 144 | 4.9/39 | 0/14 | 57.5% | 31.8% | 33.8% |
| | **TOTAL** | **339** | **3.5/39.5** | **0/14** | **57.2%** | **37.6** | **33.5%** |

For the whole otoliths, the coefficient of variation (CV), percent agreement and the standard deviation (STDEV) are plotted against MODAL age (Fig. 1.3.1.1).
The results show a decreasing trend from the lower age groups to the higher one for PA and CV and the opposite trend for the STDEV. These results could be explained by the overlapping of the growth increments in the oldest specimens (Carbonara et al., 2019) with a consequently higher difficult to recognize them. In general, for the first five age groups the agreement is around the 60% and the CV around 20%. For the age groups > 5 years, the PA decrease to 40%.

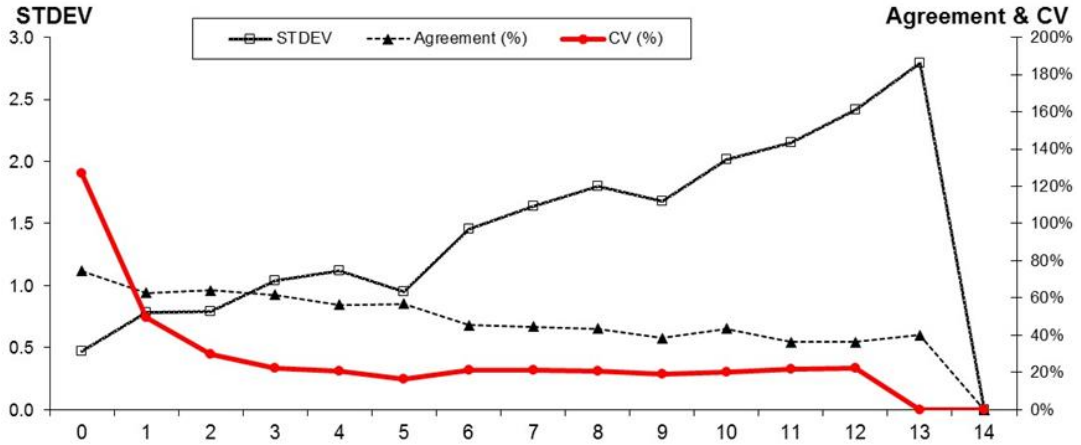



Figure 1.3.1.1 - Coefficient of variation (CV), percent agreement and standard deviation (STDEV) are plotted against MODAL age.

The percentage of agreement by readers weighed by the number of samples read are included between 48.3% and 77.7% (Table 1.3.3). Moreover the PA by age group shows a negative trend passing from 75% for the age 0 to 40% for the age 13.

Table 1.3.1.2 Percentage of agreement by reader and age group.

| | PERCENTAGE AGREEMENT | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MODAL age | Italy LC | Italy MP | Italy AB | Italy PP | Italy AM | Greece AC | ALL |
| | 0 | 100% | 78% | 56% | 42% | 81% | 92% | 75% |
| | 1 | 89% | 82% | 43% | 41% | 62% | 59% | 63% |
| | 2 | 77% | 77% | 63% | 58% | 55% | 55% | 64% |
| | 3 | 64% | 79% | 36% | 62% | 68% | 62% | 62% |
| | 4 | 79% | 76% | 35% | 41% | 59% | 47% | 56% |
| | 5 | 71% | 65% | 48% | 58% | 54% | 45% | 57% |
| | 6 | 87% | 70% | 35% | 26% | 28% | 23% | 45% |
| | 7 | 77% | 59% | 41% | 41% | 37% | 10% | 45% |
| | 8 | 57% | 52% | 56% | 40% | 33% | 19% | 44% |
| | 9 | 54% | 29% | 43% | 57% | 33% | 0% | 39% |
| | 10 | 75% | 25% | 50% | 63% | 43% | 0% | 43% |
| | 11 | 50% | 0% | 0% | 100% | 50% | 0% | 36% |
| | 12 | 50% | 0% | 0% | 100% | 50% | 0% | 36% |
| | 13 | 100% | 0% | 0% | 100% | - | 0% | 40% |
| | 14 | - | - | - | - | - | - | - |
| Weighted mean | 0-14 | 77.7% | 69.0% | 45.4% | 48.4% | 56.1% | 48.3% | 57.6% |

Relative bias can be defined as a systematic over- or underestimation of age compared to the modal age. The results of the exchange show an overestimation in the first five age groups (age 0-age 5) reaching about 0.4 year. While after the age group 7 the result show a clear underestimation that reach 2.6 year (Fig. 1.3.1.2).

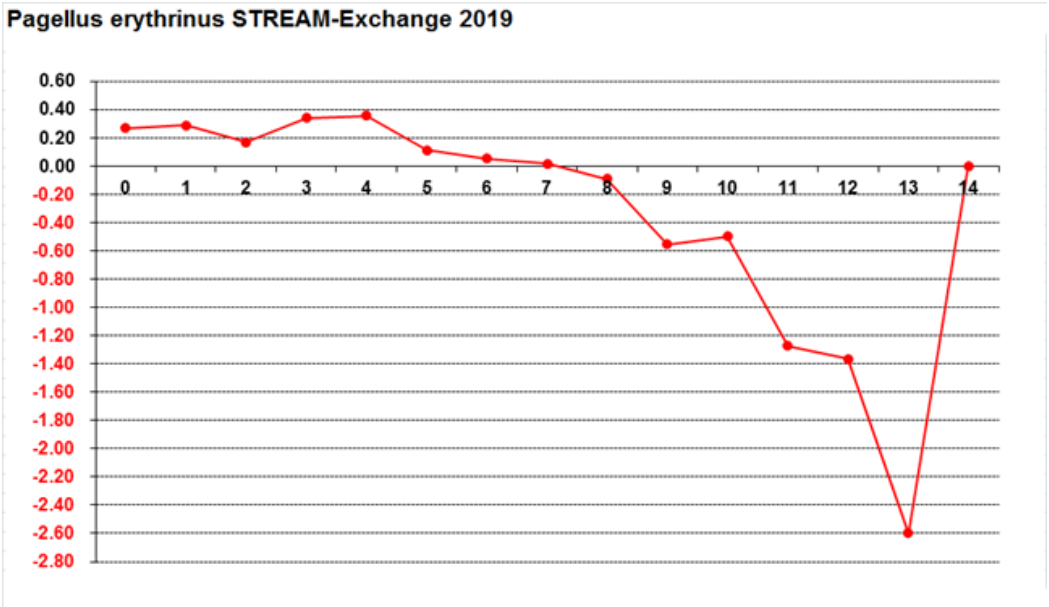



Figure 1.3.1.2 - The RELATIVE bias by MODAL age as estimated by all age readers combined

The hypothesis of an absence of bias between two readers or between a reader and the modal age estimated was tested with a one-sample Wilcoxon signed rank test (non-parametric test). The results of the test (Fig. 1.3.1.3) highlighted that there is no significant difference between the readers from the same Institute. All readers show significant difference with modal age.

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

| | Italy LC | Italy MP | Italy AB | Italy PP | Italy AM | Greece AC |
|---|---|---|---|---|---|---|
| Reader LC | | | | | | |
| Reader MP | * | | | | | |
| Reader AB | ** | ** | | | | |
| Reader PP | ** | ** | – | | | |
| Reader AM | ** | ** | ** | ** | | |
| Reader AC | ** | ** | ** | ** | ** | |
| MODAL age | * | ** | ** | ** | ** | ** |

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

Figure 1.3.1.3 - Inter-reader bias test and reader against modal age bias test. -: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)

66 images of the all sample (339 images) presented an agreement ≥ 80% (Tab. 3.3.4). These images could represent a first reference collection of common Pandora otoliths.

Table 1.3.1.4 – The number of images with an agreement ≥ 80% by modal age.

| Criterion 80% agreement | |
|---|---|
| MODAL AGE | n |
| 0 | 18 |
| 1 | 16 |
| 2 | 10 |
| 3 | 8 |
| 4 | 6 |
| 5 | 4 |
| 6 | 0 |
| 7 | 1 |
| 8 | 3 |
| 9 | 0 |
| 10 | 0 |
| 11 | 0 |
| 12 | 0 |
| 13 | 0 |
| 14 | 0 |
| 15 | 0 |
| 16 | 0 |
| 17 | 0 |
| 18 | 0 |
| 19 | 0 |
| 20 | 0 |
| | 66 |

Plotting the mean length by age group and readers (Fig. 1.3.4), it seems clear that the mean length of the first 4 age groups (from age 0 to age 4 years) is consistent among most of the readers. This could be explained by the relative easiness to recognize the first growth increments.

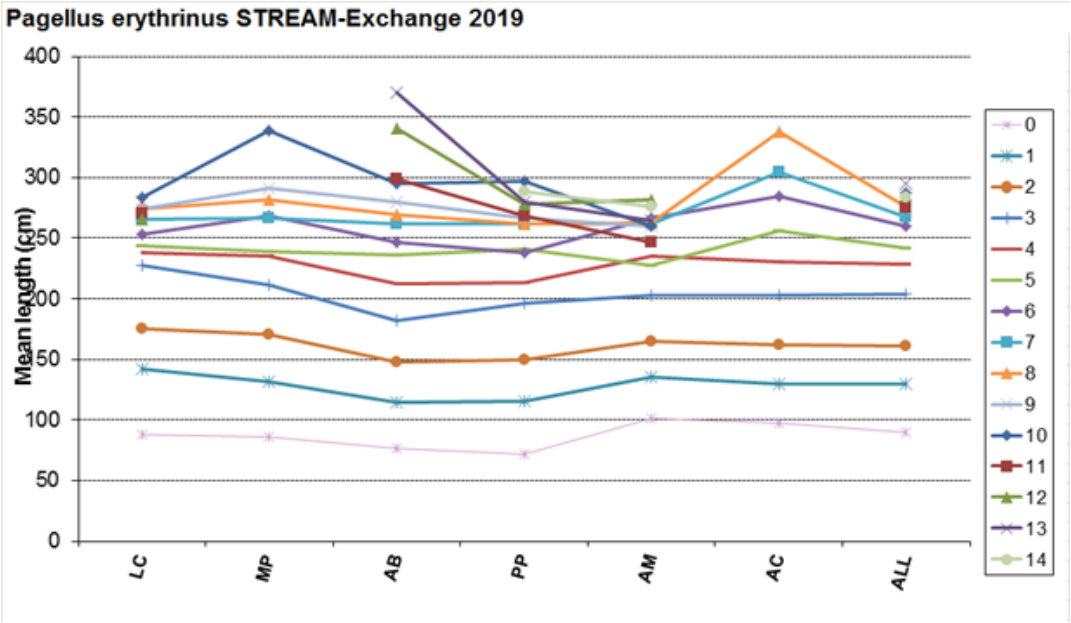



Figure 1.3.1.4 - The mean length at age as estimated by each age reader.

The Smartdot software allowed to measure the distance from the core to each marked growth increment. Figures 1.3.1.5 and 1.3.1.6 show the average distance from the centre to the winter rings for all readers together and by reader. The average distance from the centre to the winter rings show rather regular increases for the first 4 winter rings, followed by a reduction of the width of the subsequent increases. The "outliers" out of the box-whiskers usually are remarkable for some readers. This may be indicative of different interpretations of the otoliths. This pattern became worse in the age groups > 4 both among and within the readers.

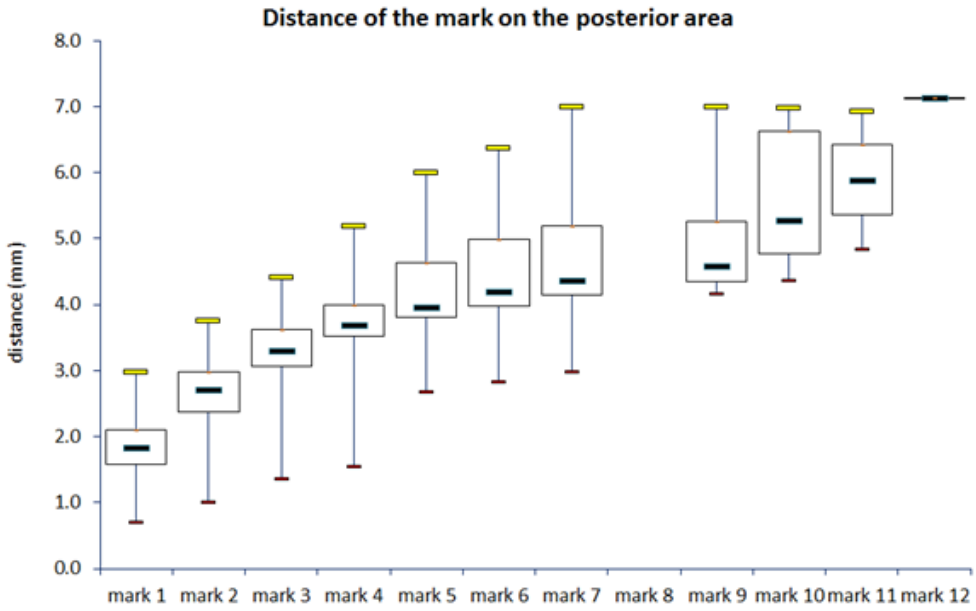


Figure 1.3.1.5 - Plot of average distance from the centre to the winter rings. The boxes represent the median, upper and lower boundaries of the interquartile range.

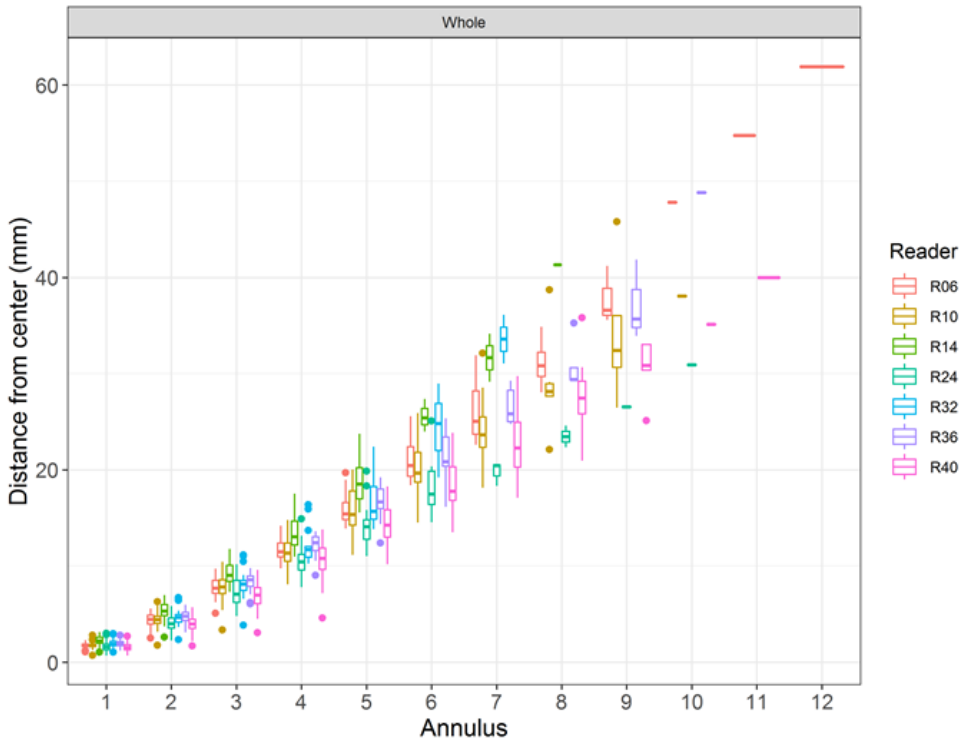



Figure 1.3.1.5 - Plot of average distance from the centre to the winter rings by reader. The boxes represent the median, upper and lower boundaries of the interquartile range, whiskers represent the minimum and maximum values and the dots represent the outliers.

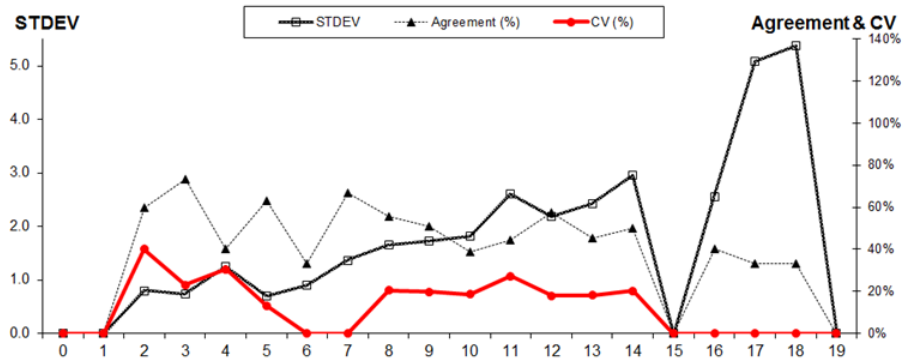
## 1.3.2 Whole and Sectioned otolith

In terms of precision, the results of the whole otolith images are 42.9%, 25.4% and 36.2% for the PA, CV and APE, respectively. For the otolith sections, they are 51.9%, 22.3% and 36.5% for the PA, CV and APE. The precision indices (PA, CV and APE) showed significant differences (Kruskal–Wallis test; p>0.05) if they were considered a sample with different preparation methods (whole and thin section) (Tab. 1.3.2.1).

Table 1.3.2.1 - Reading's precision for *P. erythrinus* by preparation method

| Species | Geographical area | Otoliths number | Length Range (cm) | Age range (year) | Percentage of Agreement | CV | APE |
|---|---|---|---|---|---|---|---|
| *P. erythrinus* | Central-Southern Tyrrhenian_whole | 49 | 22/39.5 | 1/14 | 42.9% | 25.4% | 36.2% |
| | Central-Southern Tyrrhenian_section | 49 | 22/39.5 | 1/19 | 51.9% | 22.3% | 36.5% |

**Section**




**Whole**

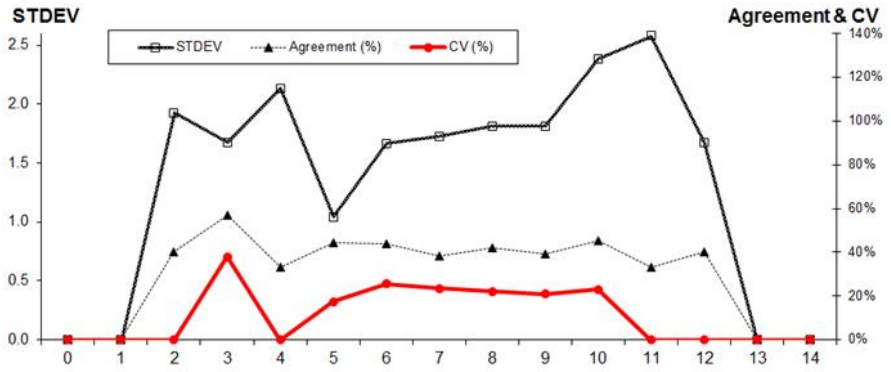


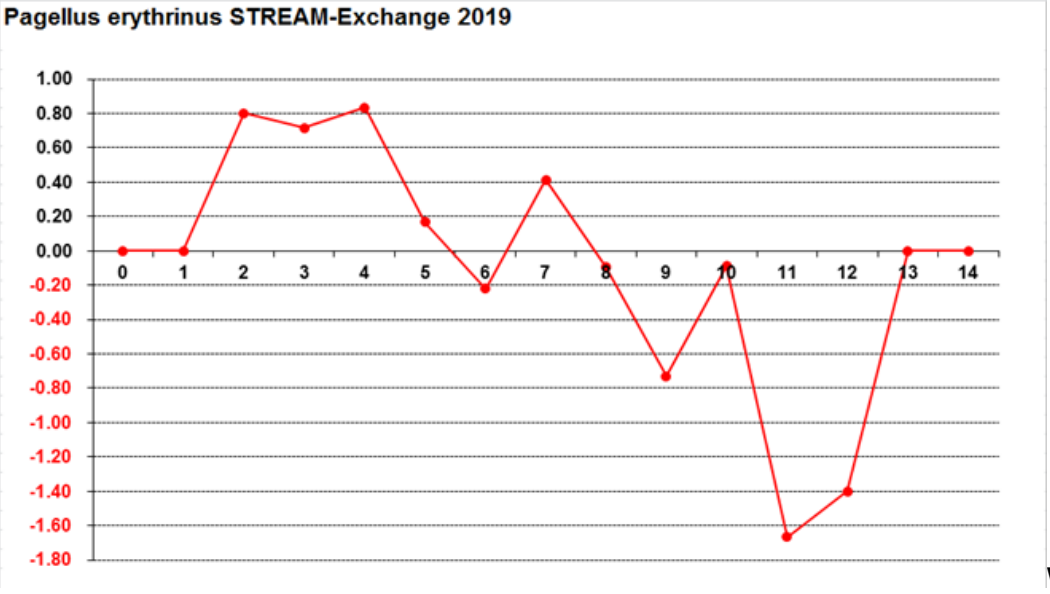

Figure 3.3.2.1 - The coefficient of variation (CV), percent agreement and the standard deviation (STDEV) are plotted against MODAL age.

The PA in the otolith sections is around the 60%, while in whole otoliths around the 40%. Moreover, 14 age groups were identified using whole otoliths, and 19 age groups in otolith sections (Fig. 1.3.2.1).

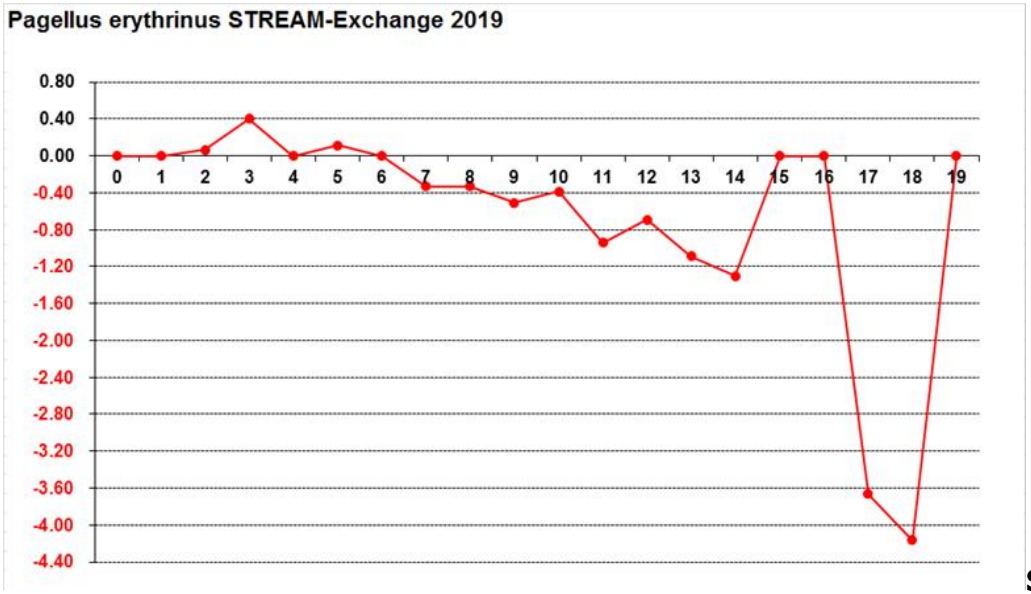
Table 1.3.1.2 Weighed percentage of agreement by reader and age group.

| | PERCENTAGE AGREEMENT | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MODAL age | Italy LC | Italy MP | Italy AB | Italy PP | Italy AM | Greece AC | ALL |
| Whole | 0 | - | - | - | - | - | - | - |
| | 1 | - | - | - | - | - | - | - |
| | 2 | 0% | 0% | 0% | 100% | 100% | - | 40% |
| | 3 | 80% | 80% | 0% | 60% | 100% | 0% | 57% |
| | 4 | 100% | 100% | 0% | 0% | 0% | 0% | 33% |
| | 5 | 67% | 67% | 33% | 67% | 0% | 33% | 44% |
| | 6 | 57% | 71% | 43% | 43% | 33% | 14% | 44% |
| | 7 | 83% | 83% | 33% | 17% | 0% | 0% | 38% |
| | 8 | 46% | 57% | 64% | 29% | 36% | 10% | 42% |
| | 9 | 29% | 38% | 63% | 63% | 25% | 0% | 39% |
| | 10 | 100% | 0% | 50% | 100% | 0% | 0% | 45% |
| | 11 | 0% | 0% | 0% | 100% | 100% | 0% | 33% |
| | 12 | 0% | 0% | 0% | 100% | 100% | - | 40% |
| | 13 | - | - | - | - | - | - | - |
| | 14 | - | - | - | - | - | - | - |
| Weighted mean | 0-14 | 55.3% | 57.1% | 42.9% | 46.9% | 38.5% | 8.1% | 43.0% |

| | PERCENTAGE AGREEMENT | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MODAL age | Italy LC | Italy MP | Italy AB | Italy PP | Italy AM | Greece AC | ALL |
| Sectioned | 0 | - | - | - | - | - | - | - |
| | 1 | - | - | - | - | - | - | - |
| | 2 | 100% | 0% | 0% | 100% | 100% | - | 60% |
| | 3 | 67% | 100% | 0% | 100% | 100% | - | 73% |
| | 4 | 100% | 0% | 0% | 50% | 50% | - | 40% |
| | 5 | 60% | 80% | 40% | 80% | 80% | 0% | 63% |
| | 6 | 100% | 0% | 100% | 0% | 0% | 0% | 33% |
| | 7 | 100% | 100% | 0% | 100% | 100% | 0% | 67% |
| | 8 | 33% | 33% | 67% | 100% | 100% | 0% | 56% |
| | 9 | 50% | 50% | 63% | 63% | 63% | 14% | 51% |
| | 10 | 100% | 33% | 0% | 33% | 67% | 0% | 39% |
| | 11 | 67% | 67% | 67% | 0% | 67% | 0% | 44% |
| | 12 | 50% | 60% | 80% | 40% | 100% | 0% | 58% |
| | 13 | 100% | 25% | 25% | 75% | 25% | 0% | 45% |
| | 14 | 67% | 25% | 75% | 75% | 33% | 0% | 50% |
| | 15 | - | 0% | 100% | 0% | - | - | - |
| | 16 | 0% | 0% | 100% | 0% | 100% | - | 40% |
| | 17 | 100% | 0% | 0% | 0% | 100% | 0% | 33% |
| | 18 | 0% | 0% | 100% | 100% | 0% | 0% | 33% |
| | 19 | - | - | - | - | - | - | - |
| Weighted mean | 0-19 | 67.4% | 42.9% | 46.9% | 61.2% | 69.6% | 3.4% | 51.5% |




**Whole**




**Sectioned**

Figure 1.3.2.2 - The RELATIVE bias by modal age as estimated by all age readers combined.

The difference in age due to the preparation method is significant; in general, the otolith sections present a higher PA and lower CV (Fig. 1.3.2.3).

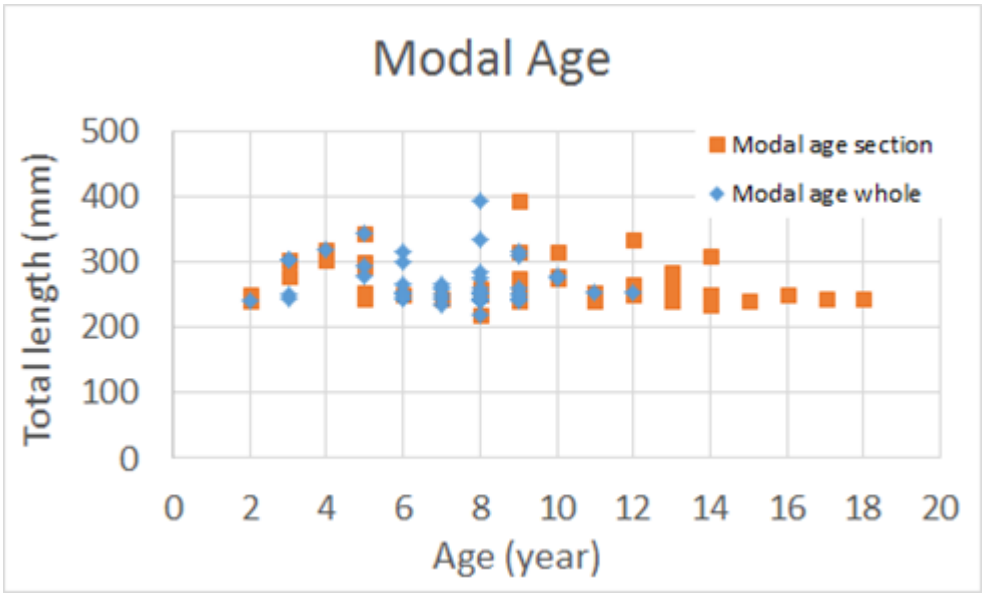



Figure 1.3.2.3 – Length at age from the whole and sectioned otoliths.

## 1.4 Remarks

The analysis of the precision indices by age group showed a negative trend from the first age group to the oldest ones. In addition, the bias analysis highlight an under-estimation for the oldest age group, while an overestimation for the first age groups (0-

5 year). These results could be explained by the difficulty to recognize the growth increment in the oldest fish (age > 5 years) due to the overlapping of the rings.
These results are confirmed by the mean length at age as estimated by each age reader. In the first 6 age groups (from age 0 to age 5 years), the mean length at age is consistent among the readers.
The comparison of the age readings among the readers and between each reader and the modal age highlighted that a group of readers follow the same age scheme.

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