Laboratory procedures and protocols for age determination in European anchovy (*Engraulis encrasicolus*) in ICES 8c and 9a North areas.

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Workshop on Age estimation of European anchovy (*Engraulis encrasicolus*) (WKARA3) On line: 23-25 November 2021

Age interpretation criteria

Followed by Uriarte et al., (2002 and 2016), ICES WKARA 2009 and WKARA2 (ICES, 2017)

The method is based on the **knowledge of the annual growth pattern** of the anchovy otoliths, of the seasonal growth of **otolith edge** by ages and of the most typical **checks**.

- A set of an **opaque** and **hyaline** zone corresponds to an **annual growth** zone (annulus).

- The **date of birth** is conventionally assumed to be the **1st of January** and the fish is assigned to a year class on this basis (if an otolith is collected during the first semester the age group correspond to the number of hyaline zones, if the otolith is collected from a fish caught during the second semester, the hyaline edge will not be considered).

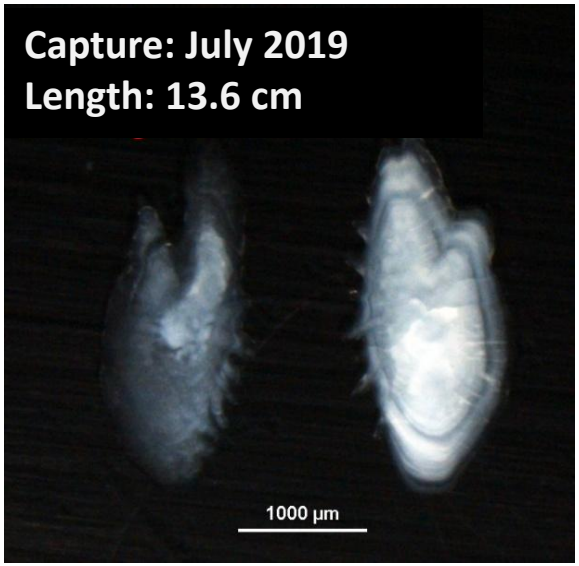
- **Maximum** otolith growth (opaque ring formation) takes place in **summer** months, and it **decreases** in **winter** time (hyaline ring formation).

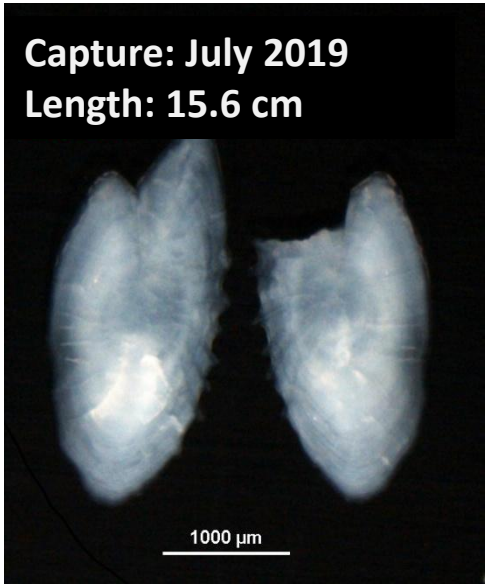
- Typical **checks** occur before and after the first winter ring is formed, during age 0 and age 1 of anchovy.

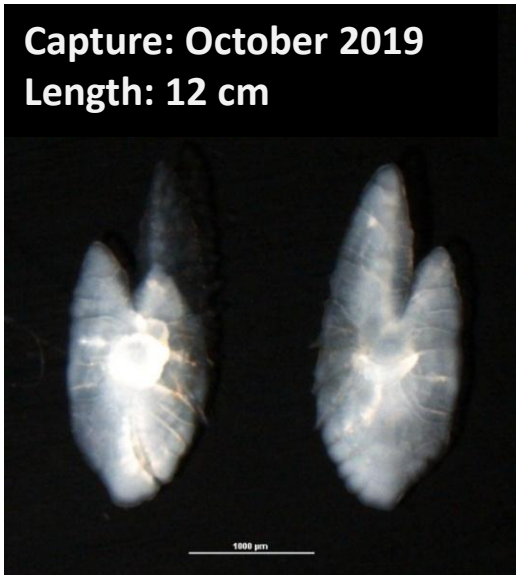
  - The checks **before** the true hyaline winter ring are generally present around the nucleus (**central check**), these checks are named **C05** and **C08**.
  - The most typical ring formed **after** the first true hyaline ring is formed during June/July in many of the 1 years old anchovy at the peak of their first spawning period (**spawning check**), these checks are named **C15** or **C18**.

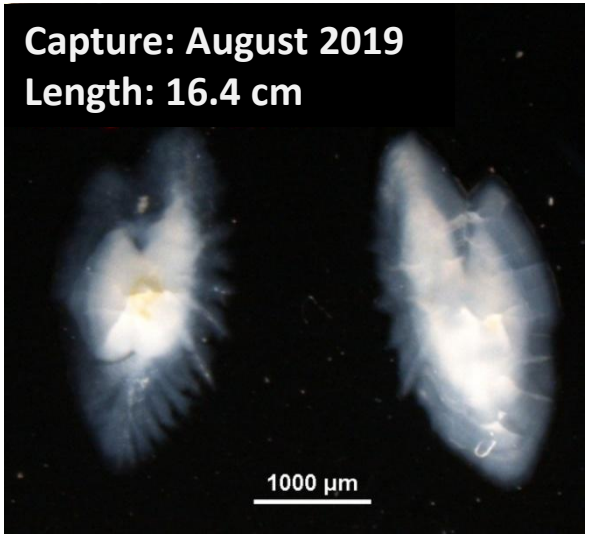
Advice about some methods

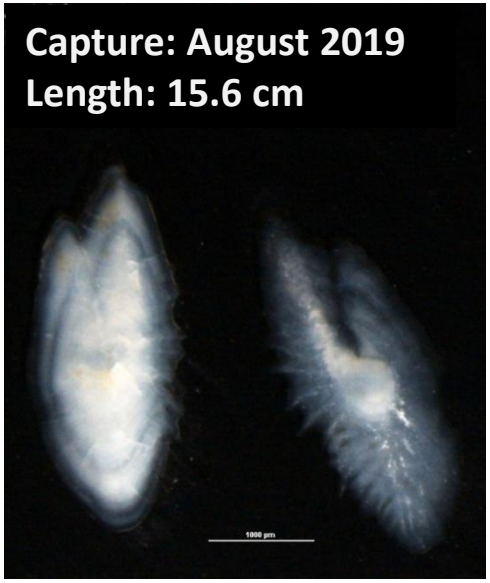
It is advisable to have both otoliths to take the readings, because sometimes one of the otoliths breaks during extraction, is amorphous or decalcified.

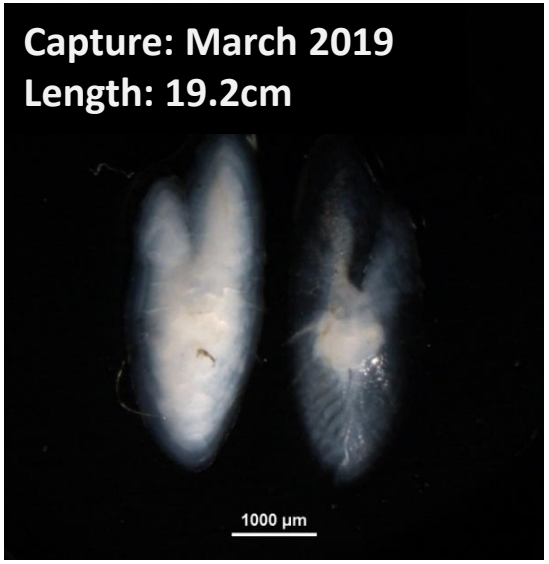










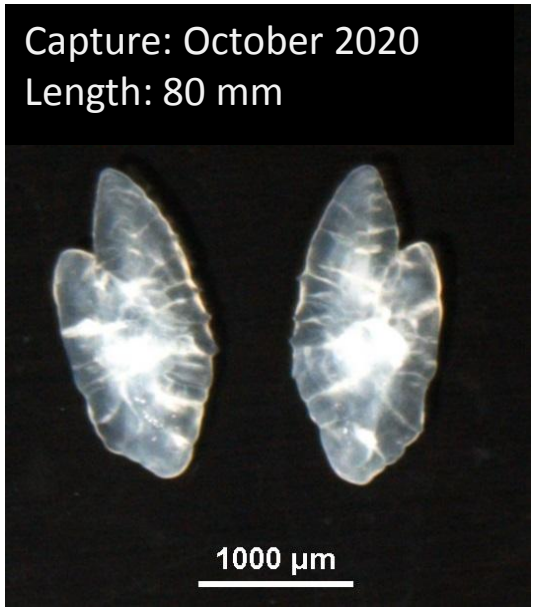


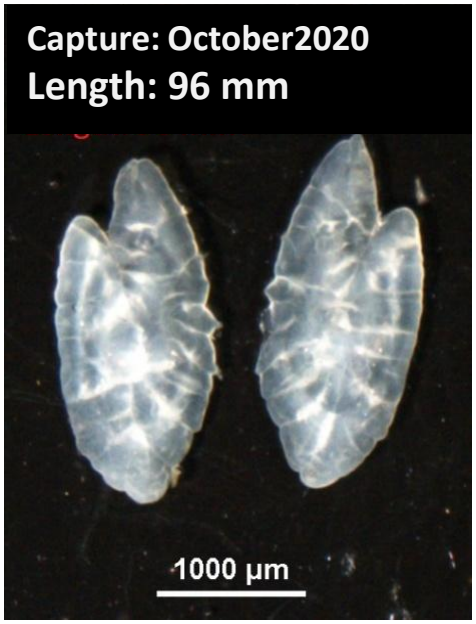
First reading otoliths submerged in fresh water.

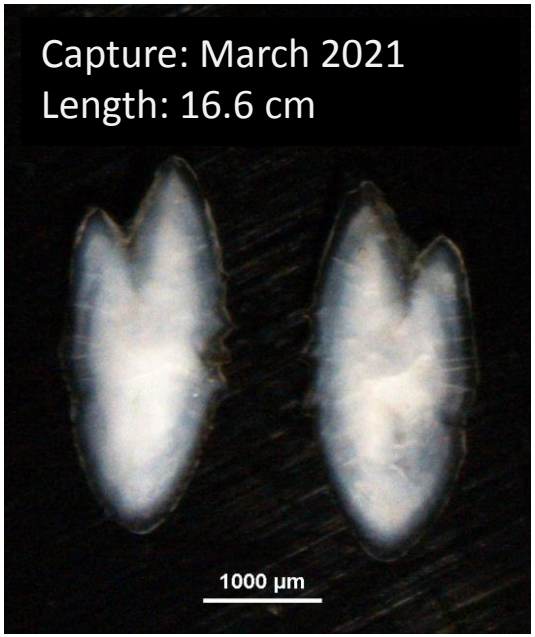
A first reading of otoliths can be made with **immersion in fresh water**, this takes advantage of to clean and eliminate the organic matter that may have adhered from its extraction.

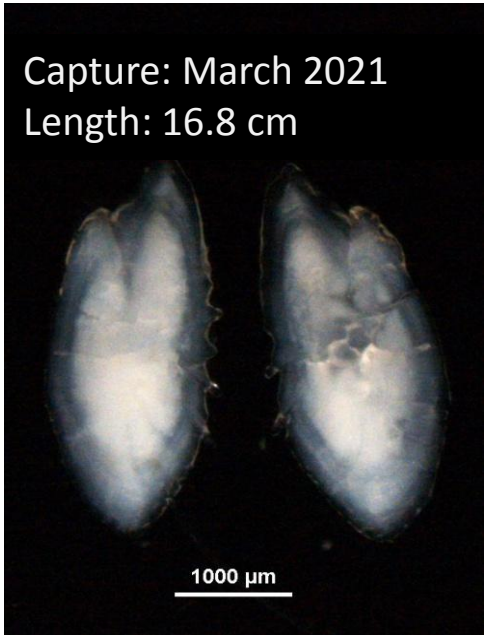
But with fresh water the sharpness of the hyaline rings is not so contrasted and otoliths edges are not clear. This makes it difficult to estimate age, so it is recommended that a **later reading** be done when the otoliths are **fixed**. This way we will have a more concise appreciation of the type of edge and a clearer vision of the rings.

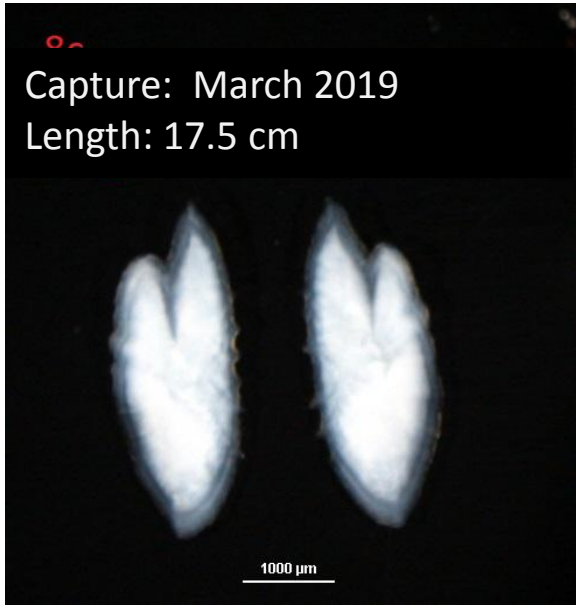
These otolith images have been taken with submerged in fresh water.











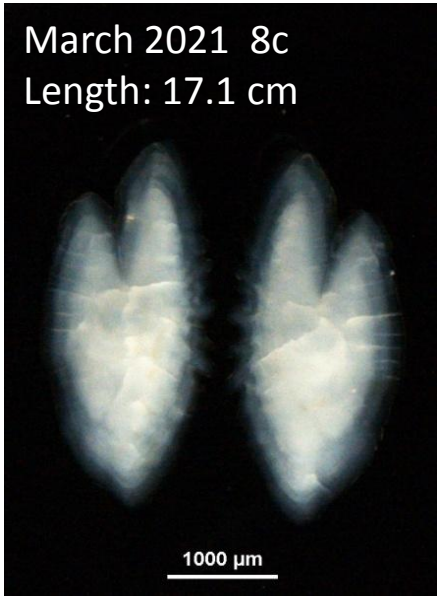
Guidelines to take into account to estimate age

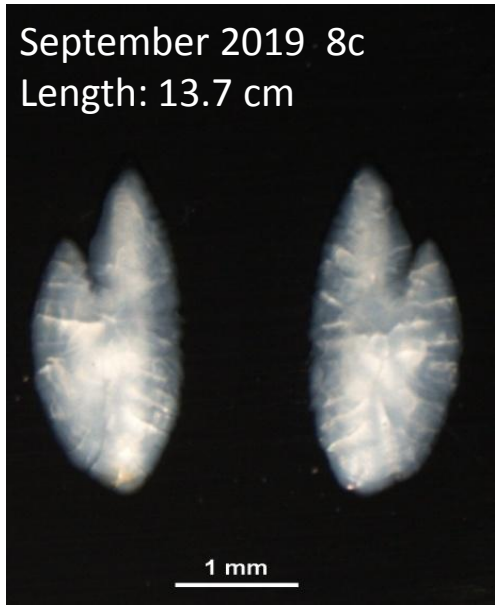
Difficulty interpreting a weak ring (poorly marked)

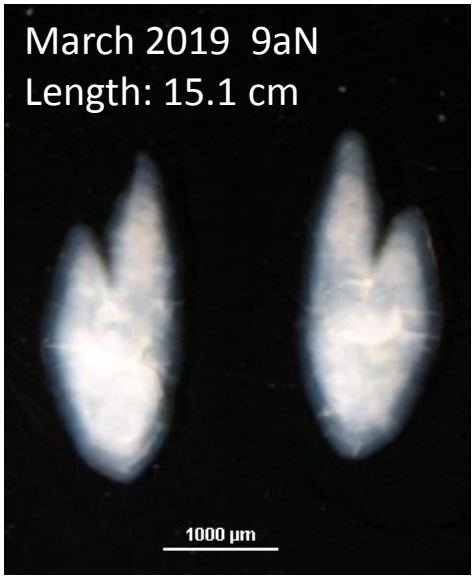
Comments for interpretation of the WKARA2 Report, 2016

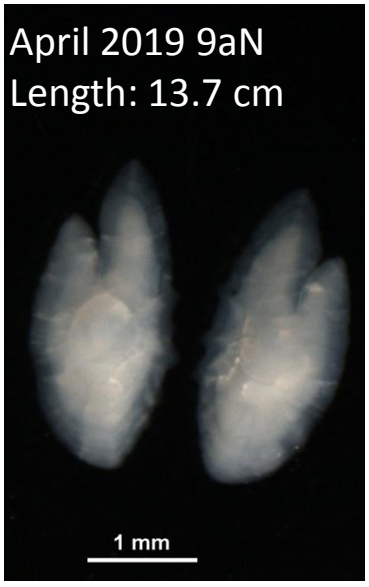
"*If we find a **faint ring**, not very marked, at a distance in which a true winter ring would be expected (whether it is observed visually or counting on the data of the measurement of the radius of the first ring according to its area), the possibility whether such a mark corresponds to a true winter ring **could be assessed** by its conformity with the **expected annual growth** and the **type of edge** for the resulting age.*"

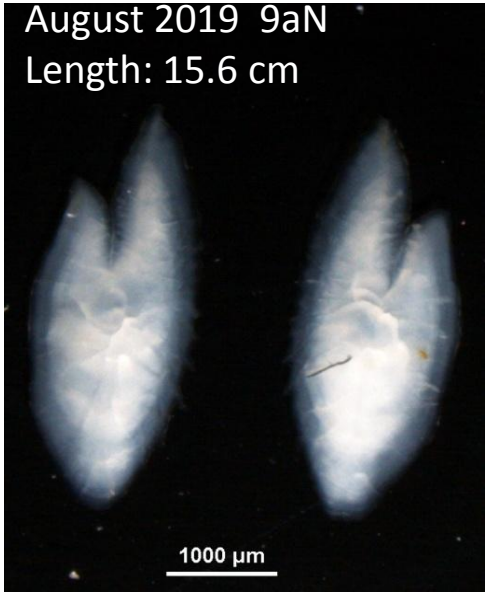
Difficulty interpreting a weak ring (poorly marked)

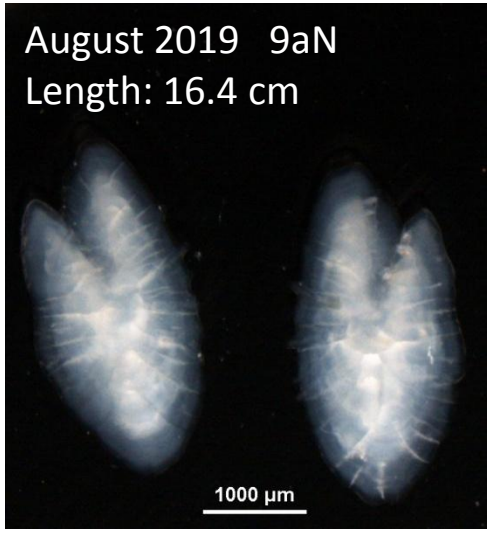












Otoliths showing little growth from the 1st winter ring to hyaline edge

WKARA2 Report, 2016

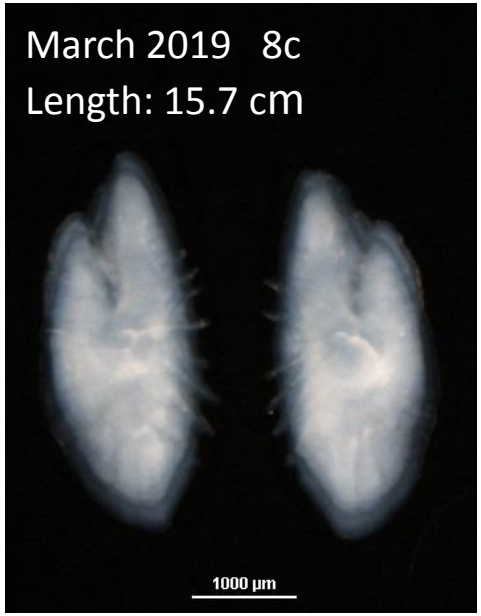
"*The **growth pattern** must always **be taken into account** when interpreting rings that can be winter compared to rings that are false*."

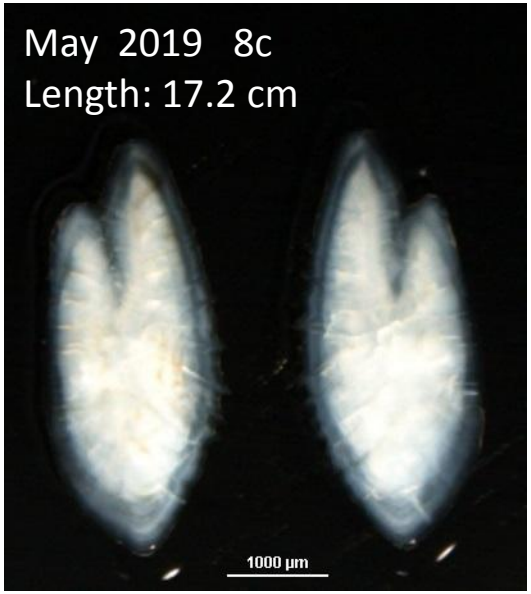
"***High growth is expected** from the nucleus to the 1st winter ring that continues with a wide and large dull growth zone until the second hyaline winter ring is formed*".

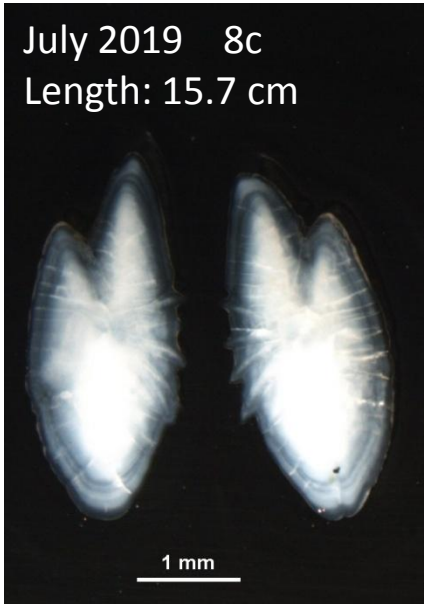
"*The most typical ring formed after the first true hyaline ring forms during June / July in many 1-year-old anchovies at the peak of their first **spawning** period, which is considered a laying check. Depending on their position in relation to the expected total annual growth, these checks are called **C15** or **C18**. Checks in the second year of life named C22 or C25 are very rare*".

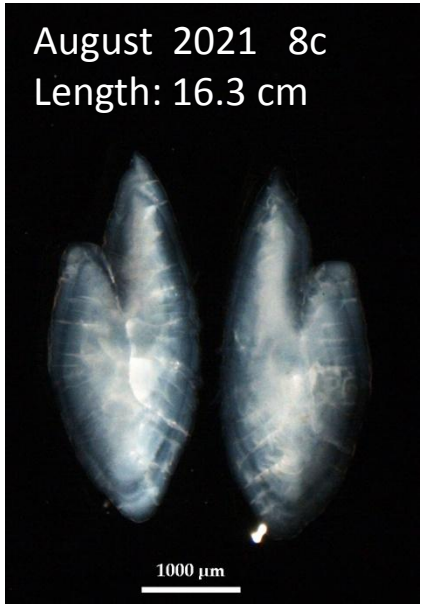
In the studied areas (8c and 9aN) there are otoliths to which I would tend to assign an older age based on their length and which show little growth from their first winter ring to the hyaline border, but taking into account the previous premises I consider that the hyaline edge of the otolith is not a winter ring.

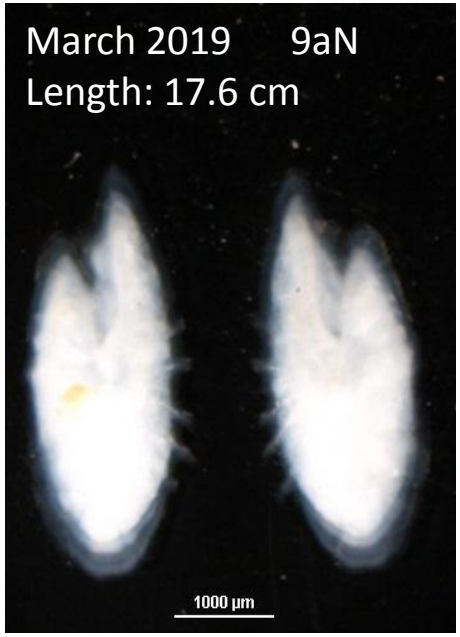
Otoliths showing little growth from the winter ring to its hyaline border.

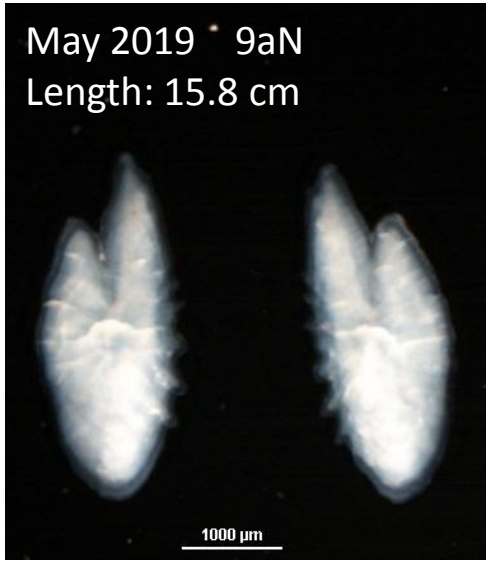


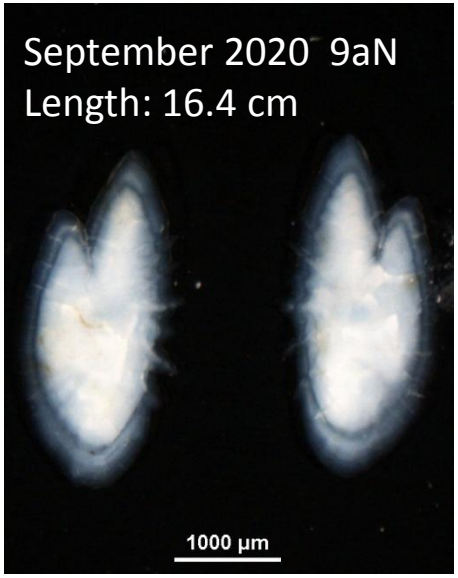


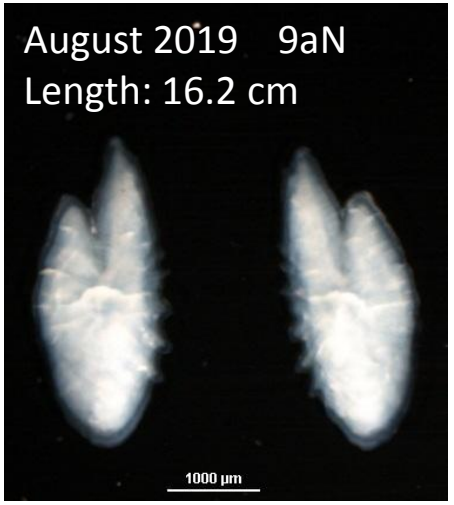












Identify a possible central check or a true winter ring

WKARA2 Report, 2016

In the Bay of Biscay zone *"according to the work of Hernández et al. (2013) to corroborate the position of the **first hyaline check** formed before their first winter annual ring, through the counts of micro-increments, it was suggested that hyaline marks placed at a distance from the core of less than **850 µm (±100µm)** should be considered as a check **C8** (not as true winter hyaline rings) R1= 1295 µm, R2= 1589 µm."*

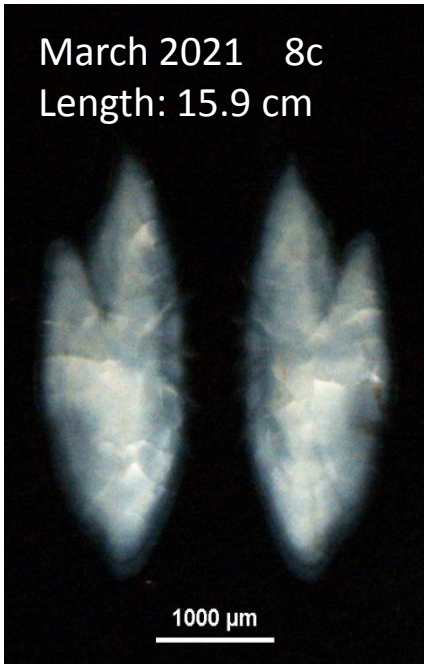
In the ICES zone Subdivision North 9a. *"The analysis of the growth rings biometric measurements (Hernandez et al., 2016): in cases where the distance from the core to the first visible ring was **<779 ± 95 µm**, this ring could be assign by the reader as the presumed check **C08** and R1= 1213 µm, R2= 1643 µm."*

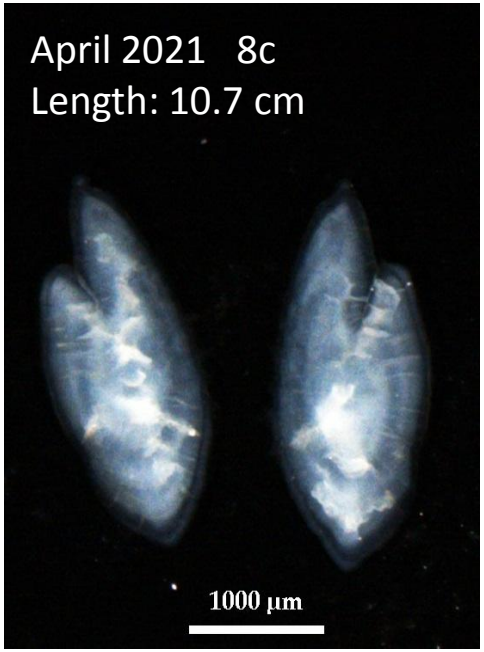
Having a micrometer installed in the magnifying glass helps a lot to interpret whether a 1st hyaline ring is the winter one or it is false, so we can measure the radius from the nucleus to the beginning of the formation of the first translucent ring, following the guidelines described above.

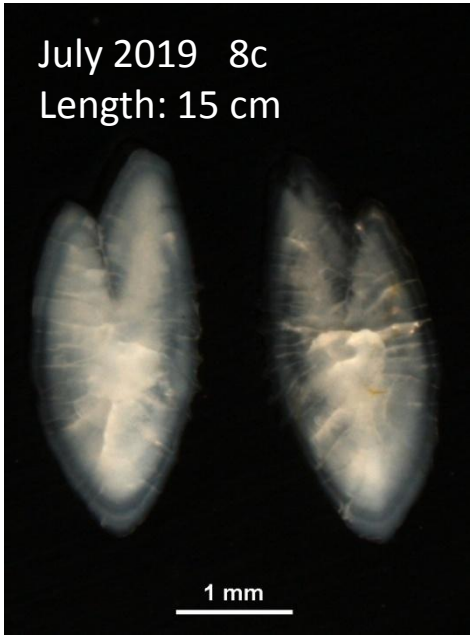
*"Usually **checks** tend to be **weaker** or more diffuse than true annual rings and often they are not completely formed all around otolith, their position will often differ from the expected position of the true annual rings."*

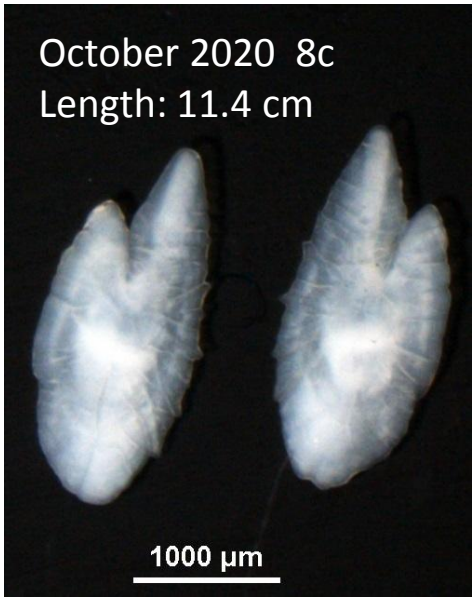
But this supposed property is not always decisive, since sometimes the nature of the otolith shows us that the way to mark the annual rings is weak and when the individual is large these rings are counted as annuals. To discern between a weak annual ring and a check we must follow the guidelines described above and measure the radius of these rings.

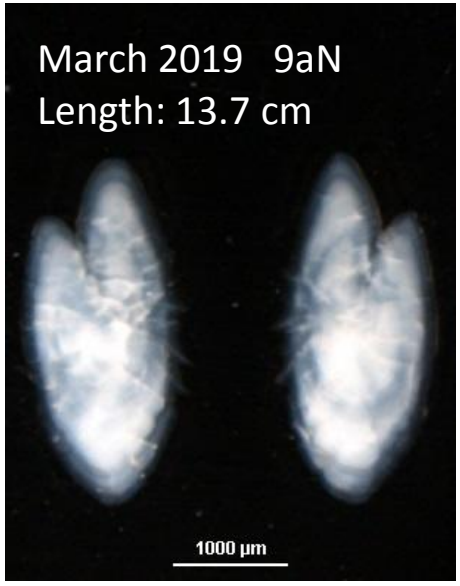
Identify a possible central check or a true winter ring

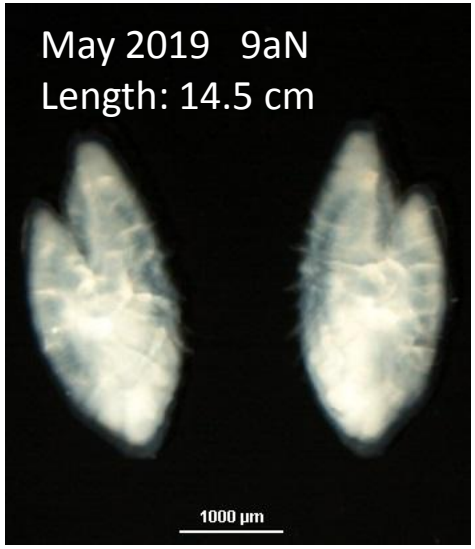


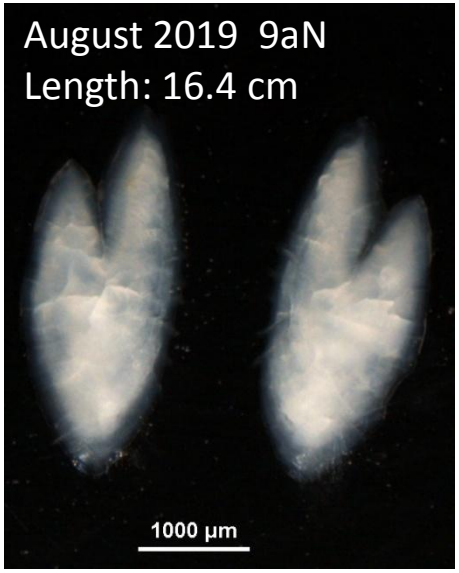


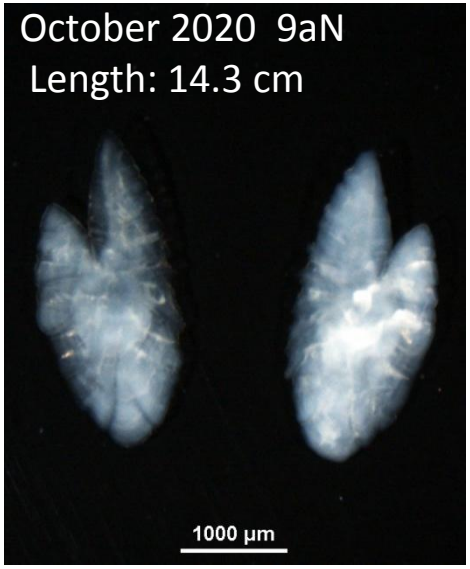












Multiples hyaline zones

WKARA2 Report, 2016

"*Some first winter hyaline zones may be composed of several close hyaline marks forming a **cluster** (two or more very close hyaline marks). Readers should be aware of such possibility for proper joint assignation to a single winter zone."*

*"In all areas readers identify **checks** and therefore in order to improve the age determination readers should become familiar with the most common checks in their area."*

*"The number of checks rings is highly variable. In some cases, checks rings do not have an annual periodicity. Anchovies lay downs **different** numbers of **checks**."*

*"Age can be assigned taking into account the most common possible checks: the most typical checks formed during the first year of life (age-0) are named **C05** or **C08** because they are formed approximately at 50 or 80% of the expected annual growth of the otolith at age-0. Other typical checks are **C12**, **C15** or **C18**, which correspond to checks formed at age-1 at ~20, 50 or 80% of the expected annual growth of the otolith for that age."*

When an otolith presents multiple hyaline zones, it is sometimes difficult for me to distinguish between them which is the first annual ring, this has important implications when I have to measure that true ring,

then according to this advice:

*The **quality** (or credibility) of the interpretation of age is assigned according to the "3-point rating system" (WKNARC Report, 2011):*

- ***AQ1** easy to read otoliths.*
- ***AQ2** otoliths difficult to read.*
- ***AQ3** otoliths whose interpretation is practically impossible.*

In these cases, the quality of reading that I assign is AQ2, since it is difficult for me to discern between them which are the winter rings, and these otoliths are discarded for taking measurements.

So is it advisable to use the AQ2 as an age quality assignment? What decision do you make?

Multiples hyaline zones