Age validation in early stages of *Sepia officinalis* and its application to age estimation of Sepia species

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VIII INTERNATIONAL SYMPOSIUM ON MARINE SCIENCES

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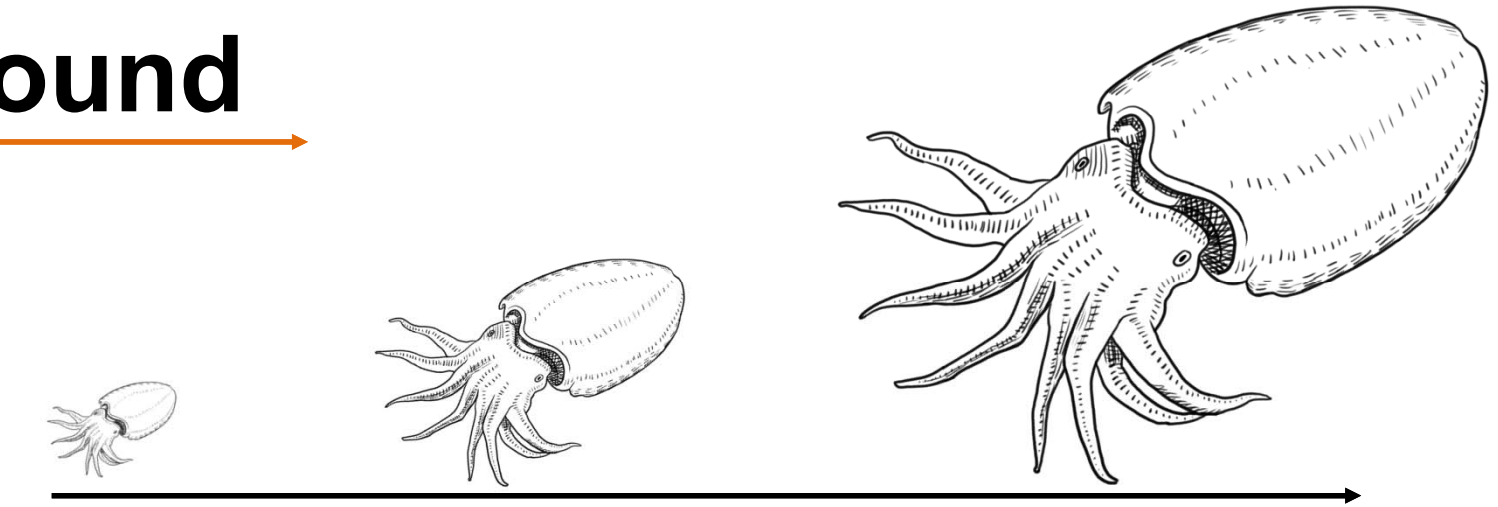
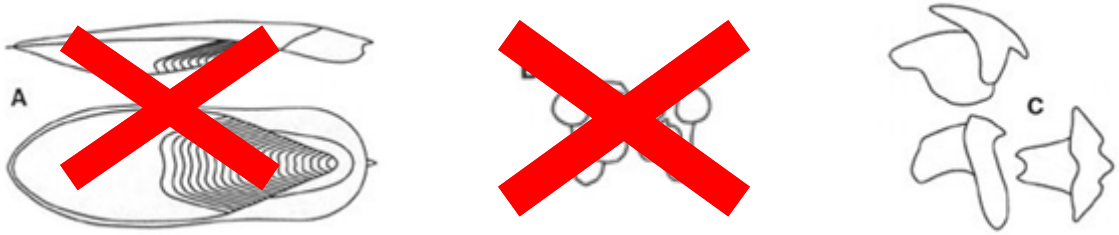
Background



Hard structures



Background

Octopus vulgaris

Hernández-López et al., 1999
Peráles-Raya et al.,2000, 2014

Octopus maya

Villegas-Bárcenas et al. 2014

Sepia officinalis

Lishchenko et al, 2018

Aim

“Validate the growth marks in early stages of *Sepia officinalis* beaks that allow us to estimate the real age in Sepia species”

Experiment design

Experiment 1

Culture at 21º C

Feed ad libitum

Sacrifices day by day, until day 5. Then every 5 days or by natural death.

Experiment 2

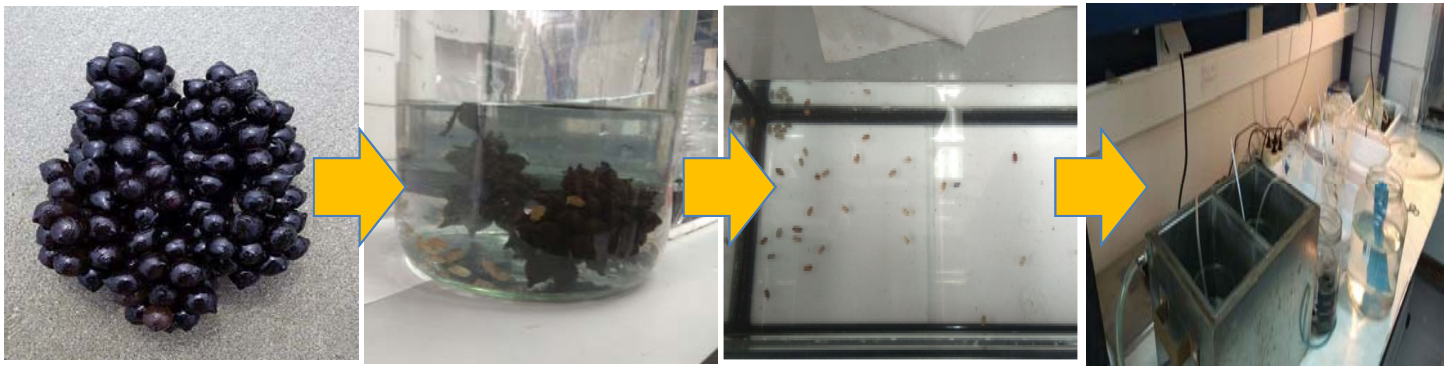
Culture at 18º C

Feed ad libitum

Sacrifices day by day, until day 5. Then every 5 days or by natural death.

The experiments were repeated twice in order to standardize the methodology.

Culture



~ 5.5 mm DML

~40 µm LRL

Phase I

29 individuals a 18ºC
73 individuals a 21ºC

Max age → 31 days

Phase II

47 individuals a 21ºC

Max age → 20 days

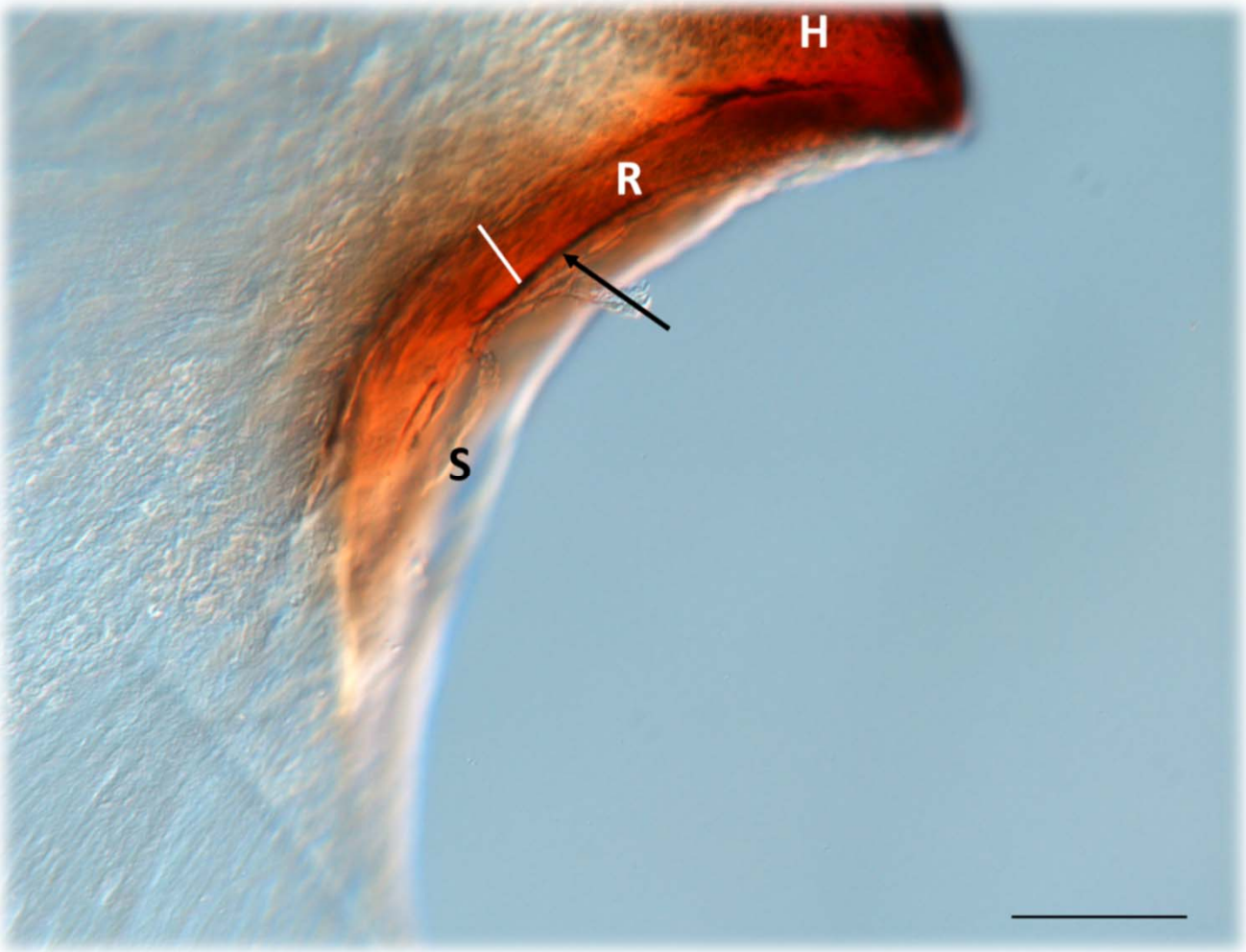
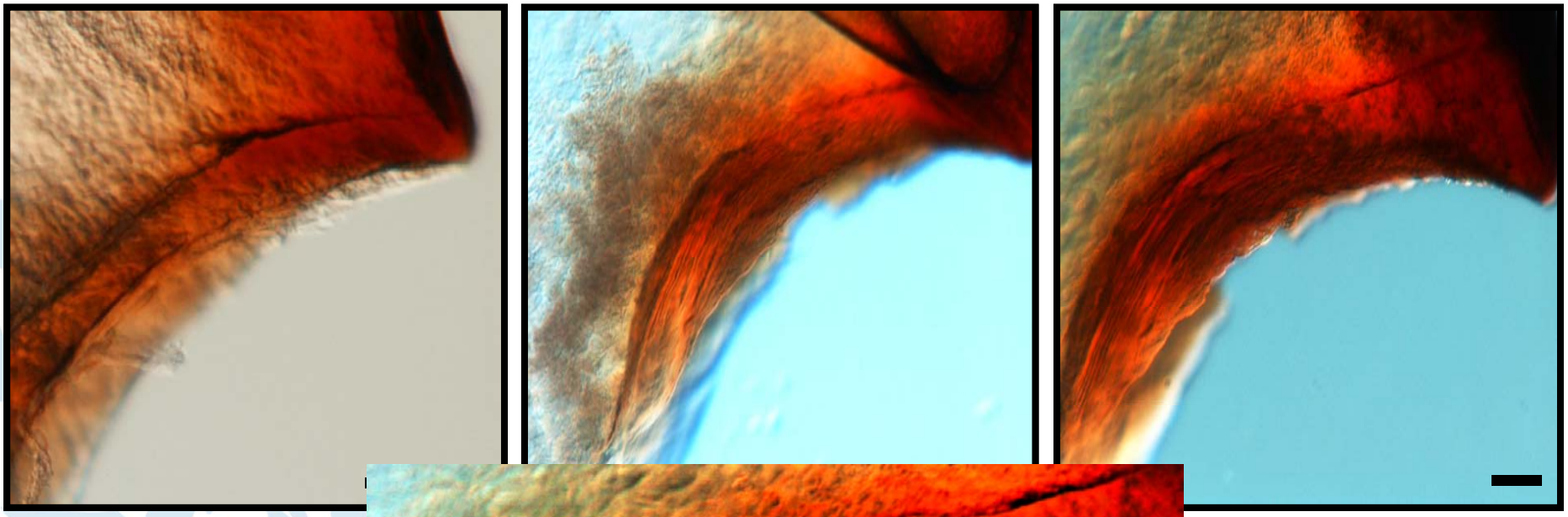


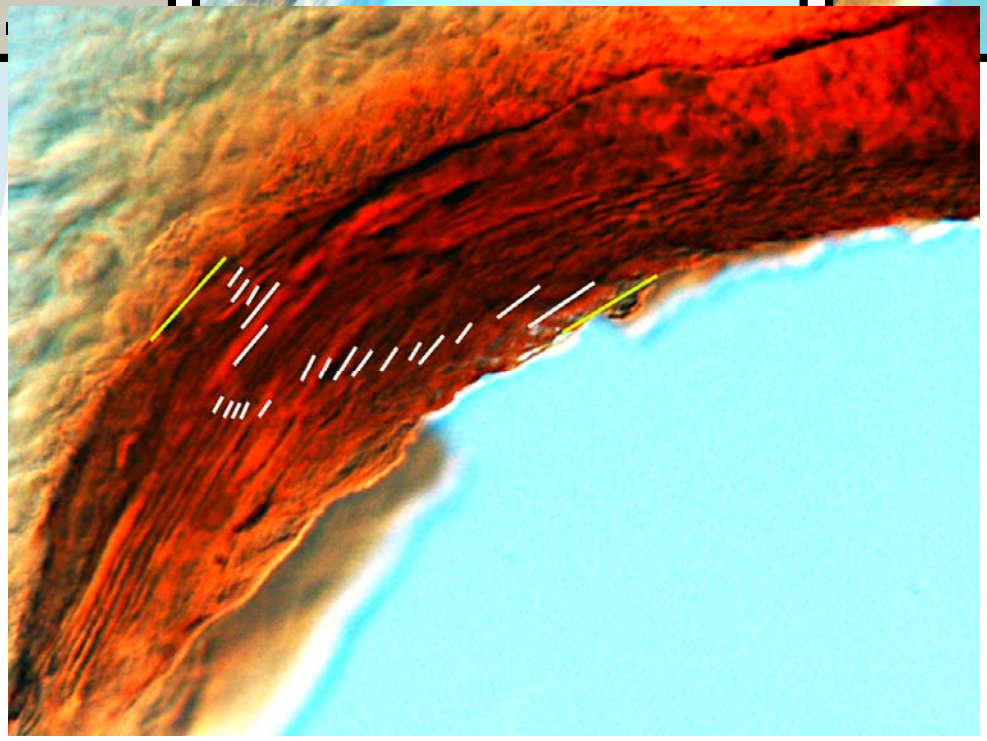
Figure 1. Anterior region (lateral view) of the lower beak : Location of the main regions (R: rostrum, H: Hood, S: Developing shoulder); the arrow indicates the first increment and the white segment indicates the width of the reading area (scale bar= 100 µm).

Hatching day

10 days

20 days





Reproducibility

The increments were counted by two readers, both reading each beak two times. When counts of both readers coincided twice, the reading was taken as valid.

Coefficient of variation (CV) (Perales-Raya, 2018) was calculated to determine the precision (reproducibility) of the counts for each reader in each individual:

$$\mathrm{CV}(\%) = \frac{100\, x\, \sqrt{(R1-R)^2 + (R2-R)^2}}{R}$$

To avoid any bias by age groups, the CVs were averaged for each age group, and when CV<7.6% were taken as valid (Campana et al., 2001).

Reproducibility

Mean reading precision (CV) from the beak reading was 2.95 ± 5.98 % for all individuals. CV in age reading in sepia cultured at 21ºC (n=120) was 3.26 ± 6.75%, and 2.01 ± 2.47% for individual cultured at 18ºC (n=25).

The methodology is validated

1 increment → 1 day of life

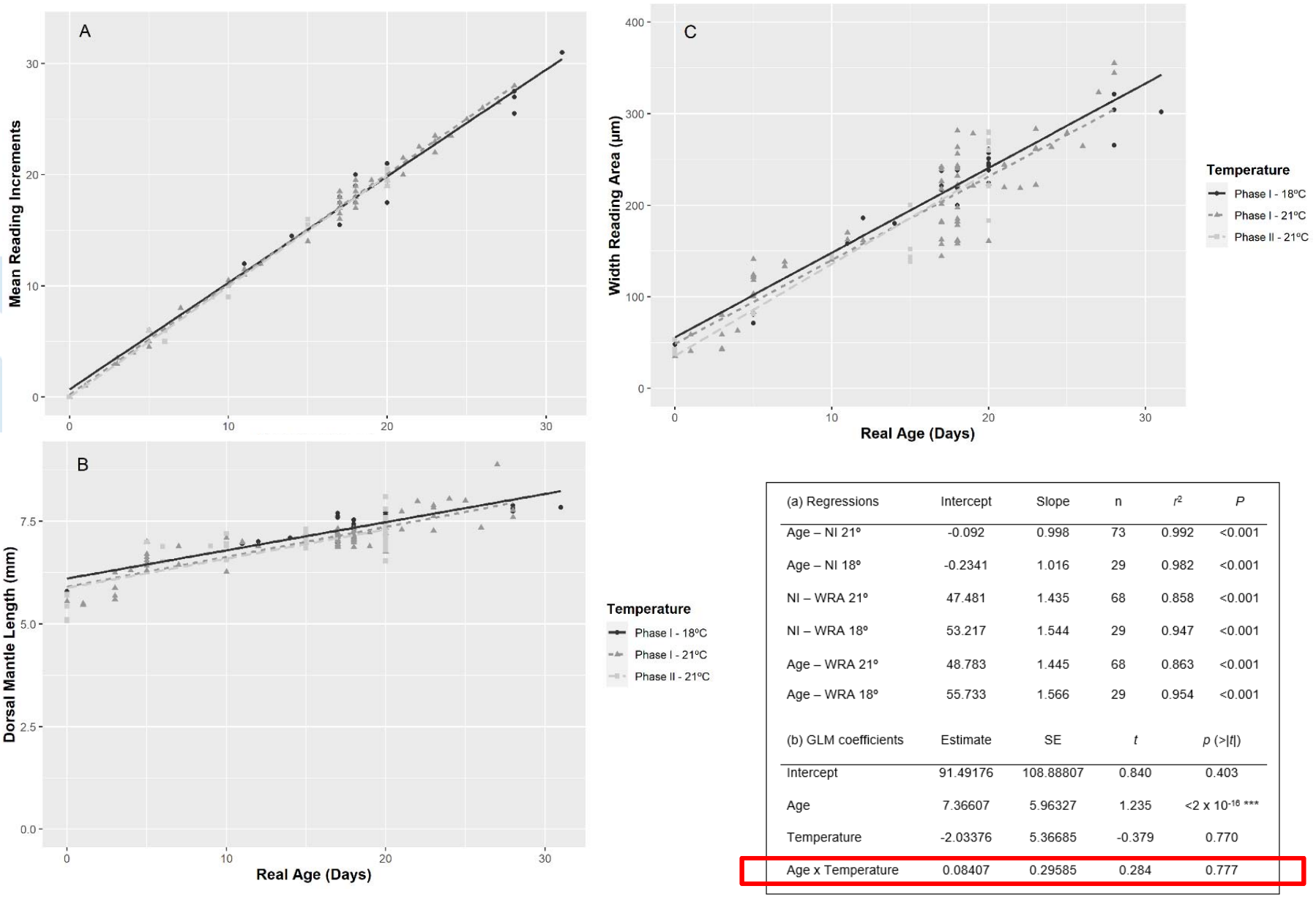


Figure 2. Growth of cultured newly hatched *Sepia officinalis*: **(A)** Relationship between the mean number of increments counted in the lower beak and the real age; **(B)** Dorsal Mantle Length and the real age; **(C)** Lower beak growth (width of the reading area; see Fig. 1) with real age. Data grouped by temperature in the different phases.

(a) Regressions	Intercept	Slope	n	r^2	*P*
Age – NI 21º	-0.092	0.998	73	0.992	<0.001
Age – NI 18º	-0.2341	1.016	29	0.982	<0.001
NI – WRA 21º	47.481	1.435	68	0.858	<0.001
NI – WRA 18º	53.217	1.544	29	0.947	<0.001
Age – WRA 21º	48.783	1.445	68	0.863	<0.001
Age – WRA 18º	55.733	1.566	29	0.954	<0.001

(b) GLM coefficients	Estimate	SE	*t*	*p* (>|*t*|)
Intercept	91.49176	108.88807	0.840	0.403
Age	7.36607	5.96327	1.235	$<2 \times 10^{-16}$ ***
Temperature	-2.03376	5.36685	-0.379	0.770
Age x Temperature	0.08407	0.29585	0.284	0.777

Table 1. Results of: (a) lineal regression analysis for the number of growth increments (NI), the age (days from hatching) and width of the reading area (WRA) by rearing temperature (21º or 18ºC) in the Phase I. (b) General lineal model (GLM) coefficients; ****p*<0.001 Regressions are divided by phases

Conclusions of Age validation

The rostrum was the best region of the beak where the sequence of growth increments was the most visible. The hatching mark was clearly identified

The high coincidence among readings indicates the reproducibility of readings of the rostral area in beaks of *Sepia officinalis.*

Daily deposition was validated in the rostral area of lower beaks from 0 to 31 days.

No differences were observed in the daily deposition and the growth reading area at different temperatures (*p=0,777*).

Applications- *Sepia bertheloti*

A total of 1123 individuals of *Sepia bertheloti* were collected from June 2018 to January 2020. They were caught by commercial trawlers operating in Morocco (552 individuals) and Guinea-Bissau (571 individuals).

A subsample of 78 individuals from Morocco and 128 from Guinea-Bissau were analysed. The subsample was randomly performed by categorizing the individuals by size range for both sexes every 5 mm of Dorsal Mantle Length (DML).

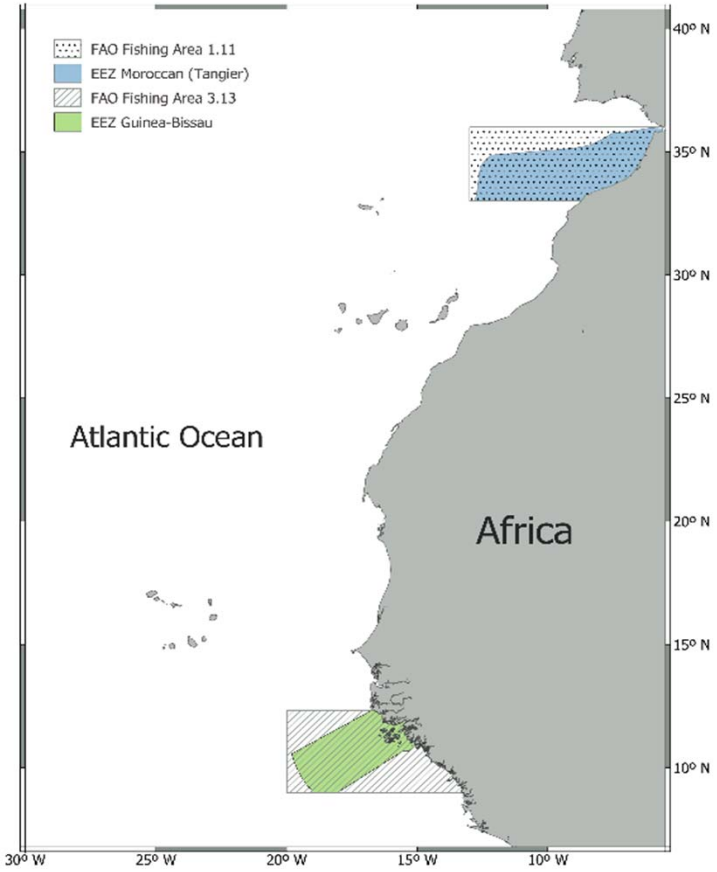
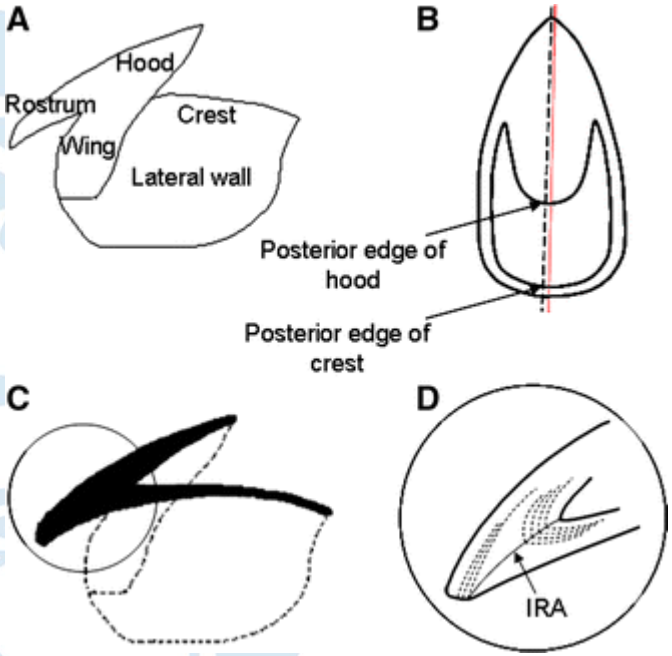
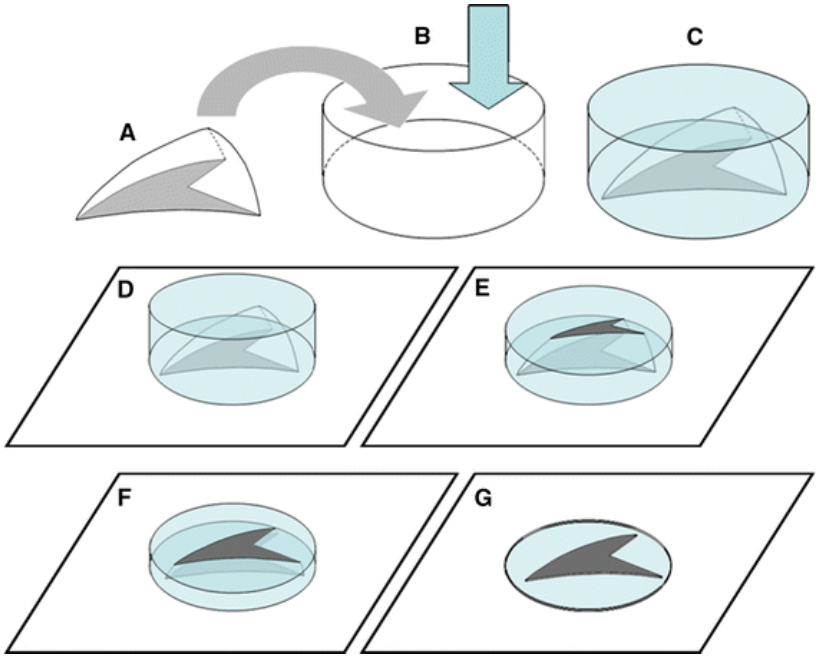


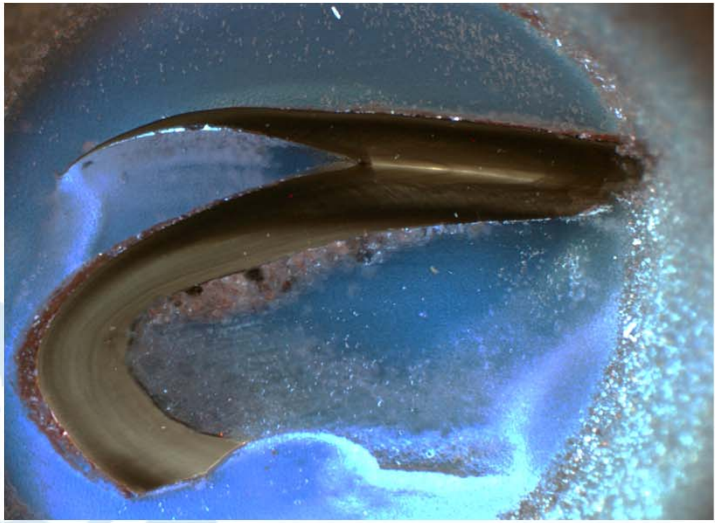
Figure 3. Sampling areas (FAO Fishing Areas) where the commercial trawlers caught *Sepia bertheloti* in Morocco (Tangier zone) (FAO 1.11) and Guinea-Bissau (FAO 3.13). Exclusive Economic Zone (EZZ) for Morocco (Tangier zone) and Guinea-Bissau in the FAO Fishing areas are shaded

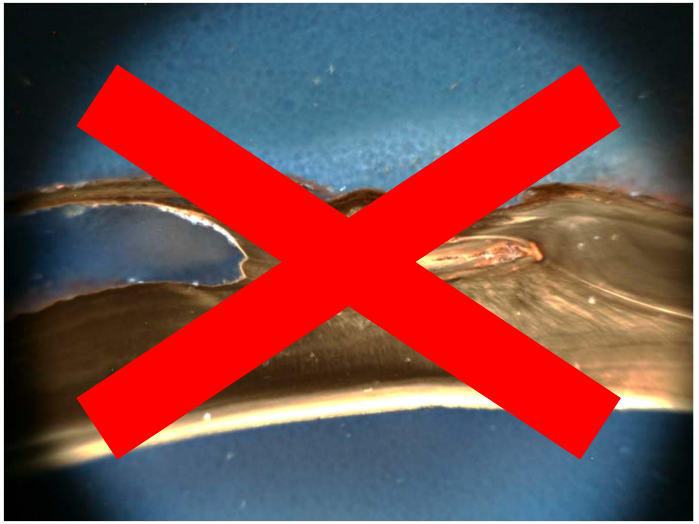
The methodology used for processing the Lower beaks was that described by Perales-Raya et al. (2010).

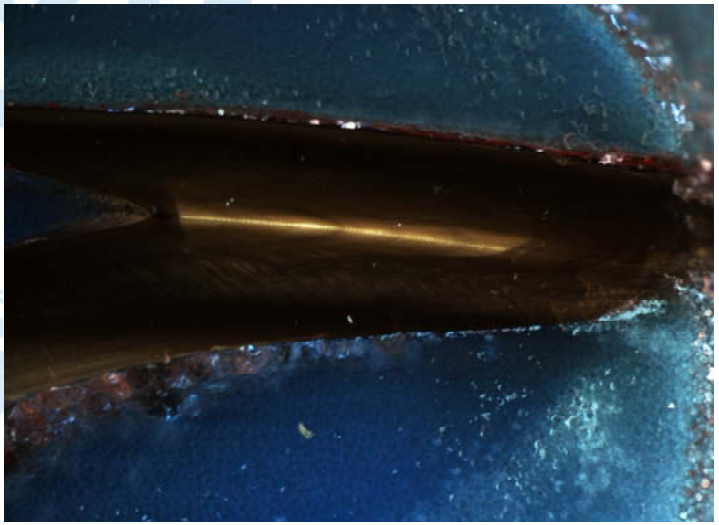


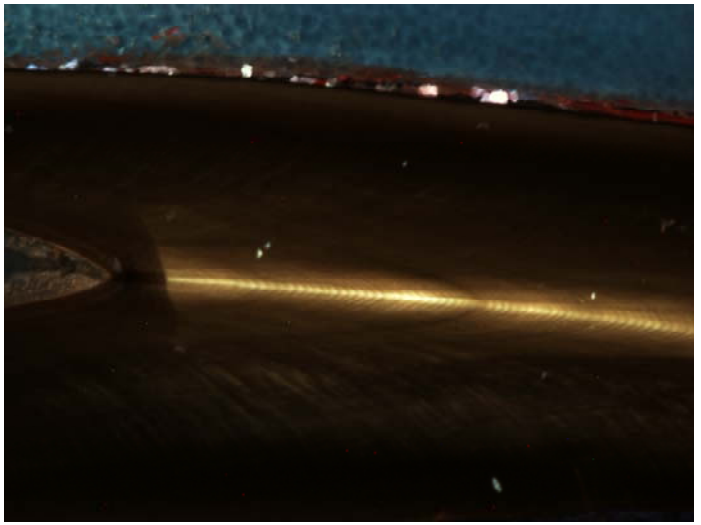


Once the beaks were processed, they were analysed using a Nikon Microscope Multizoom AZ100 with and UV-epiillumination attachment (vertical reflected light).









Results

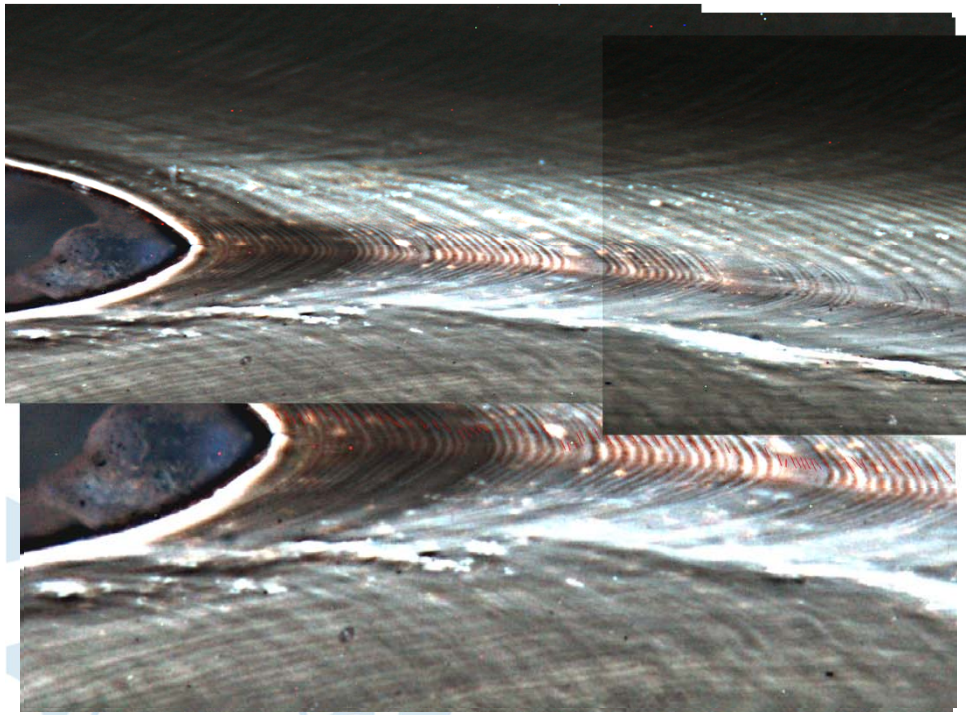
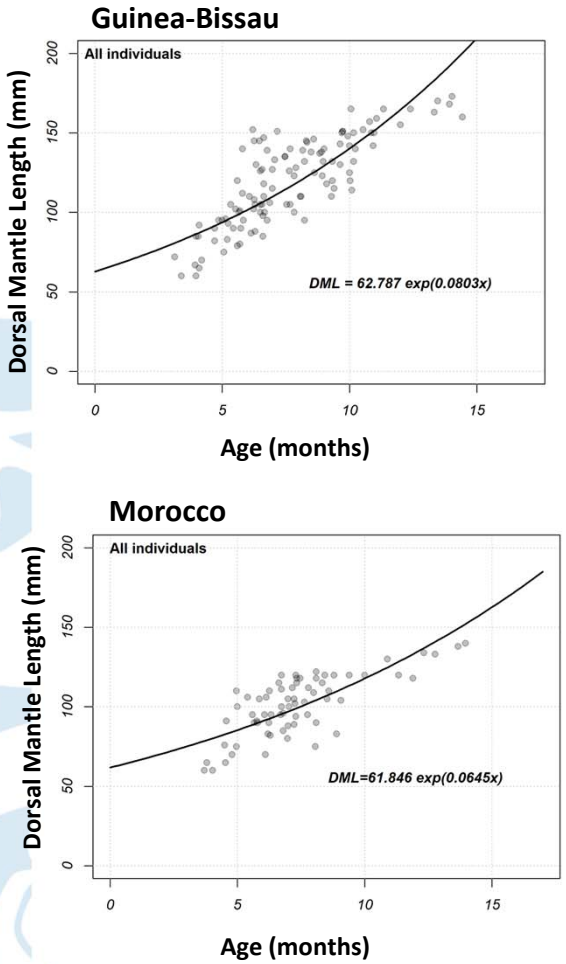


Figure 4. Appearance of growth increments in the lower beak section of *Sepia bertheloti* (300x).

Season	N	NI (days)		
		min	max	$\bar{X}$ ± SD
	13	144	267	212.62±39.12
Total				
Summer '18	42	144		
Autumn '18	10	137	383	202.5±67.96
Winter '20	17	111	410	207.94±82.30
Guinea-Bissau				
Females				
Summer '19				
Autumn '19	25	94	316	201.28±57.04
Winter '20	12	102	301	213.16±55.91
Males				
Summer '19	31	174	433	269.48±75.60
Autumn '19	25	118	400	219.84±77.35
Winter '20	21	122	372	234±74.06
Total				
Summer '19	31	174	433	269.48±75.60
Autumn '19	50	94	400	210.56±67.91
Winter '20	33	102	372	226.48±67.88

Cuttlefishes from Guinea-Bissau showed an estimated age between 94 (72 mm DML) and 433 days (160 mm DML), with a mean age of 231.19 days. Estimated age for individuals from Morocco ranged from 111 (60 mm DML) to 419 days (140 mm DML), with a mean value of 220.53 days.



Age class (Days)	Morocco DML $\bar{X}$ ± SD	Morocco G	Morocco AGR	Guinea - Bissau DML $\bar{X}$ ± SD	Guinea - Bissau G	Guinea - Bissau AGR
Females						
<100				72.00±0.00	-	-
101-190	81.78±16.27	-	-	94.38±15.24	**0,402**	0,332
191-280	100.71±13.89	**0,147**	0,141	107.5±12.29	0,182	0,183
281-370				129.25±16.40	0,288	0,340
Males						
101-190	89.6±15.51	-	-	93.52±21.45	-	-
191-280	108.52±9.97	**0,281**	0,277	130.42±13.58	**0,455**	0,505
281-370	123.67±6.62	0,129	0,149	146.79±12.77	0,144	0,199
>371	137.00±3.61	0,137	0,179	166.50±4.76	0,131	0,205

Table 2. Dorsal mantle length growth-rates for each age-class of *Sepia bertheloti* females and males from Morocco and Guinea-Bissau. G: instantaneous relative growth rate (% DML d^{-1}); AGR: absolute growth rate (mm d^{-1}). $\bar{X}$: average; SD: standard deviation.

Conclusions

Assuming the initial hypothesis of 1 increment – 1 day of life, **according to the first experiment in *Sepia officinalis*,** a maximum life expectancy of 433 days was obtained for Guinea-Bissau and 419 days for Morocco.

Specimens from Guinea-Bissau showed larger sizes than individuals from Morocco for the same age classes.

The differences in growth patterns seem a priori to be related to the different oceanographic conditions of both areas (individuals from Guinea Bissau grow faster due to its warmer waters).

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Thanks for you attention!

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