Study of the growth of *Octopus vulgaris* in the Moroccan Mediterranean Sea by direct age estimation through the analysis of upper beaks

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Abstract

Beaks are one of the most important sclerochronological structures used to study the age and growth of cephalopods, in particular *Octopus vulgaris* Cuvier, 1797. The present study provides results of ageing of 128 *O. vulgaris* (56–239 mm dorsal mantle length, DML; 121–5974 g total weight, TW) collected in the southern Moroccan Mediterranean coasts between Fnideq and Jebha. The number of increments corresponding to the age (days since hatching) varied from 137–368 in females and from 129–382 in males. There was a significant correlation between beak and somatic growth. The correlation coefficients of the growth curves DML-Age and TW-Age were similar for both power and exponential models: $DML = 0.185Age^{1.188}$ ($R^2 = 0.547$), $DML = 35.933e^{0.005Age}$ ($R^2 = 0.546$), $TW = 0.00002Age^{3.260}$ ($R^2 = 0.532$), $TW = 29.56e^{0.014Age}$ ($R^2 = 0.541$). The average width of the increments was similar between females and males. It varied significantly with season and stage of sexual maturity. Comparison of the growth curve with those estimated by other authors showed that Moroccan Mediterranean *O. vulgaris* grew faster than that of Sardinia (Italy) and slower than that of the Mauritanian coast.

Introduction

The common octopus is characterized by rapid non-asymptotic growth (Alford & Jackson, 1993), with high individual variability (Semmens *et al.*, 2004). This variability has been found both in aquaculture (Iglesias *et al.*, 2004) and in the wild (Domain *et al.*, 2000). The growth rate of cephalopods is influenced by diet (Forsythe & van Heukelem, 1987; García García & Cerezo Valverde, 2006; Cerezo Valverde *et al.*, 2008) and temperature (Aguado Giménez & García García, 2002). However, a comparative study of age between wild and cultivated *O. vulgaris* paralarvae showed that temperature and the age × temperature interaction significantly influenced the deposition of daily increments, whereas diet had no influence (Perales-Raya *et al.*, 2017).

Ageing methods based on the count of growth increment on age-registering structures (Lipinski & Durholtz, 1994; Bettencourt & Guerra, 2000; Arkhipkin *et al.*, 2018) are considered the most appropriate for cephalopods. Distinctive increments, possibly related to daily growth, have been observed on statoliths (Young, 1960), eye lenses (Gonçalves, 1993), the inner rostral area (Raya & Hernández-González, 1998) and lateral walls (Hernandez-Lopez *et al.*, 2001) of *Octopus vulgaris* beaks and inner shell (stylets) (Sousa-Reis & Fernandes, 2002; Doubleday *et al.*, 2006; Leporati *et al.*, 2008). However, among all of them the reading of growth increments on beaks seems to be the most reliable method for octopods. A summary of the studies on growth increments on *O. vulgaris* beaks, some of which validated the daily deposition of these increments, is presented in Table 1.

The main aim of this study is to assess growth of the common octopus on the Moroccan Mediterranean coast.

Materials and methods

Materials

A total of 128 octopus specimens were used for the age study using octopus beaks, with sizes between 56–239 mm and weights between 121–5974 g. This sample is composed of 58 females and 70 males, derived from the catches of artisanal boats and trawlers, between 7 March 2019 and 13 September 2019 in the maritime area Fnideq-Jebha (Figure 1).

Processing and measurements

In all octopuses, the DML was measured with an ichthyometer to the nearest 1 mm, the TW to the nearest 1 g, and sexual maturity was assessed using the Idelhaj (1984) scale: immature, maturing, mature and spent (females only). Upper and lower beaks were preserved in distilled water below 6°C as recommended by Perales-Raya *et al.* (2010). Rostrum length (RL), hood length (HL) and crest length (CrL) were measured by a calliper to the nearest 0.01 mm for

Table 1. Validating *Octopus vulgaris* age studies based on beaks, carried out by several authors

| Authors name, year	Technique used	Conclusions
Raya & Hernández González (1998)	Rostrum sagittal section (RSS) of the beaks, particularly the upper beaks.	Validation of daily deposition of growth increments needs the analysis of a larger sample, covering a larger size range.
Hernandez-Lopez et al. (2001)	Sagittal section with scissors of the lateral wall surfaces (LWS) of the upper beaks so as to obtain two symmetrical half-beaks from each one.	Provides an easy new method of determining *Octopus vulgaris* age based on the upper beak microstructure, validated for the paralarval period.
Perales-Raya et al. (2010)	Compared RSS and LWS.	This study recommends the use of the Hernandez-Lopez et al. (2001) method as the most appropriate technique for age estimation of octopus.
Canali et al. (2011)	Thermal shock marking procedure	Results support evidence of the daily periodicity hypothesis of growth increments in the upper beak of *Octopus vulgaris* (56 individuals with body weight range 160–610 g).
Perales-Raya et al. (2014a)	Chemical (Calcofluor injection) and environmental (temperature and containment) marking techniques and also RSS and LWS of the upper beaks.	Using for the first time a full age range of *O. vulgaris*: 49 marked wild animals kept in aquaria with weight range, 158–3521 g and 24 captive-reared known-age individuals (paralarvae, 0–98 days old; adults, 200–734 days old), this study validates the daily deposition of increments in the beaks for LWS and RSS.

Figure 1. Study area between Fnideq (35.86°N 5.34°W) and Jebha (35.20°N 4.59°W) in the Moroccan Mediterranean.

upper beaks (Figure 2), the weight of upper and lower beaks was estimated within the nearest 0.001 mg.

Age estimation

We used the growth line reading approach of Hernandez-Lopez et al. (2001). It consists of making a sagittal section with scissors of the upper beaks' lateral walls to obtain two symmetrical half-beaks from each one, cleaned with water. Then the sectioned lateral walls were placed dried after washing under a stereo-microscope: concave (inner) part on top and convex (outer) part on the bottom. The presence of water droplets or humidified areas on the lateral walls of the half-beaks hinders the good visibility of the growth increments.

During age estimation, the half-beaks tended to dry out quickly and became concave. The rehydration of the half-beaks was essential before continuing the counting. Using an oily
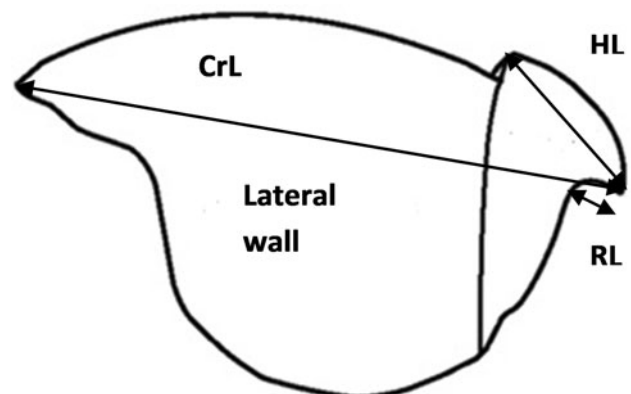
Figure 2. Side view drawing of *Octopus vulgaris* upper beak with measured lengths (HL: Hood length, RL: Rostrum length, CrL: Crest length).

solution (glycerol) to impregnate the half-beaks, reduce the desiccation time, increase the reflection of light rays and improve the visibility of the growth increments, has been unsuccessful, as it blurred growth lines instead of increasing their visibility.

A stereo-microscope (NIKON SMZ 745T) connected to a computer with NIS-Elements F imaging software, with 40× magnification for adult individuals and 50× for young ones, was used to count upper beak growth increments. The 20× magnification was used to follow the trajectory of the growth increments when the 40× did not provide clear visibility of the increments.

The use of an external white reflected light was necessary to illuminate the half-beaks, from the inner side, by placing the light source very obliquely. As counting the increments proved to be an almost impossible task directly with the naked eye, several photos per half-beak were taken (3–5 photos for young specimens and up to 8 for older ones) to count on the screen. The direction of counting and taking the pictures started from the rostral tip to the posterior edge of the lateral wall.

Statistical analysis (processed by XLSTAT 2021.2.1. and R software) was carried out using regressions (linear, power, exponential models): RL/HL/CrL-DML, Upperbeak weight/Total beak weight-TW and DML/TW/HL/RL/CrL/Upper beak weight/Total beak weight-Increments number. Due to a better distinction between stress marks and growth increments during counting, the second count values (L_2) were adopted for calculations and analyses. The normality and homogeneity hypotheses were checked by the graphs diagnosis generated by the R software in addition to the Shapiro–Wilk and Lilifors (Kolmogorov–Smirnov) tests. A non-parametric Mann–Whitney test was used to determine whether there were significant differences in the increments number between males and females.

Precision is defined as the reproducibility of repeated measurements (age readings) on a given structure, whether those measurements are accurate or not (Kalish et al., 1995). Nevertheless, there are two widely used and statistically valid measures for assessing the precision of age readings: the average percentage error (APE) and the coefficient of variation (CV) (Campana, 1995). However, the use of APE as a measure of precision is not advisable as it varies widely both between species and between ages within a species. A study can be considered valid when the CV of age readings is <7.6% (Campana, 2001).

In the second growth increment counting, 1043 streak width measurements (μm) were taken from photographs – each scaled in μm – of 112 upper beaks using an image processing and analysis program (Image J 1.46r. https://imagej.nih.gov/ij). The widths were measured at the area rostral tip–posterior end of the lateral wall with an average of 9 measurements. A three-way ANOVA was performed between increment width (dependent variable) and sex, maturity stage and increment season (qualitative explanatory variables). In fact, it would be possible to deduce the season of each increment formation knowing the capture date of octopus individuals, their age (increments number) and the sequence number of the increment.

To compare octopus growth of the current study in the Moroccan Mediterranean with that from three other studies in Sardinian, Mauritanian and Brazilian coasts, the monthly sea surface temperature (sst) was provided for the period of each study from the database: NOAA NCEP EMC CMB GLOBAL Reyn_SmithOIv2 monthly sst: Sea Surface Temperature data, https://iridl.ldeo.columbia.edu/maproom/Global/Ocean_Temp/Monthly_Temp.html.

Results

Biometric relationships: beak and body measurements

The residuals normality (H_0) and homogeneity hypotheses are valid for the length variables (DML, RL, HL, CrL) but not for the weight ones (TW, Upper beak weight, Total beak weight) (Appendix 1). The Shapiro–Wilk tests showed that H_0 is correct for relationships CrL-Age ($w = 0.987$, $P = 0.29$), HL-Age ($w = 0.98$, $P = 0.49$), RL-Age ($w = 0.98$, $P = 0.15$), Total beak weight-Age ($w = 0.98$, $P = 0.08$) at $\alpha = 0.05$, and for DML-Age ($w = 0.98$, $P = 0.04$) at $\alpha = 0.01$, respectively. The Lilliefors (Kolmogorov–Smirnov) test showed that this H_0 is correct for the relationship Upper beak weight-Age ($D = 0.081 < D_{critical}$, $P = 0.052$). $D_{critical} = 1.031/\sqrt{n} = 0.092$ at $\alpha = 0.01$ (Rakotomalala, 2011).

The power regressions in Appendix 2 showed that there was a very good correlation between the length measurements (RL/HL/CrL-DML) on the one hand and the weight measurements (Upper beak weight/Total beak weight-TW) on the other. Coefficients of determination R^2, demonstrated that the power model was more appropriate than the linear (Appendix 3). Beak growth is, therefore strongly proportional to somatic growth.

The increments: morphology, counts and precision

Stereomicroscopic observation shows that the growth increments are perpendicular to the counting line (tip of the rostrum – posterior end of the lateral wall) with an undulating trajectory. The width of each growth increment varies very little along its trajectory (Figure 3A). Figure 3B–E shows the sequence of growth rings from the anterior region (rostral tip) to the posterior one of the lateral wall of the upper beak.

Counting increments was possible for 125 specimens of 128 in total, i.e. 97.7% of the individuals sampled. Two counts per beak were performed. The calculated coefficients of variation of the two readings have ranged from 0–0.7% (mean CV = 0.13%). A third counting was therefore considered not necessary.

The increments number (age in days) ranged from 137–368 days for females and from 129–382 days for males. The oldest individual (382 days, DML = 225 mm, TW = 5318 g, male, maturity stage 3) was not the largest (277 days, 239 mm, 4662 g, female stage 3) and the youngest individual (129 days, 62 mm, 174 g, male stage 1) was not the smallest (150 days, 56 mm, 124 g, female, maturity stage 1) (Appendix 4).

The non-parametric Mann–Whitney test showed no significant differences in the increments number between males and females ($U = 1600$, $P = 0.094$ at $\alpha = 0.05$).

Growth curves: octopus individuals and beaks

Figure 4 showed that regression equations have provided a satisfactory correlation between DLM and Age (DML = $0.185 Age^{1.188}$, $R^2 = 0.55$), and between TW and Age (TW = $29.56 e^{0.014 Age}$, $R^2 = 0.54$). The statistical results of both power and exponential models are presented in Table 2. For beak measurements, the best regression equations (Appendix 5) were RL = $0.0015 Age^{0.337}$ $R^2 = 0.621$ and HL = $2.802 e^{0.004 age}$ $R^2 = 0.618$ (Table 2).

The octopus measurements (DML, TW, CrL, HL, Upper beak weight) estimated by equation growth curves (Appendix 6) lead to conclude that, Moroccan Mediterranean Octopus vulgaris was larger than that of the Brazilian and Sardinian coasts and smaller than that of the Mauritanian coasts.

Increment width

The three-way ANOVA showed that there were significant differences in increment width variation by increment season ($P = 0.002$), and sexual maturity stage ($P < 0.0000$). There was no significant difference in increment width by sex ($P = 0.72$) (Table 3).
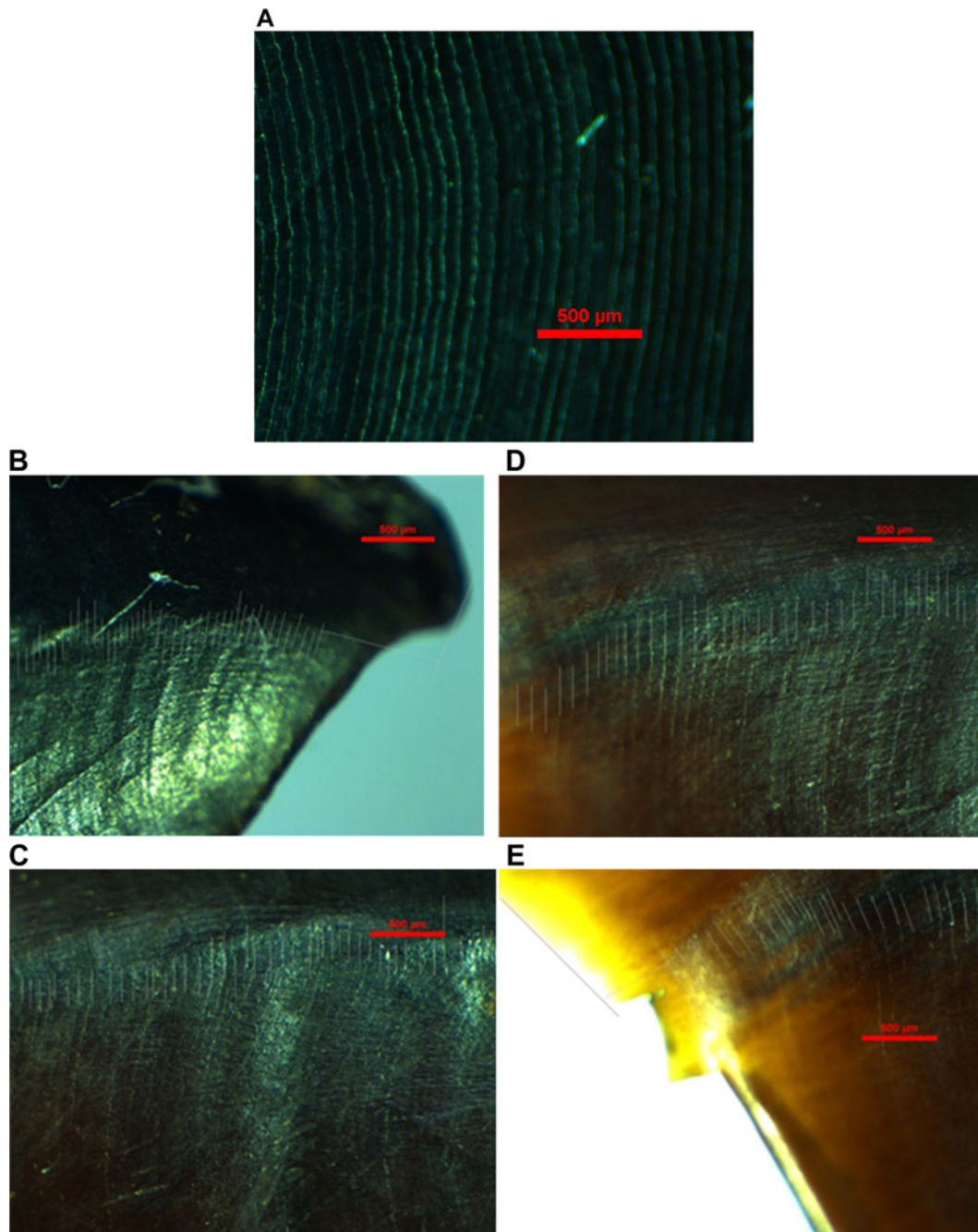
Figure 3. (A) Width of *Octopus vulgaris* growth increments. Growth increments of a male specimen (DML = 82 mm, TW = 321 g, maturity stage 1); (B) anterior region showing the first increment with the extrapolated part; (C–D) increments of the middle region; (E) posterior region showing the last increment with the extrapolated part (×50).

The mean increment width was similar between females and males though juvenile males at stage 1 generally had wider increments than juvenile females (Kruskal–Wallis test, $P = 0.0066$) (Figure 5A). The narrowest increments for combined sex were formed in spring (59.4 μm) whereas the widest was formed in summer (72 μm) (Figure 5B). The same result was found for all males, all females and mature males but not in mature females considered separately (narrow increments in spring and wide in autumn) (Table 4).

In general, the growth increment widths of the *O. vulgaris* upper beaks varied between 17–136 μm from the 37th to the 352nd increment with no clear trend (Figure 5C). The high individual variability of increment widths (SD = 22.5) seems to be due to the low number of increment widths measured by individual. The mean increment width per sequence number showed a trend similar to the bell-shaped curve (Figure 5D) from the 40th to around the 290th increment. After, the remaining points were scattered randomly.

Discussion

The growth increments in common octopus beaks consist of two parts: a thick one that appears clear under reflected light and a thin, deep one that appears dark (Raya & Hernández-González, 1998). Counting these increments is generally more difficult in the rostral tip area as they are frequently discontinuous (Hernandez-Lopez *et al.*, 2001) and generally narrower than those in the medial and posterior region of the beak.

Hernandez-Lopez *et al.* (2001) and Perales-Raya *et al.* (2014a, 2017) had proven according to studies on *O. vulgaris* paralarvae
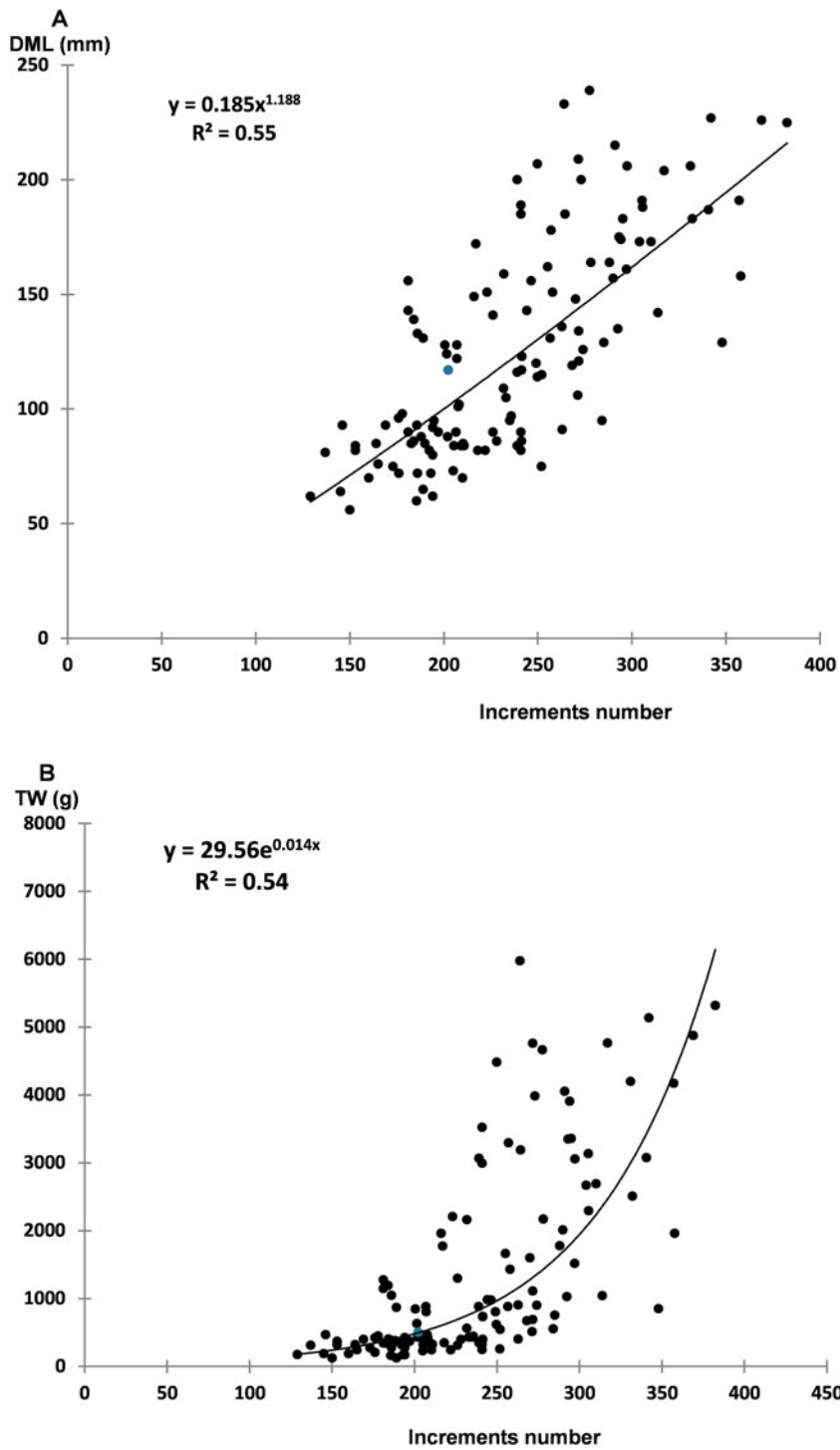
Figure 4. (A) Relationship between octopus size DML and Increments number; (B) relationship between octopus weight TW and Increments number. N = 125.

Table 2. Statistical analysis results of regressions: size (DML), weight (TW), beak measurements according to the age (Increments number)

Relation	Number N	F Statist.	P value	Power model Equation	R^2	Exponential model Equation	R^2
DML (mm)-age	125	144.6	<0.0001	$y = 0.185 \times {}^{1.188}$	0.547	$y = 35.933e^{0.005x}$	0.546
TW (g)-age	125	105.7	<0.0001	$y = 2 \times 10^{-5} \times {}^{3.260}$	0.532	$y = 29.56e^{0.014x}$	0.541
CrL (mm)-age	124	172.7	<0.0001	$y = 0.083 \times {}^{1.006}$	0.582	$y = 7.139e^{0.004x}$	0.595
HL (mm)-age	124	198.1	<0.0001	$y = 0.052 \times {}^{0.900}$	0.604	$y = 2.802e^{0.004x}$	0.618
RL (mm)-age	124	186.3	<0.0001	$y = 0.0015 \times {}^{1.337}$	0.621	$y = 0.589e^{0.006x}$	0.608
Upper beak weight (mg)-age	121	124.7	<0.0001	$y = 5 \times 10^{-6} \times {}^{2.644}$	0.552	$y = 0.602e^{0.011x}$	0.562
Total beak weight (mg)-age	111	122.5	<0.0001	$y = 3 \times 10^{-6} \times {}^{2.828}$	0.582	$y = 0.906e^{0.012x}$	0.594

Table 3. Three-way ANOVA statistical result of upper beak increments widths by sex, sexual maturity stage and increment season (Statistical language R).

	Sum Sq	Df	F value	P value
Increment season	4489	1	9.2179	0.002456 **
Sex	64	1	0.1322	0.716196
Maturity stage	11,499	1	23.6133	0.000001359 ***
Residuals	505,945	1039		

'**': 0.01, '***': 0.001.

Table 4. Mean width (μm) by season, sex (female: F, male: M) and mature individuals (stage 3) of *O. vulgaris* in the Moroccan Mediterranean

	Autumn	Winter	Spring	Summer
F	63.4 ± 23.4	63.8 ± 21.8	55.1 ± 19.6	67.5 ± 21.8
F stage 3	74.1 ± 20.3	71.6 ± 24.6	56.2 ± 20.3	71.3 ± 20.5
M	70.2 ± 22.7	66.4 ± 20.1	60.0 ± 20.7	75.4 ± 22.4
M stage 3	70.8 ± 22.5	68.6 ± 18.7	66.4 ± 21.9	75.6 ± 24.7

that the daily deposition of growth rings on the lateral wall of the upper beak begins on the first day after hatching. This would allow confirmation of the deposition time of the first increment, essential to validate the age deduced from any hard structure (Campana, 2001). True increments can only be observed when the darkening process starts to occur, and those noted on the lateral walls during the embryonic phase could be considered false increments (Miserez *et al.*, 2010). To ensure a good estimation of the common octopus age, it is always necessary to determine the age of the formation of the
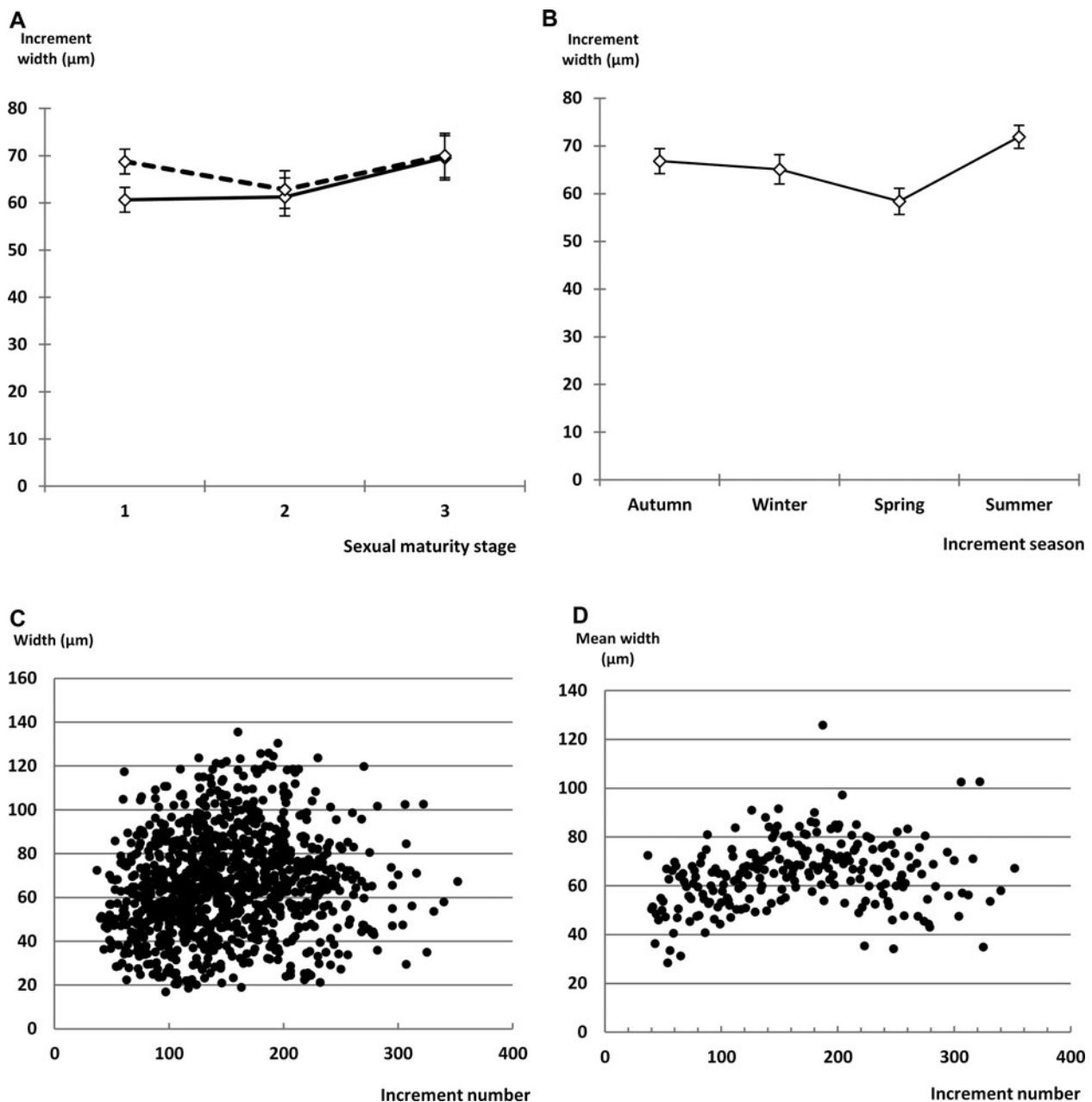

Figure 5. (A) Estimated means of upper beak increment widths by sex and sexual maturity stage with standard error bars. ▬ ▬ ▬ ▪: Males. ▬▬▬▬: Females; (B) estimated means of upper beak increment widths per season with standard error bars; (C) evolution of the octopus upper beak increment widths according to their sequence number (N = 1043); (D) evolution of the octopus upper beak increment mean widths by sequence number (N = 232).

Measures	Present study	Other authors	Area
DML (mm)	$y=0.185\,x^{1.188}$, $R^2=0.547$, N=125 $y=35.933e^{0.005x}$, $R^2=0.546$, N=125	Castanhari and Tomás (2012). $y=0.876x^{0.871}$, $R^2=0.620$, N=120	Brazilian coasts
		Cuccu et al (2013). $y=47.06e^{0.003x}$, $R^2=0.722$, N=128 (Males). $y=41.05e^{0.003x}$, $R^2=0.770$, N=93 (Females).	West Central Sardinian Coast (Mediterranean)
TW (g)	$y=2E\text{-}05\,x^{3.260}$, $R^2=0.532$, N=125 $y=29.56e^{0.014x}$, $R^2=0.541$, N=125	Castanhari and Tomás (2012). $y=0.001x^{2.424}$, $R^2=0.631$, N=120	Brazilian coasts
		Cuccu et al (2013). $y=45.92e^{0.010x}$, $R^2=0.804$, N=128 (Males). $y=31.39e^{0.011x}$, $R^2=0.827$, N=93 (Females)	West Central Sardinian Coast (Mediterranean)
		Perales-Raya et al (2010). Age=$27.978TW^{0.2491}$, $R^2=0.754$, N=30	Central-eastern Atlantic Waters (Mauritania)
CrL (mm)	$y=0.083x^{1.006}$, $R^2=0.582$, N=124	Castanhari and Tomás (2012). $y=0.210\,x^{0.790}$, $R^2=0.529$, N=120	Brazilian coasts
HL (mm)	$y=0.052x^{0.900}$, $R^2=0.604$, N=124	Perales-Raya et al (2010). $y=0.188\,x^{0.737}$, $R^2=0.75$, N=30	Central-eastern Atlantic Waters (Mauritania)
Upper beak weight (mg)	$y=0.602e^{0.011x}$, $R^2=0.562$, N=121	Perales-Raya et al (2010). $y=0.0006x^{2.4091}$, $R^2=0.763$, N=30	Central-eastern Atlantic Waters (Mauritania)

Figure 6. Comparisons of *Octopus vulgaris* growth curves DML/TW/Crl/HL/Upper beak weight-Age (Increments number).

first increment in the lateral walls of the beak (Armelloni et al., 2020).

Erosion of the rostrum area of the lower and upper beak, due to predation on armoured prey such as bivalves or crustaceans, may result in an underestimation of the number of increments (Perales-Raya et al., 2010). To minimize any underestimation, Perales-Raya et al. (2010) proposed counting increments in the dorsal area of the rostrum, less affected by erosion. The presence of false increments, as their formation may be induced by environmental or metabolic conditions independently of age, might overestimate this counting (Canali et al., 2011). All studied lower beaks were more eroded than the upper beaks, all of which were very little eroded. Large width consisting from several fused primary increments may underestimate the age during the counting. Perales-Raya et al. (2014b) observed, in the daily increments sequence of rostrum area, checks or discontinuities (assimilated to large widths) that may record periods of perturbation or stress in the octopus life.

The maximum age of *O. vulgaris* observed in this study was slightly more than 12 months (12.7 for males and 12.3 for females) as was recorded by Hernandez-López et al. (2001), Perales-Raya et al. (2014b), Smale & Buchan (1981) in the Atlantic, Castanhari & Tomás (2012) on the Brazilian coast and Cuccu et al. (2013) on the Sardinian coast (Mediterranean). Those recorded by Domain et al. (2000) on the Senegalese coast and Nafkha et al. (2019) on the Tunisian coast (Mediterranean), estimated at 14–20 months and 24–30 months, respectively were different.

Pastor et al. (2018) found that the lowest sea temperature in Mediterranean is in winter (typically February) with a steep increase in spring towards the highest values in summer (August). From autumn onwards, temperatures are decreasing throughout the winter. Considering that the spring season is the most important spawning period in the Mediterranean (Otero et al., 2007; Cuccu et al., 2013; Sieiro et al., 2014) and mature male and female stage 3 (Table 4) pre-spawning individuals, sexual maturity might explain the narrowest increment growth in spring (Table 4). Likewise, the high seawater temperature could explain the highest increment growth noted in summer.

Estimating octopus measurements of 180 and 270 days old for example, through the growth curve equations of the present study and other authors (Perales-Raya et al., 2010; Castanhari & Tomás,

2012; Cuccu et al., 2013), allows comparing the octopus growth in the four areas studied. Because the average annual SST on Moroccan Mediterranean, Sardinian and Mauritanian coasts estimated from the NOAA database of Reynolds et al. (2002) was respectively 18.8, 19 and 21.2°C, the Moroccan Mediterranean octopus grew faster than that of Sardinian and slower than that of Mauritanian coasts (Figure 6).

In conclusion, the direct age estimation of *Octopus vulgaris*, by reading growth increments on the lateral walls of the upper beaks, for the first time provides information on growth of the population of the Moroccan Mediterranean. Growth rates of this stock were found to be intermediate between more northerly octopuses (Sardinia) and more southern stock in Mauritania. Sardinian coasts are generally nutrient-poor regions (Caddy et al., 1995) whereas biological productivity of the Moroccan Mediterranean, where the Atlantic Ocean and the Mediterranean Sea meet and mix, is greater than those of other parts of the Mediterranean (Vargas-Yáñez et al., 2009). The coastal upwelling area off Mauritania is one of the most biologically productive regions of the world oceans (Kock et al., 2008; Bonino et al., 2019). In addition to the influence of sea surface temperature, this would explain the difference in *O. vulgaris* growth from Moroccan Mediterranean, Sardinian and Mauritanian coasts.

Author contributions. AF, HC, and MMI conceived and planned the experiments. AF conducted all the required experiments, found all the results, both experimental and statistical and wrote the manuscripts. HC, MMI, and JB provided critical feedback and corrections to all the results found, supervised and contributed to the final version of the manuscript.

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