Age validation in *Octopus maya* (Voss and Solís, 1966) by counting increments in the beak sections of known age individuals

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The present study was carried out to validate the daily deposition and age estimation by using beak growth increments of cultivated *Octopus maya* (Voss and Solís, 1966). This study validates for first time the periodicity of beak increments by using animals of known age. We analyzed the rostrum sagittal sections (RSS) of upper and lower beaks in 40 juveniles of *O. maya* divided into four age groups (63, 87, 105 and 122 days) with 10 individuals per group. The animals were fed with a soft diet allowing obtaining age estimations not affected by the beak erosion. At the same time 50 animals were sampled every 20 days until 120 days old to obtain an age-body wet weight (BW) curve which could be compared with the age-BW curve obtained using age estimations from beaks. Co-variance analysis showed no statistical differences between both curves. The number of increments present in the beaks corresponded with the number of days from hatchling. Therefore, it was possible to validate that a growth increment corresponds to a day of life in *O. maya*, confirming that, up to 122 days old, the beaks counts can be used to determine the age of *O. maya*

Keywords: *Octopus maya*, beak, age, growth increments, age validation.

1. Introduction

Knowing the chronological age of organisms is highly relevant in the ecological study of populations and for implementing strategies of conservation and management of fisheries (Thorrold et al, 2001, Gillanders, 2002). There are different direct and indirect methods for estimating age in cephalopods; indirect methods approximate the octopus´s age by length frequencies, gonadosomatic index, morphometric analysis of the beak and its proportion with respect to the whole body. These tools can give complementary information in the analysis of age, but this is an unreliable method for absolute age estimation because of the intrinsic biological variability. Animals included in the same cohort, even born in the same reproductive event, may vary widely in size. One of the most promising techniques (because of its easy preparation and its potential applicability in any cephalopod species) is the use of growth increments in the beaks; this is a technique that allows recognizable conspicuous marks in the rostrum sagittal sections (RSS); however it is important to mention that samples from small octopuses (less than 87 days of life) can be difficult to read because the growth increments are difficult to recognize. In 2010, Perales-Raya and collaborators estimated the age of wild *O. vulgaris* counting growth increments in beak sections; this method required validation because these studies were made using wild octopuses of unknown ages (Raya and Hernández-Gonzalez, 1998; Perales-Raya et al., 2010).

Preliminary studies using chemical or environmental markers in statoliths of *Loligo vulgaris reynaudii* (Lipinski et al., 1998), as well as *Octopus vulgaris* beaks (Oosthuizen, 2003; Canali et al., 2011; Perales-Raya et al., unpublished results) and stylets (Hermosilla et al., 2010) have suggested that the deposition rate of material has a daily periodicity and results in the formation of one increment per day.

The red octopus *Octopus maya* (Voss and Solís, 1966) is an endemic species that lives in shallow water of the Yucatan peninsula in Mexico (Solís, 1967; Van Heukelem, 1983). *O. maya* is an octopus that shows holobenthic development, which facilitates its culture; the Octopus Experimental Division at the Faculty of Sciences of the UNAM has managed to cultivate red octopus successfully and therefore has known age organisms. This is the first attempt of a validated *Octopus maya* ageing study based on the beak microstructure using animals of known age. Although this study was based in the methods previously described for the age study of *O. vulgaris* using beaks (Perales Raya et al., 2010) the present study validate for first time the beaks marks using animals of known age.

2. Materials and methods

Octopus maya juveniles analysed in the present study were born and cultivated in the Octopus Experimental Division at the Faculty of Sciences of the Universidad Nacional Autónoma de México (UNAM) in Sisal, Yucatán, Mexico. Hatchlings with 0.12 $\pm$ 0.02g were placed in six 4m diameter outdoor ponds at a density of 28 animals m^{-2} and maintained at 28ºC. Tanks were connected to a re-circulatory sea-water system coupled to anthracite vertical filter and protein skimmer (Domingues, et al., 2012). Each tank was provided with 3 *M. corona bispinosa* shell per animal. During culture time, animals were fed *ad libitum* two times a day (09:00 and 18:00 h) with squid paste (70% squid meat, 30% shrimp meat plus 5% gelatin without flavor; Rosas *et al.,* 2008), at a ratio of 100% of octopus wet weight. Before feeding (08:00h), tanks were cleaned and remaining food removed using a siphon. Seawater in the tanks was maintained at 26 $\pm$ 2°C, 8 $\pm$ 0.5 pH, dissolved O_2 > 5.5 mg/L, nitrite < 0.05mg/L and ammonia< 0.5 mg/L; a natural photoperiod of 10-14 h light-darkness, respectively was maintained during the culture. From the ponds we obtained 40 octopuses divided into four age groups (63, 87, 105 and 122 days) with 10 individuals per group, that were weighed (wet body weight=BW) and their beaks removed, cleaned and preserved in distilled water. Beaks were kept at 4°C. Upper and lower jaws were weighed (g) and morphometrically analyzed measuring hood length (HL, mm), height (H, mm), crest length (CL, mm) and rostral length (RL, mm) (Clarke 1986) (Fig. 1). Simultaneously, 50 octopuses maintained in the same culture conditions were weighed at day 1, 20, 40, 60, 80, 100 and 120 to obtain an age-BW curve which could be compared with the age-BW curve obtained using age estimations from beaks. A co-variance analysis was performed to compare both curves.

Increments on the beaks were obtained using the technique developed by Raya and Hernández-González (1998) and implemented by Perales-Raya et al. (2010). This part of study was done in the Instituto Español de Oceanografía (IEO), in Santa Cruz de Tenerife, Spain. Slices from the RSS of the beaks were obtained and encapsulated in polyester resin (Fig.2). Beaks were grounded with 1200 grit carborundum sandpaper and polish with 1 µm diamond past. Samples were then observed with ultraviolet light in an epi-illumination microscope with a magnification range of 300X-450X. Pictures of the increments in beaks were analyzed and counted with an Image Analysis System. Two counts were made in each individual and age precision was assessed with the coefficient of variation (CV), calculated as the ratio of the standard deviation over the mean (Chang 1982; Campana 2001).

Exploratory analysis was applied to all data to verify its normal distribution, and we calculated standard deviation and mean. ANOVA, linear or power regression analysis were performed to model the relationships between variables (age vs. increment number; BW vs. Increment number).. Data were log transformed and analyzed (Zar, 1984) and the statistical analysis was performed using the STATISTIC 7.0.

3. Results

The detailed sampling results from the red octopus *Octopus maya* beaks are shown in table 1. The Increments observed in the beaks were more conspicuous in animals with between 105 to 122 d age because the size of the beak are bigger (Fig. 3); in samples of octopuses younger than 105 days of life, definition was lost while getting closer to the final segment of the beak. In this portion of the beak the segments fold on each other so it was more difficult to count. This was reflected mainly in the youngest group, which has a higher value of CV (lower precision), as it is shown in Table 2. This table also shows the mean CV (95% confidence) calculated from the RSS readings of each age group. These results indicate that both readings were similar, therefore either could be selected. The --- reading was used as age estimation (number of increments in Table 1) from beaks.

The number of beak increments obtained in the present study was lineally related with the age (Fig. 4). Values of r^2 and slope close to 1 were obtained (p<0.00005). Fig. 5 shows age-BW relationship for two data sets, one with ages obtained from beaks analysis and a second group with true ages. Co-variance analysis showed that there were no statistical differences between curves obtained both types of data (p>0.05); for that reason an exponential equation (r^2=0.97) was calculated for the relationship between BW and age using both data sets (Fig 5). This equation was calculated for a BW interval of 0.13 to 152g.

4. Discussion

In the present study the use of beaks of the cultivated octopus of known age, allowed to validate the readings in rostrum sagittal section of the beak (RSS) as a method for obtaining absolute ages in *O. maya*. According with the results obtained in the present study one mark is one day of life of this octopus species. In the present study animals came from culture conditions where were fed with a soft diet during their growth, that reduces the beaks erosion. Previous studies made on *O. vulgaris* beaks underestimated the age of animals due to the loss of daily marks produced by beaks erosion during feeding (Perales-Raya et al., 2010). As many octopus species, *O. maya* and *O. vulgaris* have a diet rich in crustaceans that have hard exo-skeletons which must be bite, eroding the beak rostrum and in consequence affecting the age determination (Guerra, 1978; Boucaud-Camou y Boucher-Rodoni, 1983; Hanlon and Messenger, 1996). To avoid that Perales-Raya *et al.* (2010) have proposed reading the increments in the beak´s lateral wall (LWS), as an alternative of the rostrum because in that zone erosion could be negligible. Following that recommendation Canali *et al.* (2011) made a study on *O. vulgaris* beaks analyzing the daily increments in LWS in animals with between 160 and 610 g BW. Although a LWS was validated as a zone to studying the age of octopus, further research is necessary to explain the significant differences that were found between the numbers of increments when LWS or RSS were analyzed. At the date there are two hypothesis to be tested (Perales-Raya et al, 2010): (i) feeding erosion of the rostral tip, even in the dorsal-posterior area of the hood (where first increments were counted), could have biased increment count toward underestimation; or (ii) increment number is underestimated in the RSS because growth increments start depositing in the rostrum several weeks after hatching. This study demonstrated that when the beak rostrum is not eroded an accurate age determination is obtained from RSS beaks. Moreover, the coincidence between ages from hatching and the number of increments in not eroded RSS beaks indicates that increments are laid down in the rostrum from hatching, and therefore the second hypothesis can be rejected. Further studies including known-age animals of the complete age range and fed with hard diet will be interesting to develop a correction factor for accurate age estimations in the wild.

An exponential function was obtained from the relationship between BW and age of *O. maya*, allowing calculates the octopus BW using data of age. Although it is widely recognized that age and BW of wild octopuses are not necessarily related, using data obtained from octopus maintained in outdoor tanks at a density of 25 animals m^{-2} we observed that the equation obtained in the present study gave wet weight values close to those obtained from the ponds suggesting that, at least within this BW range, the actual equation could be useful for *O. maya* in culture conditions. Coefficients of this equation (Fig. 5) are higher than those obtained for wild octopuses of other species such as *O. pallidus* (Leporati et al., 2008) or *O. vulgaris* (Hernández-López et al., 2001; Sosa Reis and Fernández, 2002; Perales-Raya et al., 2010; Canali et al., 2011; Cuccu et al., 2012). It can be partially explained by the cannibalism in the culture tanks of *O. maya* that kills smaller individuals.

It is interesting to note that the equation obtained for *O. maya* was exponential for a wide interval of living BW suggesting that from juveniles to pre-adults maintained into the outdoor ponds, growth was maintained without changes along the culture time. Similar results were observed in *O. pallidus* where animals maintained an exponential growth curve along its life span (Semmens et al., 2011). *Octopus maya*, as other species exhibited exponential growth from 1 to 120 days age. Along that age interval BW showed greater size dispersal, varying widely even amongst organisms from the same spawning (André et al., 2009; Briceño et al., 2009). Results obtained in the present study demonstrate that animals maintained in outdoor ponds and sampled for beaks analysis showed a high dispersion even for animals of the same age; animals with 120 d age had an interval of BW between 62 and 264 g showing that the model obtained in the present study is useful only when a mean age value is needed (i.e. when recruitment models are constructed) but has limitations when trying to use these values to fisheries management programs that seek to exploit certain age classes. Other integrative models for *O. maya* fisheries programs should be done to maintain the fisheries of this important cephalopod species.

5. Conclusion

The number of increments present of the 4 age groups, correspond with the number of days from hatching, therefore, it was possible to validate the hypothesis 1 increment-1 day from beak sections of *O. maya* (in the range of ages analyzed) and that deposition starts at hatching. Since the organisms analyzed in the present study were fed with soft diet, the underestimations of age reported in other studies could be due to the erosion by feeding preys with hard structures.

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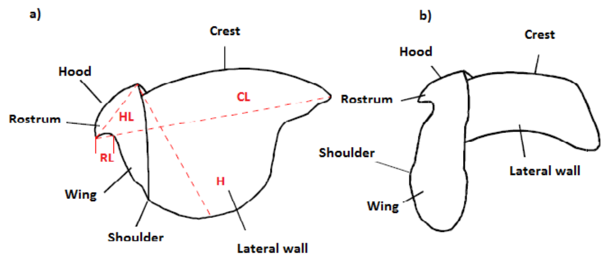

Fig.1. Illustration of upper (a) and lower (b) beak jaws. Main lengths are in red. Segments measured in *O. maya* (hood length (HL), height (H), crest length (CL) and rostral length (RL)). Adapted from Perales-Raya (2001).

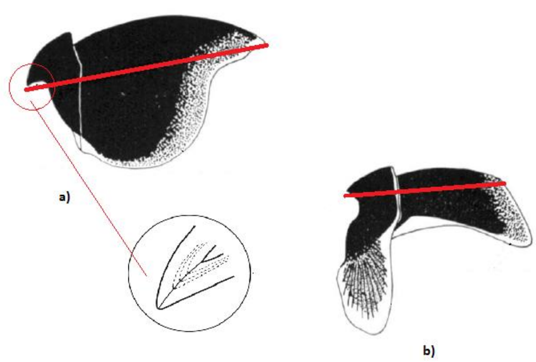

Fig.2. Illustration of upper (a) and lower (b) beak sagittal section. Inside the red circle is the reading area, where the rostral section and the increments are shown. Adapted from Perales-Raya (2001).

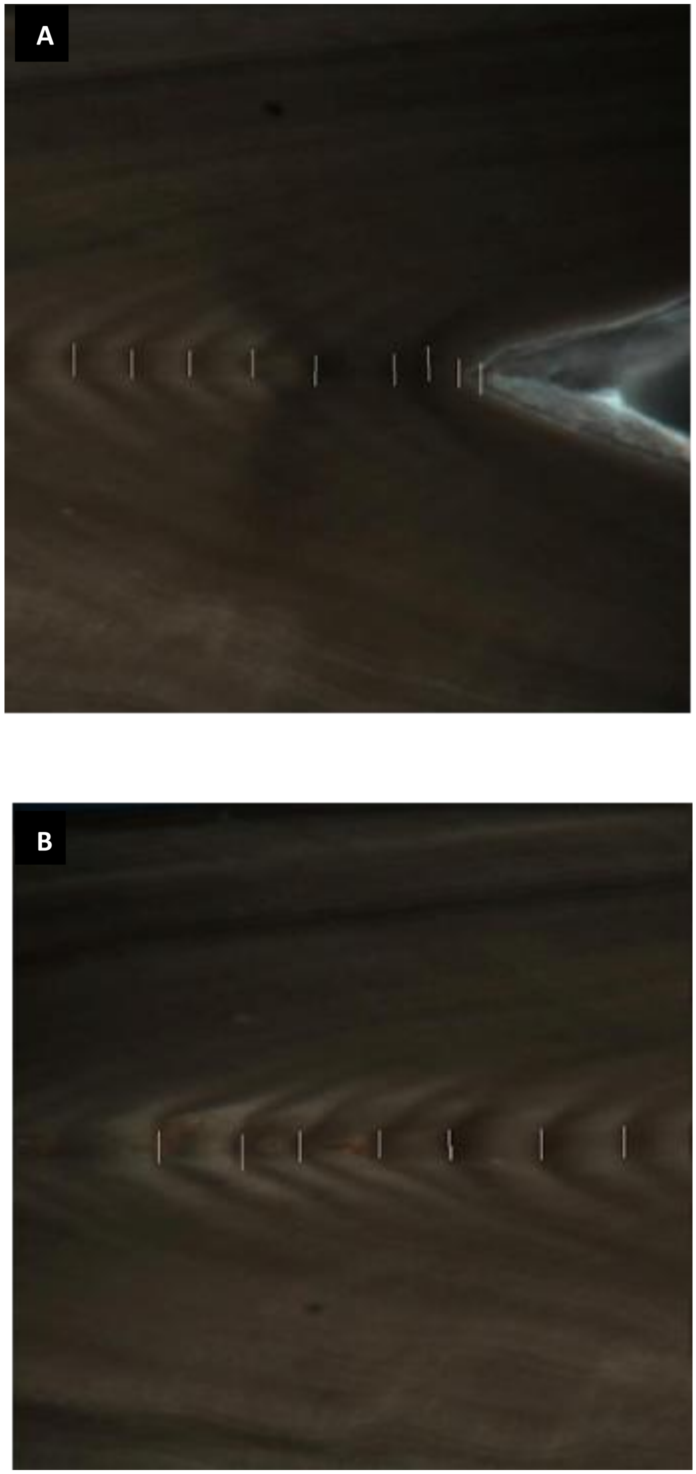

Fig.3 Sagittal sections of anterior region (A), and medium region (B) of octopus beak of 122 days old (300X, UV light). Increments are marked in white.

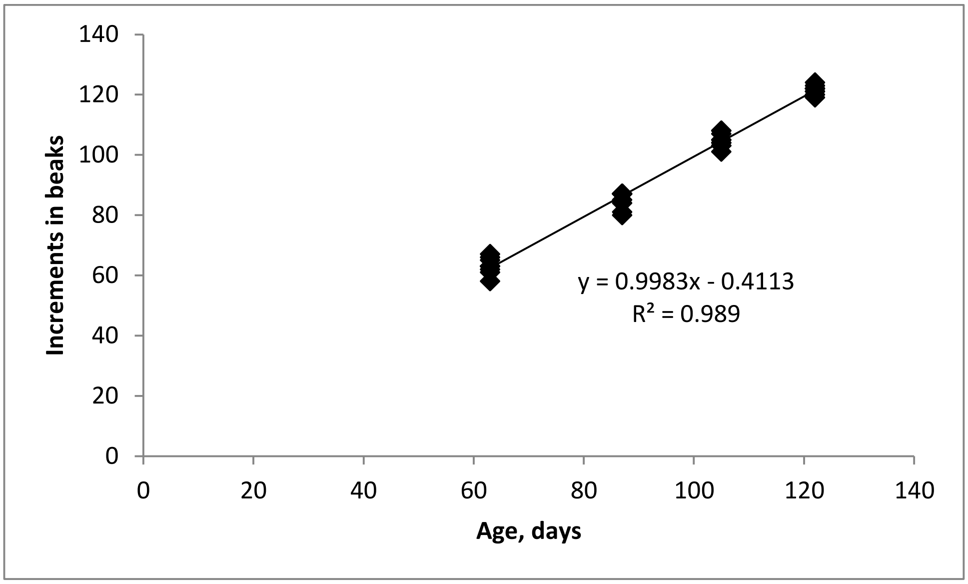

Fig.4**.** Relationship between age (days) and the number of increments in the beak sagittal sections of *Octopus maya.* Numbers close to line are the octopus age in days.

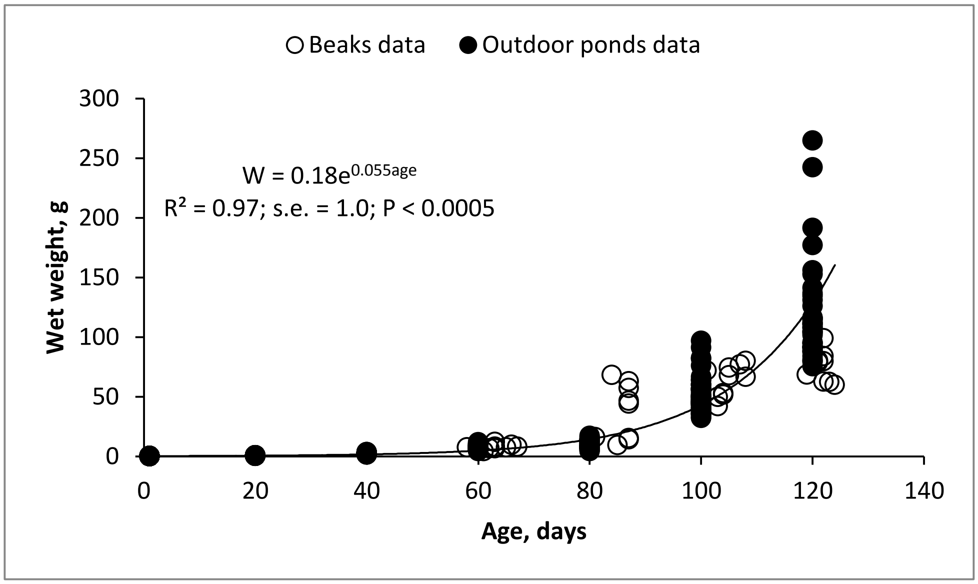

Fig.5. Relationship between age (days) and wet body weight (g) for two data sets, one with ages obtained from beaks analysis (open circles, n=40) and a second group with true ages (black circles, n=50) of cultivated *Octopus maya*. Equation for both data groups together.

Table1. Morphometric characteristics (total weight) and reading results in upper (1) an lower (2) beak.

Total Weight, g	Age, days	Number Increments	Jaw (upper: 1; or lower: 2)
7.6	63	63	2
7.6	63	65	1
6.6	63	62	2
7.9	63	67	1
12	63	63	2
7.5	63	58	1
6.6	63	63	2
9.6	63	66	1
7.9	63	63	2
4.4	63	61	1
14.2	87	80	2
14.2	87	87	2
9.3	87	85	1
16.1	87	81	2
15.3	87	87	1
57.3	87	87	2
46.6	87	87	1
62.9	87	87	2
68.3	87	84	1
44.2	87	87	2
67.9	105	105	1
42	105	103	2
52.9	105	104	1
51.6	105	104	2
77.1	105	107	1
74.5	105	105	2
49.8	105	103	1
66.6	105	108	2
71.9	105	101	1
80.1	105	108	2
83.9	122	122	1
79.2	122	120	2
78.8	122	121	1
62.6	122	122	2
99.1	122	122	1
60	122	124	2
62.4	122	123	1
68.5	122	119	2
80.2	122	121	1
79.8	122	122	2

Table 2. Precision of counts for sagittal sections of beaks, in the four groups of age in the *O. maya*. CV (coefficient of variation), **n** (number of samples).

Age group (days)	Mean CV	Confidence interval (±95%)	n
63	3.42	1.34	10
87	1.49	0.80	10
105	1.71	1.11	10
122	0.90	0.68	10