

BEAK INCREMENT COUNTS AS A TOOL FOR GROWTH STUDIES OF THE COMMON OCTOPUS *Octopus vulgaris* IN SOUTHERN BRAZIL

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ABSTRACT

The common-octopus *Octopus vulgaris* Cuvier, 1797 has high commercial value, and it is the most important octopus species on landings all over the world. Age estimation is vital for the comprehension of species life cycle and population dynamics, and further elaboration of an adequate management planning of its exploitation. In this study, 120 beaks of *O. vulgaris* had its growth increments counted and, assuming a daily basis periodicity, growth curves were adjusted, taking into account the relation between estimated age (a) and dorsal mantle length (DML), total weight (Wt) and upper beak crest length (CrL). The octopuses analyzed ranged from 50 to 163 mm of DML (162 and 290 marks, respectively) and 55 to 1,498 g of Wt (164 and 356 marks, respectively, consider a one year life expectancy). The best fit for all the analyzed situations was found using power equations: $DML = 0.876a^{0.871}$, $WT = 0.001a^{2.424}$, $CrL = 0.210a^{0.790}$, considering individual with number of marks from 118 to 356. Counting increments on the upper beak of octopus appears to be a relatively simple and useful way of estimating its age.

Key words: Cephalopods; fisheries; ageing; daily marks

CONTAGEM DE INCREMENTOS EM BICOS COMO UMA FERRAMENTA PARA OS ESTUDOS DE CRESCIMENTO DO POLVO-COMUM *Octopus vulgaris* DO SUDESTE-SUL DO BRASIL

RESUMO

O polvo-comum, *Octopus vulgaris* Cuvier, 1797, apresenta alto valor comercial e é a espécie de polvo mais capturada mundialmente. Estimativas de idade são vitais para a compreensão do ciclo de vida e dinâmica populacional de uma espécie e futura elaboração de um plano de gerenciamento adequado de sua exploração. Neste estudo, incrementos encontrados nas mandíbulas foram contados e, considerando uma deposição diária de incrementos, curvas de crescimento foram ajustadas, considerando a relação entre a idade estimada (a) com o comprimento dorsal de manto (DML), peso total (Wt) e comprimento da mandíbula superior (CrL). Os indivíduos analisados possuíam entre 50 e 163 mm de DML (162 e 290 incrementos, respectivamente) e entre 55 e 1.498 g de Wt (164 e 356 incrementos, respectivamente, na expectativa de 1 ano de vida). O melhor ajuste em todas as situações analisadas foi encontrado utilizando equações potenciais: $DML = 0,876a^{0.871}$, $WT = 0,001a^{2.424}$, $CrL = 0,210a^{0.790}$, para indivíduos com número de incrementos entre 118 e 356. A contagem de incrementos na mandíbula superior de polvos se mostrou uma forma relativamente simples e bastante útil na estimativa de idade dos mesmos.

Palavras chave: Cefalópodes; pesca; idade; marcas diárias

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INTRODUCTION

The common-octopus, *Octopus vulgaris* Cuvier, 1797 (Cephalopoda, Octopodidae), is a species of high commercial value, shows a world-wide distribution and is one of the most studied species of cephalopods. However, there are still considerable lacks on the knowledge of the species, especially related to growth and ageing techniques.

The octopus growth rates are strongly influenced by many environmental factors, such as temperature, quality and availability of food, individual length and reproductive activity, which makes quite hard the comparison of results obtained on rearing experiments with what would happen on nature (MANGOLD, 1983; RAYA and HERNANDEZ-GONZALEZ, 1998). Even small temperature changes cause strong variations on growth rates of cephalopods, especially on the initial phases of life cycle (JACKSON, 2004; SEMMENS *et al.*, 2004). FORSYTHE (2004) mentions that squid *Loligo forbesi* may increase three fold its size after 90 days if reared within a temperature only 1 °C higher.

Furthermore even under controlled environmental conditions, there are a wide range of individual variation on growth rates (DOMAIN *et al.*, 2000). WELLS *et al.* (1983) noticed differences of the order of 50% on oxygen consumption on identical size *O. vulgaris* in French coast, under the same rearing conditions, which indicates great individual variety on metabolic rates. In the Brazilian coast between Cabo Frio (23°S) and Cabo de Santa Marta Grande (29°S), reproduction occurs all over the year, with no evidence of seasonal peaks of reproductive activity, which complicates the identification of cohorts (TOMAS and PETRERE-JR, 2005). Those attributes, associated to a non-asymptotic growth (MANGOLD, 1983; JACKSON and CHOAT, 1992; SEMMENS *et al.*, 2004) and the soft nature of cephalopods (JACKSON *et al.*, 2000) makes difficult the use of length frequency methods for determining age composition and growth rates. Some authors, such as GUERRA (1979), PEREIRO and BRAVO-DE-LAGUNA (1979) and ARREGUÍN-SANCHEZ (1992), had used this kind of methods for adjusting von Bertalanffy model for octopods. However, many other authors point

out that these analysis may result on overestimated longevity and do not show an adequate adjust of growth (MANGOLD, 1983; JACKSON and CHOAT, 1992; ALFORD and JACKSON, 1993; DAY and TAYLOR, 1997; JACKSON *et al.*, 2000; SEMMENS *et al.*, 2004).

Statoliths are the most commonly used structures for ageing cephalopods (BETTENCOURT and GUERRA, 2000; SEMMENS and MOLTSCHANIWSKYJ, 2000), but more appropriate for squids, once in octopus they are quite small and fragile. HERMOSILLA *et al.* (2010) successfully estimated *O. vulgaris* age by counting increments on stylets and validated a daily deposition, proving them as a reliable structure for ageing octopus (SOUSA-REIS and FERNANDES, 2002; DOUBLEDAY *et al.* 2006; LEPORATI *et al.* 2008). Other tools, as the use of lipofuscin (DOUBLEDAY and SEMMENS, 2011), are more expensive.

About the use of beaks, CLARKE (1965) analyzed the deposition of increments on the beaks of squid *Moroteuthis ingens* and found cycles of increments, although the periodicity of increments deposition was not estimated. RAYA and HERNANDEZ-GONZALEZ (1998) and HERNANDEZ-LOPEZ *et al.* (2001) noticed the deposition of increments on *O. vulgaris* beaks. RAYA and HERNANDEZ-GONZALEZ (1998) mentioned a regular pattern of deposition of increments of similar width on beaks internal rostral area, which should be related to the age of individuals. HERNANDEZ-LOPEZ *et al.* (2001) validated daily deposition of increments on the lateral wall of upper beaks of *O. vulgaris* paralarvas. PERALES-RAYA *et al.* (2010) used both methods for counting age increments on *O. vulgaris* beaks and found readings on upper beaks lateral walls provided better estimative of age. CUCCU *et al.* (2012) also had positive results on estimating octopus age by counting increments on the lateral wall of upper beaks. CANALI *et al.* (2011) validated a daily deposition of increments on the beaks, for octopus from 160 to 610 g of total weight collected in the Mediterranean Sea. Thus, although daily deposition of increments on the beaks have not yet been proved for all ranges of size in adults populations, there seems to be a consensus among all authors mentioned that this deposition occurs, in fact, on a daily basis.

The main goal of this study is to evaluate the correlation of length and weight with the increments found on the beaks of the common octopus *Octopus vulgaris* in the southern Brazil, as a first step to consider the possibility of using beaks increments as ageing tool, similarly to what been done for other populations. To that, increments from 120 beaks of *O. vulgaris* were counted (65 females and 55 males) for the first time in a Brazilian octopus population.

MATERIAL AND METHODS

Samples of octopus beaks used for this study were obtained between 'Cabo Frio' (23°S) and 'Cabo de Santa Marta Grande' (29°S) (Brazil) by the double-otter trawl commercial fishery from June 1999 to July 2000. The region is dominated by a coastal water mass over the shelf break with insertions of a cold water mass (South Atlantic Central Water), which flows northwards below the tropical water mass (Brazil Current), that can cause influence in the recruitment and consequently in the growth. After extraction, the upper beaks were maintained on a 70% glycerol solution. Total weight (Wt, in g), dorsal mantle length (DML, in mm), crest length (CrL, in mm) and gender (male or female) were taken for each individual.

Two methodologies were tested on preparing the beaks for reading: the first developed by RAYA and HERNANDEZ-GONZALEZ (1998) and another following HERNANDEZ-LOPEZ *et al.* (2001), which is of much simpler preparation and allowed better visualizations of the increments, thus being adopted on this study (as recommended by PERALES-RAYA *et al.*, 2010).

Beaks were taken to stereomicroscope with an increase rate up to 50 times, connected to a computer. The increments were counted direct from the stereomicroscope, from the edge of the inner part of lateral wall to the tip of rostrum (Figure 1). It was usually not possible to follow a straight line, once the beaks presented many eroded areas, due to abrasive movement of chew, in which the increments could not be seen. At these points, the adopted procedure was searching the extension of increments for an area of better visualization.

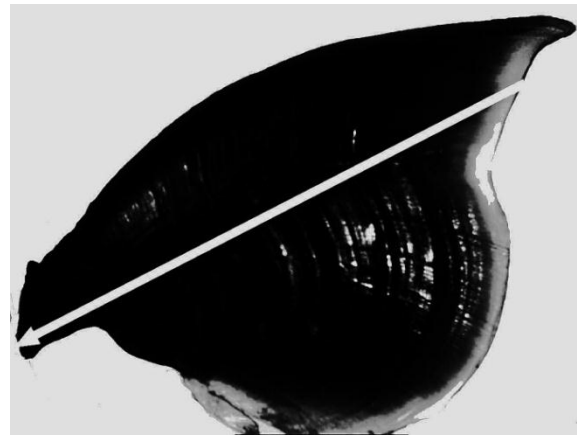


Figure 1. Representative scheme of the line and direction of age increments counting on the lateral wall of the upper beaks of *Octopus vulgaris*, caught between Cabo Frio (23°S) and Cabo de Santa Marta Grande (29°S), Brazil.

The increments were counted twice by the same observer. Coefficient of Variation (CV) and Average Percent Error (APE) were estimated from each of the individuals. For those animal whose rates of CV and APE were higher than what was suggested for CHANG (1982) and CAMPANA (2001) (CV > 7.6; APE > 5.5), a third counting was made and values of the first counting were discarded. A paired *t* test was used to check precision between valid counting. Furthermore, a linear regression was made to check similarity on counting.

As results showed a high correlation between valid counting, the average between them was calculated and these values were considered age for all other analysis.

Total weight, crest length and total length were related to number of increments (estimated age), analyzing which model would offer the best fit (potential, exponential or logarithmic), using the determination coefficient (r^2). The coefficients of each equation were also fitted with the iterative method Solver presented as a routine at MS Excel. Differences on these relations due to gender were statistically tested by covariance analysis, after data linearization.

RESULTS

The sample was composed by individuals from 50 to 163 mm of DML (162 and 290

increments, respectively) and from 55 to 1,498 g (164 and 356 increments, respectively). The youngest individual showed 118 increments (71 mm and 129 g) and the oldest 356 increments (152 mm and 1,498 g). The sample comprised a satisfactory number of animals on each class of estimated age and a good range of estimated

ages, from young animals until those nearly reaching the life expectancy for the species (around one year) and the median number of increments in each class of DML showed a rising tendency, which corroborates with the increase on number of daily increments as individuals grows (Table 1).

Table 1. Absolute frequency and respective percentiles (numbers within brackets) of individuals on each age class (months) by class of DML (mm) of *Octopus vulgaris* caught between Cabo Frio (23°S) and Cabo de Santa Marta Grande (29°S), Brazil. (Bold numbers indicate the most frequent month class in each DML class).

DML (mm) Class	Age (months)									
	4	5	6	7	8	9	10	11	12	Total
50		4 [100]								4
60		3 [38]	4 [50]	1 [12]						8
70	1 [6]	4 [25]	4 [25]	6 [38]	1 [6]					16
80		2 [10]	11 [58]	2 [10]	1 [5]	2 [10]	1 [5]			19
90			2 [12]	6 [35]	7 [41]	2 [12]				17
100			1 [6]	6 [33]	6 [33]	4 [22]	1 [6]			18
110				2 [20]		3 [30]	3 [30]	2 [20]		10
120				1 [7]	4 [29]	6 [43]	2 [14]	1 [7]		14
130						2 [40]	2 [40]	1 [20]		5
140					1 [25]	2 [50]	1 [25]			4
150						1 [33]	1 [33]		1 [33]	3
160							1 [50]	1 [50]		2
Total	1	13	22	24	20	22	12	5	1	120

The increments presented as furrows on lateral wall, which follows the beak design in all its extension. Often, among the increments it was possible to notice grooves due to wear while feeding, which could be mistaken by a ring. On these situations, analyzing the continuity of the ring and how it follows the beak design can be an efficient way to separate increments from grooves. The rostral area was specially eroded and must be analyzed with extreme caution, being advisable to begin the counting on the opposite side of lateral wall, finishing it on the rostral area. Both reflected and incident lights were used and the continuous adjust of lightening was of high importance on an adequate ring visualization. In general, the

visualization of increments (Figure 2) was relatively clear, after some training. The counting was possible in about 70% of the specimens sampled.

The paired *t* test showed no significant difference between valid counting on the level of 95%. Coefficient of Variation was 7.350 and APE 5.197. Linear regression between valid counting showed an adequate fit ($r^2 = 0.883$). The potential model showed the best fit for all relations analyzed (Figures 3 to 5), and those relations presented an adequate adjust (Table 2). Covariance analysis showed statistically significant variations due to season (ANCOVA, $p = 0.000$) but none to gender (ANCOVA, $p = 0.612$) (Table 3).

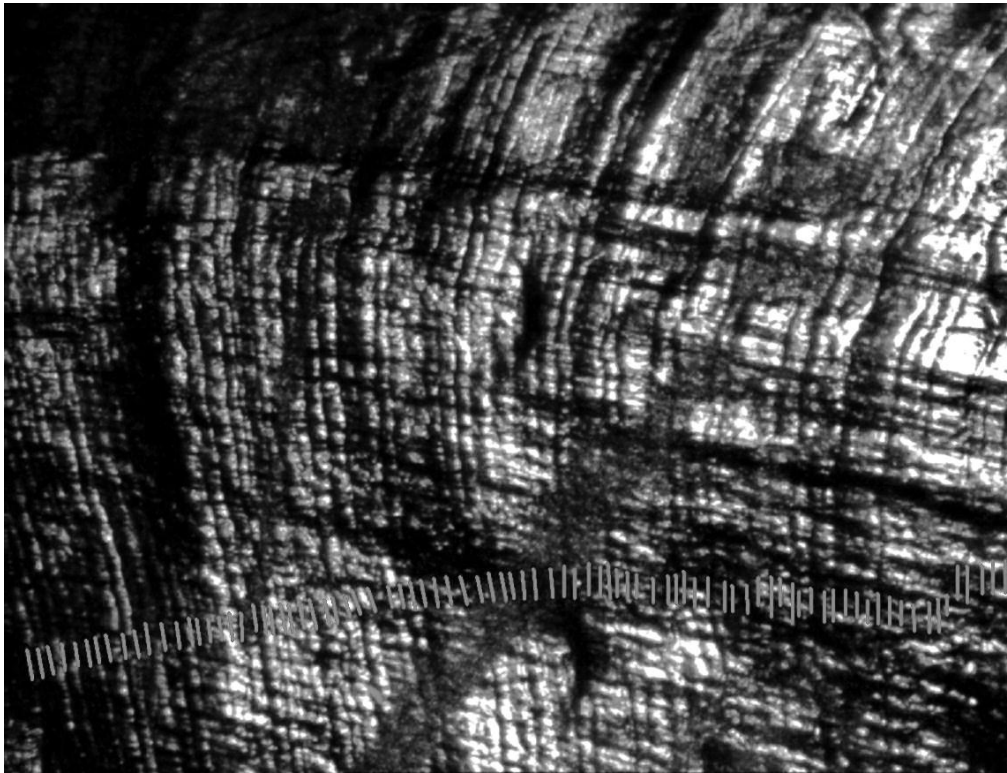


Figure 2. Increments on upper beaks of *Octopus vulgaris* caught between Cabo Frio (23°S) and Cabo de Santa Marta Grande (29°S), Brazil (photo obtained under stereomicroscope with an increase rate of 40 x).

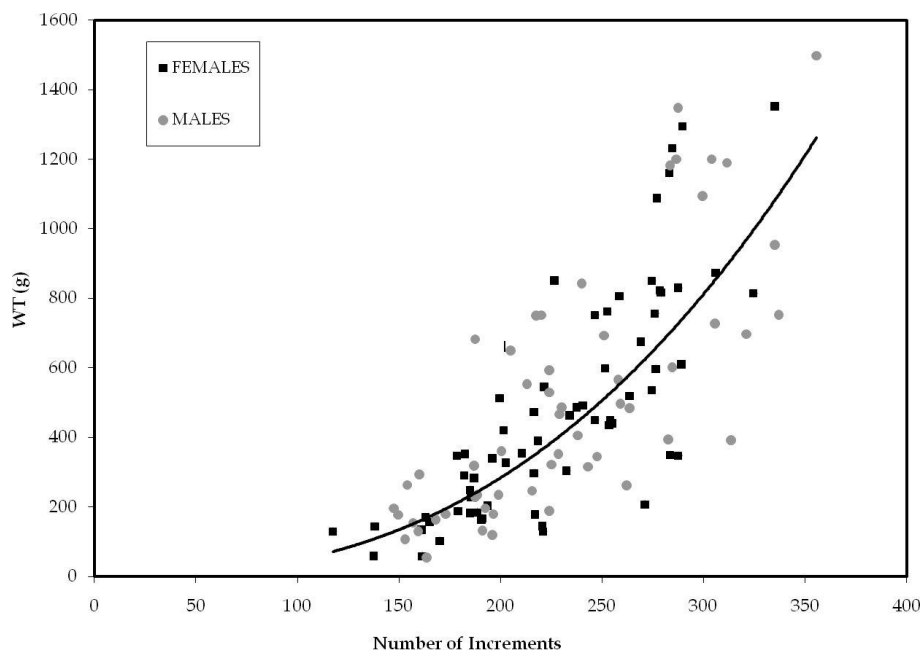


Figure 3. Total weight (TW, in g) and estimate age (in number of increments) of *Octopus vulgaris*, caught between Cabo Frio (23°S) and Cabo de Santa Marta Grande (29°S). $TW = 0.001a^{2.424}$ ($r^2 = 0.631$; $p < 0.001$; $n = 120$).

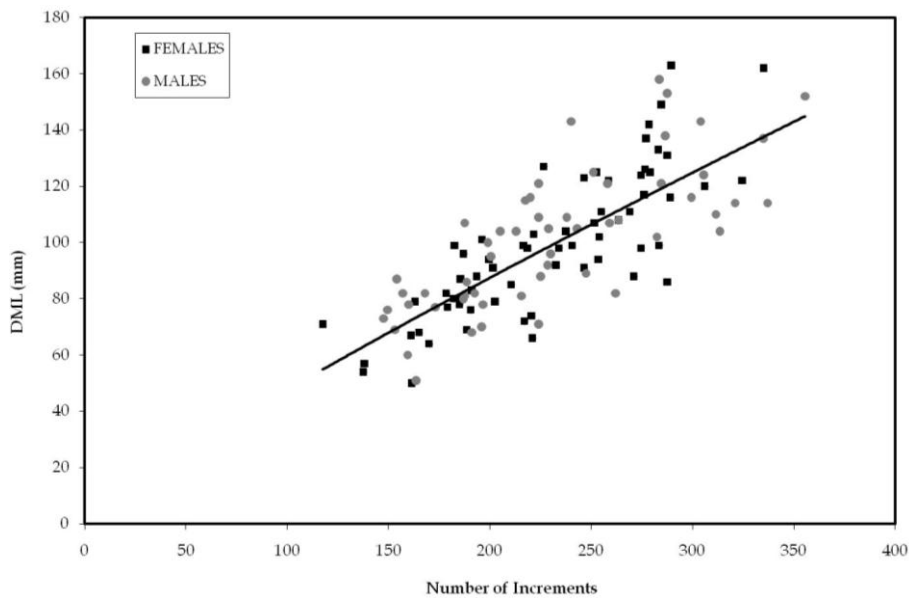


Figure 4. Dorsal mantle length (DML, in mm) and estimate age (in number of increments) of *Octopus vulgaris*, caught between Cabo Frio (23°S) and Cabo de Santa Marta Grande (29°S). $DML = 0.876a^{0.871}$ ($r^2 = 0.620$; $p < 0.001$; $n = 120$).

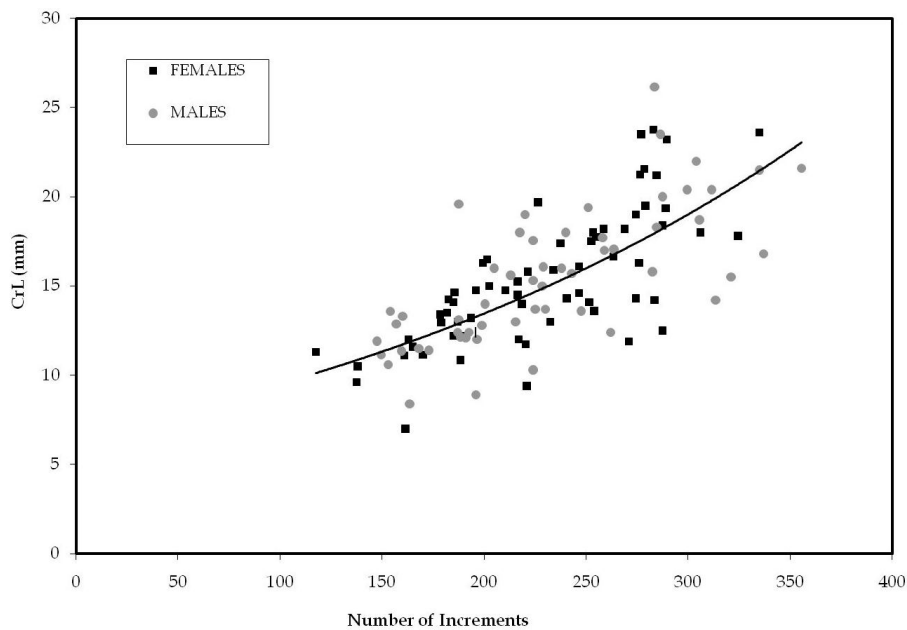


Figure 5. Crest length (CrL, in mm) and estimate age (in number of increments) of *Octopus vulgaris*, caught between Cabo Frio (23°S) and Cabo de Santa Marta Grande (29°S). $CrL = 0.210 \times a^{0.790}$ ($r^2 = 0.529$; $p < 0.001$; $n = 120$).

Table 2. Dorsal mantle length (DML, in mm), crest length (CrL, in mm) and total weight (WT, in g) and estimated age (days) (a: number of increments) *Octopus vulgaris* caught between Cabo Frio (23°S) and Cabo de Santa Marta Grande (29°S), Brazil ($n = 120$).

Relationship	Equation	r ²
WT x Age	WT = 0.001 x a ^{2.424}	0.631
CrL x Age	CrL = 0.210 x a ^{0.790}	0.529
DML x Age	DML = 0.876 x a ^{0.871}	0.620

Table 3. Covariance results of the weight (WT) and the dorsal mantle length (DML) relationship, with gender and season as factors of *Octopus vulgaris* caught in the southeastern Brazil. Dependent Variable: ln(WT), $n = 120$, $r_{\text{adjusted}} = 0.998$, $r^2_{\text{adjusted}} = 0.996$.

Source	SS	Df	MS	F	P
Ln (DML)	1129.650	1	1129.650	7203.958	0.000
Gender	0.041	1	0.041	0.259	0.612
Season	5.671	6	0.945	6.027	0.000
Error	17.563	112	0.157		

DISCUSSION

Most incirrate benthic octopods, among them *Octopus vulgaris*, have a digestive gland that alternatively produce digestive enzymes or absorbs nutrients (WELLS 1978) and have a regular daily pattern of activity and resting cycles (KAYES, 1974; WELLS *et al.*, 1983), which probably originate daily marks on its hard structures, such as increments (RAYA and HERNANDEZ-GONZALEZ, 1998). During resting period the animal would make more substances available to beak growth than on periods of activity, when the body demand for energy is higher. The hypothesis of daily increments on *O. vulgaris* beaks was validated for paralarva by HERNANDEZ-LOPES *et al.* (2001) in Canarian Islands (Spain), and for adults from 160 to 610 g of total weight in the Mediterranean Sea by CANALI *et al.* (2011). The lack of validation of the daily periodicity of the marks for the Brazilian populations is as a matter of great importance for future studies. In spite of this, there is no reason to believe that this pattern could not be used for estimating age of local population, as have been made in many other studies (eg. RAYA and HERNANDEZ-GONZALEZ, 1998; HERNANDEZ-LOPES *et al.*, 2001; PERALES-RAYA *et al.*, 2010; CANALI *et al.*, 2011; CUCCU *et al.*, 2012).

Supporting this assumption, the oldest individual on this study (356 increments) was nearly reaching the life expectancy for the species, which would be around one year (JACKSON and CHOAT, 1992; DOMAIN *et al.*, 2000; HERNANDEZ-LOPES *et al.*, 2001; TOMAS and PETREIRE-JR, 2005). Yet, HERNANDEZ-LOPES *et al.* (2001) and CANALI *et al.* (2011) mention that ages obtained by counting upper beaks increments could be underestimated, once there are many eroded areas on the beaks, which can cause some initial increments to disappear.

A relevant matter on this study was the use of stereomicroscope, with an increase rate between 30 and 50 times instead of an optic microscope. At first, an optic microscope was used, with an increase rate of 400 times and the visualization of the increments was highly affected, due to excessive amplification which focused vision on a smallest area of the beak. Thus, the identification of the ring as a whole and the difference between increments and grooves, as well as the way to follow on eroded area, were a lot harder to see (HERNANDEZ-LOPEZ³, *pers. comm.*).

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Distance between increments showed variation, as was also observed by CLARKE (1965), HERNANDEZ-LOPEZ *et al.* (2001) and CANALI *et al.* (2011), which appear to be related to differences on environmental conditions that reflect on individual growth rates.

The adjusted curves relating total weight, dorsal mantle length and crest length versus the number of increments on the beaks seems to be adequate, although there is a rather high dispersion of data. Values of coefficient of determination (r^2) found on growth curves were similar to what was found by HERNANDEZ-LOPEZ *et al.* (2001) and from PERALES-RAYA *et al.* (2010), and can be considered acceptable, as it is easily explained by the high variety on individual growth rates on cephalopods and the strong influence of environmental conditions on growth, such as food availability and temperature, especially on the first stages of life cycle (DOMAIN *et al.*, 2000; HO *et al.*, 2004; CANALI *et al.*, 2011; CUCCU *et al.*, 2012). Gender did not show significant influence on any of considered analysis. The potential model fit for the growth curve was the best option to describe *O. vulgaris* growth, which was already expected as all individuals on this study were from commercial fishing, thus being already on benthic stage.

It was concluded that the counting of increments of *O. vulgaris* beaks is a relatively simple and of great potentiality way of estimating growth on *O. vulgaris* in Brazilian populations and must be encouraged.

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