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GROWTH LINES WITHIN THE BEAK MICROSTRUCTURE OF THE OCTOPUS *OCTOPUS VULGARIS* CUVIER, 1797

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Octopus vulgaris Cuvier, 1797 is commercially the most important species in the central-eastern Atlantic cephalopod fishery. The estimation of growth parameters in wild octopus populations is essential to management of the fishery. As there are problems with methods based on length-frequency modal analyses for describing growth from field data and there is a lack of age determination techniques for octopod species, estimation of the growth rates of natural populations is currently difficult. The present work describes the use of beaks to determine the age of *O. vulgaris*, using a new method based on a sample of beaks from 25 animals caught in the area in 1993. After sectioning different planes of the beaks embedded in resin blocks, they were analysed to select the best with which to observe growth increments. The sagittal plane (from outer to inner) revealed a pattern of increments of similar width in the internal rostral area of all the beaks analysed that are possibly related to the age of the animal. The number of these increments does not exclude daily deposition.

Octopods, as do other cephalopods, constitute economically significant fisheries in many areas of the world. The type species of the genus, *Octopus vulgaris* Cuvier, 1797, is widely spread throughout the world in tropical, subtropical and temperate waters (Mangold 1983), and it is the main resource caught in the north-west African cephalopod fisheries. Age determination of octopods is critical to understanding their life history and to modelling the dynamics of their populations, both of which are essential for assessment and management purposes.

Most information on octopod growth has come from laboratory rearing studies. Some growth studies from captive animals have been done on *Octopus vulgaris* (Nixon 1969, Mangold and Boletzky 1973, Smale and Buchan 1981, Villanueva 1995). However, comparison of growth quantification from laboratory experiments with growth of the species under natural conditions is questionable and has not been possible owing to the lack of methods of estimating the age in wild animals. Methods based on tag-recapture and length frequency modal analyses involve problems related to the unknown age of octopuses (Robinson and Hartwick 1986), the size selectivity of gear, the lack of a reliable relationship between size and age (Mangold 1983) and the prolonged breeding season (Mangold 1963, Wodinsky 1972, Fernández-Núñez *et al*. 1996), all of which complicate the interpretation of such field data. Therefore, growth studies of octopus populations in nature are essential; the most important tool needed is the development of age determination techniques (Forsythe and Van Heukelem 1987).

Since Young (1960) found signs of concentric rings on statoliths of *Octopus vulgaris*, no other reference to periodic ring deposition on these structures has been made. The growth of the beaks and radula in *O. vulgaris* has been investigated to estimate the mass of the animal from which they were taken, but no information about the age of the animals was given (Clarke 1962, Nixon 1973, Smale *et al.* 1993). Gonçalves (1993) mentioned the presence of concentric rings in the internal shell and in the lens of *O. vulgaris* from the Azores, but no deposition rhythm was proposed. Clarke (1965) described cycles and rings on the lateral walls of the lower beak in the squid *Moroteuthis ingens*, but as yet no satisfactory structure has been found from which the age of a wild octopus can be assessed, because the few available hard parts have not revealed easily estimable growth increments. The present study is the first attempt to describe a method of age determination of an octopod species (*O. vulgaris*) based on beak microstructure. Upper and lower beaks have shown a regular pattern of micro-increments that may indicate a constant temporal basis.

MATERIAL AND METHODS

Specimens of *Octopus vulgaris* were obtained from Spanish freezer trawlers operating off the coast of North-West Africa (21–26°N). Sampled specimens were caught during January, June, August, September, November and December 1993 by trawl on fishing grounds at depths of 30–200 m. Total (*TM*) and eviscerated body mass were determined (±0.1 g), and each

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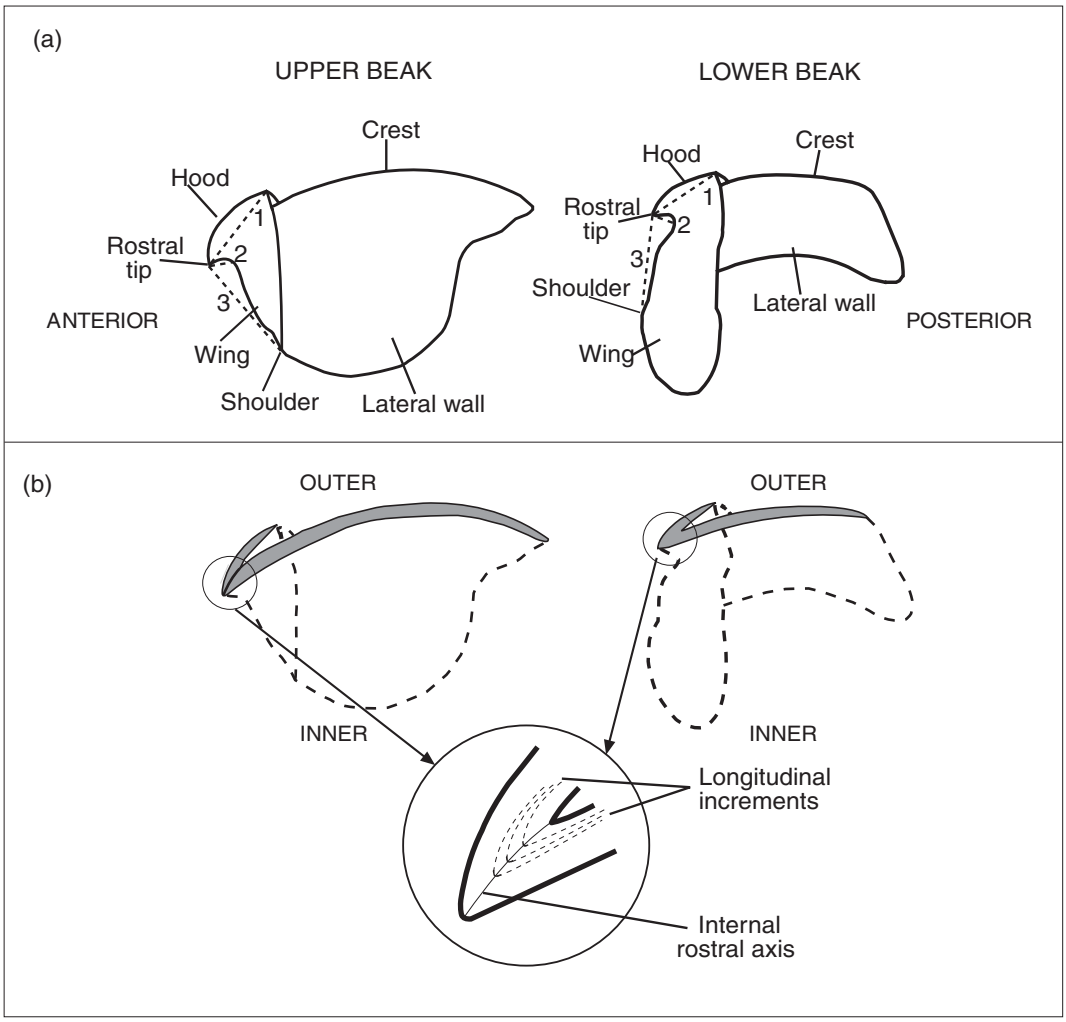

Fig. 1: Diagrams of the upper and lower beaks of *Octopus vulgaris* showing (a) the terms used in the text and (b) medial surfaces (grey) of the upper and lower beak after sagittal sectioning. The rostral area, showing the counting axis, is magnified in (b)

individual was sexed and assigned a maturity stage based on the appearance of the gonads and of the accessory reproductive organs (Día 1988). For a total sample of 524 individuals, the following body measurements were taken to the lower mm: dorsal mantle length (*DML*), mantle width, cephalic length and width, funnel length and width, and the length of the four left arms. The beaks of each animal were removed and stored in 70% ethyl alcohol. After cleaning with a 3% H_2O_2 solution, the beaks were dried and the upper and lower ones of each specimen weighed (±0.1 mg). The hood length (*HL*), rostral length (*RL*) and shoulder-tip length (*STL*) were measured (±0.01 mm) using terms defined by Clarke (1962), as shown in Figure 1a.

A subsample of beaks of different sizes was selected for sectioning from several planes in order to select the best for identifying bands. The sagittal plane was finally selected for presentation in this study. The upper and lower beaks of 25 specimens (males and females) covering the whole size range and sample period (Table I) were prepared and analysed. Sagittal

Table I: Data collected for the sample of *O. vulgaris*. Size (*DML*), total mass (*TM*), sex and maturity for each animal, hood length (*HL*), rostral length (*RL*), shoulder-tip length (*STL*) and mass for the upper and lower beaks. For the beaks analysed, the number of increments and the length of the internal rostral axis (*IRA*) are presented

Sample	Month of capture	*DML* (mm)	*TM* (g)	Beak analysed	*IRA* (mm)	Number of increments	Sex	Maturity	Upper beak				Lower beak			
									HL (mm)	*RL* (mm)	*STL* (mm)	Mass (mg)	*HL* (mm)	*RL* (mm)	*STL* (mm)	Mass (mg)
1	January	82	588.5	Upper	1.80	103	♀	2	7.2	2.1	5.6	78.4	4.8	1.3	5.4	63.3
2	January	75	423.2	Lower	1.80	84	♂	1	6.3	1.7	4.6	59.8	4.6	1.8	4.4	54.7
3	January	99	919.4	Upper	2.87	160	♀	3	8.1	3.0	5.9	77.0	5.6	2.3	5.9	65.2
4	January	113	1 317.1	Upper	3.00	163	♀	1	9.2	3.0	5.8	150.1	6.6	2.3	6.0	125.3
5	January	70	468.4	Upper	1.73	96	♀	2	6.5	2.2	3.9	44.5	3.8	1.5	4.4	36.8
6	January	120	1 709.4	Upper	3.39	186	♀	2	9.1	3.2	7.3	146.7	6.5	1.9	5.1	113.8
7	January	134	2 047.6	Upper/Lower	3.33/3.80	182/193	♂	3	9.9	3.0	7.2	155.2	7.3	2.0	7.7	130.7
8	January	166	4 170.1	Lower	3.66	177	♀	3	10.7	3.3	9.3	285.5	8.8	2.1	8.8	267.2
9	June	54	165.5	Upper	2.14	128	♀	2	4.7	1.6	2.6	14.6	3.4	1.1	3.3	12.2
10	June	64	259.5	Upper	2.34	118	♀	1	5.1	1.2	3.1	17.4	3.7	0.9	3.1	13.8
11	June	101	923.9	Upper	2.79	167	♂	2	8.4	2.8	5.2	102.3	6.0	1.7	6.1	78.6
12	June	133	2 069.7	Upper	3.08	164	♂	2	9.9	3.0	6.0	157.6	6.9	3.0	7.7	122.6
13	June	174	4 470.6	Upper	3.28	172	♂	3	11.8	4.5	11.7	375.4	9.2	3.6	9.9	339.6
14	September	92	743.6	Upper	3.25	144	♂	1	6.6	2.2	4.9	46.6	3.9	1.4	2.6	36.6
15	September	112	1 279.7	Lower	2.26	107	♂	2	8.1	2.3	4.6	70.9	5.4	2.1	5.5	60.2
16	September	124	1 697.0	Upper	2.94	164	♂	3	9.3	3.3	5.3	119.0	7.1	2.1	7.2	96.9
17	September	152	4 302.3	Upper	4.42	246	♀	2	9.3	3.4	6.3	180.8	7.3	2.2	7.2	134.0
18	November	48	157.5	Upper	1.86	108	♂	1	4.6	1.1	2.2	14.6	3.1	0.8	3.1	11.6
19	November	105	1 027.6	Upper	2.33	120	♀	2	7.4	2.7	6.9	65.3	5.5	2.3	3.3	57.9
20	November	118	1 545.1	Upper	3.01	123	♂	2	9.6	2.4	5.4	137.5	7.6	2.1	6.8	127.8
21	November	149	2 471.9	Upper	3.14	152	♀	3	9.1	3.3	6.8	163.3	7.4	1.9	7.6	143.1
22	December	58	197.2	Upper	2.48	128	♀	1	4.4	1.0	3.0	17.0	3.3	1.0	3.6	12.9
23	December	85	452.3	Upper	1.91	96	♀	1	6.1	0.8	3.1	29.3	3.7	1.1	3.7	23.0
24	December	109	1 822.1	Upper	2.85	166	♂	2	9.4	3.0	6.0	144.6	6.9	1.2	5.5	124.0
25	December	145	2 359.9	Upper	3.94	209	♂	3	9.6	3.1	7.5	240.9	7.7	2.9	8.2	216.5

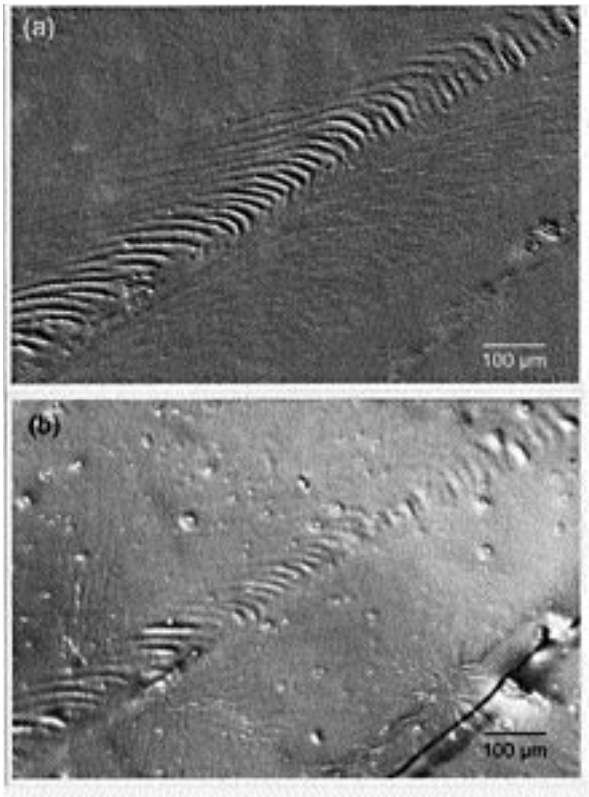

Fig. 2: Central-sagittal section of an *Octopus vulgaris* upper beak showing increments from the origin (left) to the outer part (right) along the internal rostral axis – image obtained under (a) reflected light and (b) from an acetate replication of the same beak under transmitted light

parallel sections (0.5–0.8 mm) from the outer to the inner part of each beak (Fig. 1b) were obtained after embedding the beaks in Polyfix resin and sectioning with a Disgrape diamond slitting saw. Sections were mounted on slides, ground with fine-grain (1 200 grade) carborundum paper and polished with 3- and 1-µm diamond paste. The polished surfaces were etched with a 8.5% di-sodium ethylenediaminetetraacetate (EDTA) solution with exposure times between 6 and 48 h or with 37% HCL (exposure times 5–10 minutes) prior to microstructure examination.

The selected section of each beak was analysed using an image analysis system as follows: the images for microstructure examination were obtained from a Nikon photomicrography camera mounted on a Nikon binocular microscope (75 × magnification) under oblique reflected light. The final magnification of the micrographs was 100 ×. The slides were scanned with a Minolta QuickScan 35 film scanner (150 pixels per inch resolution) and the images enhanced using the "Emboss" filter of the Adobe Photoshop 4.0 software (Adobe Systems Inc.) before counting bands and measuring distances using the Optimas Version 5.1 software (Bioscan, Inc.). The linear axis selected for counting is the longest, with maximum increment width, and it usually has the best increment clarity. That axis, defined as the contact line between the inner side of the hood and the rostral part of the crest, seems to be a constant deposition axis, hereafter referred to as Internal Rostral Axis (*IRA*), as indicated in Figure 1b. Several slides (2–4) covering the *IRA* were needed for each individual. The increments were semi-automatically counted, and the total transect length and the distance from the line origin to each successive mark were displayed in calibrated units (µm). The bands in some small distances where the increments were poorly seen were extrapolated using the mean increment width of the next 10 increments. All these distances were automatically exported to a file where increment width (± 0.01 µm) was calculated.

Acetate replications were obtained from some beaks after etching and a three-minute exposure to acetone and observed under transmitted light. A subsample of eight beaks was also selected for analysis by scanning electron microscopy (SEM) and prepared as follows: after etching, each section was removed from the slide, attached to a SEM stub with double-sided tape and the surfaces were coated with a thin continuous layer of gold before examination.

RESULTS AND DISCUSSION

Identification of increments

The upper and lower beaks of the 25 individuals studied revealed a pattern of bands on the grey-coloured surface shown in Figure 1b, after cutting in a central sagittal plane from the outer to the inner part of the beak, where a pattern of bands can be seen on the medial surfaces (Clarke 1965) facing this plane. The increments were usually more prominent along the *IRA* but they continue from the anterior to the posterior part of the hood and of the laterall wall. The term "Longitudinal Increments" is used for these thin and closed portions of the bands which are deposited parallel to the beak edges (Fig. 1b).

Comparing the upper and the lower beak images from the 25 individuals showed similar microstructure appearance with respect to both spatial and density patterns of the increments. The lower and the upper

beak were analysed in Specimen 7 (see Table I) and the number of increments counted were 182 and 193 respectively. The upper beak was generally used to count bands because the *IRA* exhibits a more complete increment sequence along this axis, although the lower beaks were used when the upper one was poorly prepared.

Statoliths are the hard structures most commonly used for cephalopod age estimation (e.g. Lipiński 1986, 1993, Jereb *et al*. 1991, Arkhipkin 1993, Jackson 1993, Raya *et al*. 1994), and the right or the left are generally used indiscriminately because both statoliths show the same morphology. As pointed out by Natsukari *et al*. (1988) for *Photololigo edulis*, right and left statoliths are mirror images of each other. The case of the beaks is different, because neither their morphologies nor their functions are identical: the lower beak supports the muscles that control the movements of the upper beak, cutting the food by means of the movements of the latter over the former. The lower beak also injects paralysing saliva, which contains cephalotoxin (Nixon 1985), into the prey (Altman and Nixon 1970, quoted from Gonçalves 1993). It is also possible that there is some erosion of the rostral area during the life of the animal, although no evidence of incomplete increments has been observed on the surface of the central sagittal plane.

Observation of increments

Of the three observational techniques employed (directly under oblique reflection light of the binocular microscope, acetate replicating tape and SEM), the first was selected to analyse the sample because visualization and detection of the bands was much better than with the other methods. Only when the surface is reflected by the incident light (as with a "mirror" effect) are the bands clearly revealed (Fig. 2a) from the origin to the outer part of the axis. Under reflected light, each increment taken into account is composed of two units of deposition: a "light band" and a thinner and deeper "dark band" or "valley". These valleys were counted by the image analysis system and used as the increment count.

In the acetate replication, only the more prominent bands appear on the sheet under transmitted light (Fig. 2b). Better results were obtained with this technique and SEM for viewing the longitudinal increments along the hood and the lateral wall. An electron micrograph of Specimen 5 (see Table I) is presented in Figure 3a; it shows longitudinal increments in the hood of the lower beak. These increments were rejected for the analysis, because the number of bands varied depending on the counting axis selected in the same beak. The axis for counting those bands is shorter than

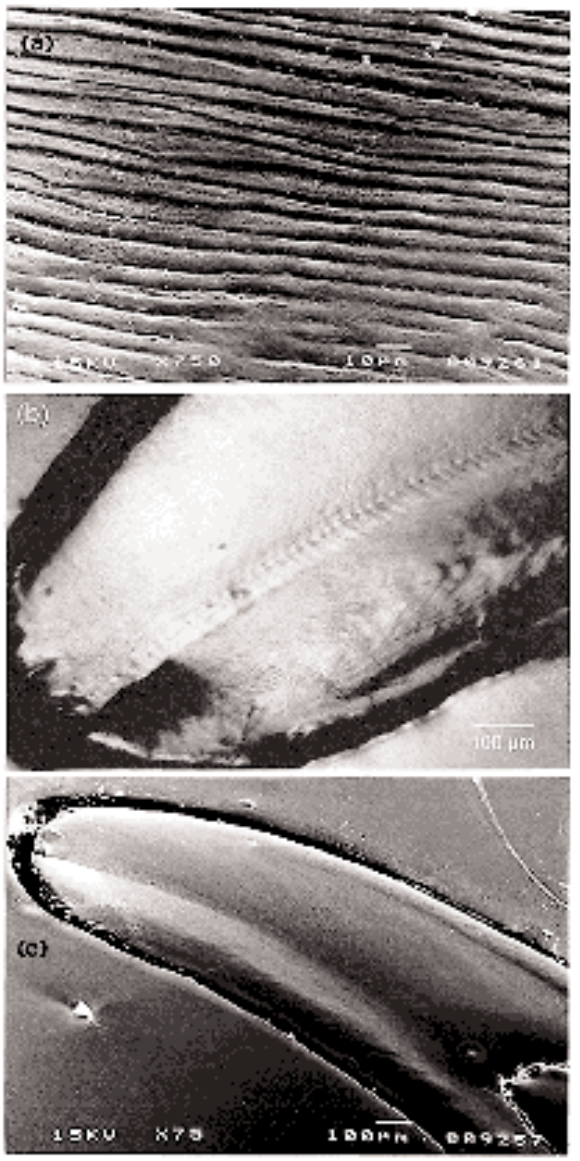

Fig. 3: (a) Longitudinal increments in an upper beak section of *Octopus vulgaris* as viewed with scanning electron microscopy; (b) increments revealed along the internal rostral axis under reflected light and (c) appearance of the same beak under the scanning electron microscope. All scans orientated from the origin (right) to the outer part (left)

IRA and usually exhibits an incomplete increment sequence. As pointed out by Campana (1992) for otolith microstructure examination, axis length and

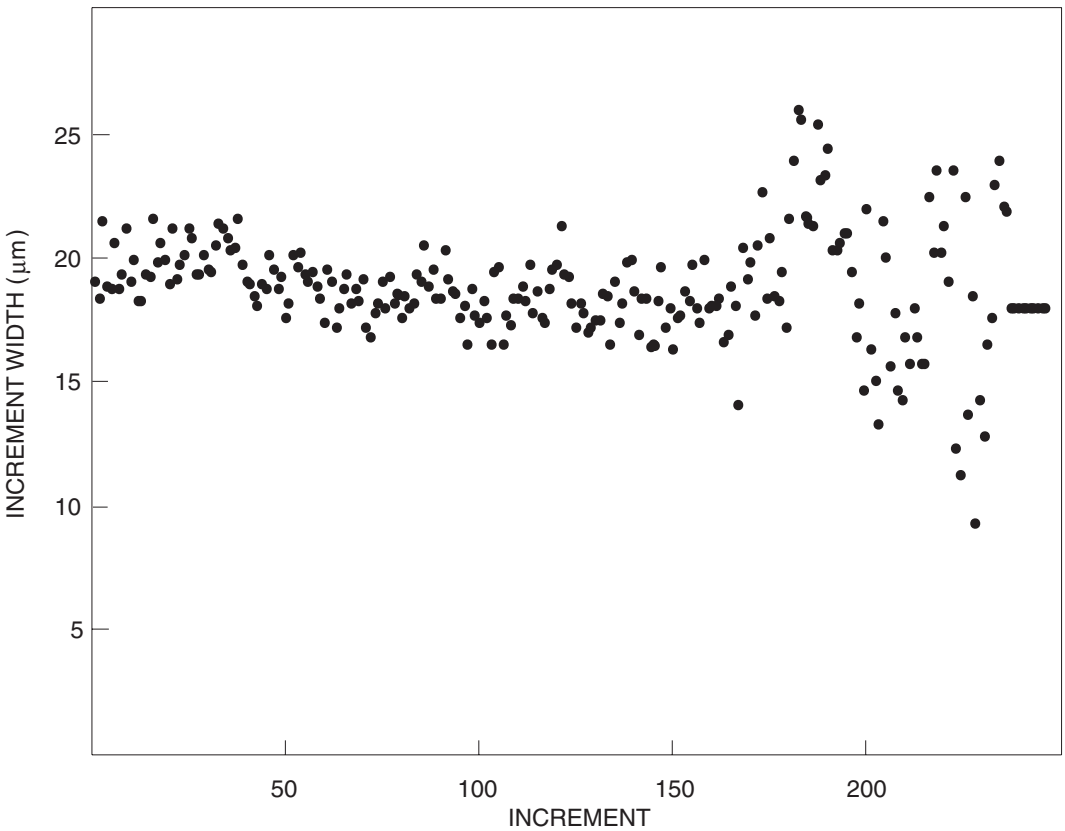

Fig. 4: Trend in increment width (average of each increment for the 25 specimens analysed) along the internal rostral axis of *Octopus vulgaris*

increment clarity should be the two criteria considered in the selection of axis process.

The bands observed along the *IRA* were more visible using the binocular microscope under oblique reflected light (Fig. 3b) than using SEM (Fig. 3c). Orientation of the specimen in the electron beam seems to be a key factor for visibility of the bands along the *IRA*, because increments are seen only when the beam of electrons bombards the beak surface with a minimum of 45°. Even when changing the angle and producing the signal by secondary and backscattered electrons, the eight samples analysed with SEM revealed only a few increments. These results are difficult to interpret because several factors may be involved, such as beak composition and/or a non-constant relief pattern of the bands throughout the section (some of the valleys seem to be orientated in oblique planes and not perpendicularly to the surface along the *IRA*). As pointed out by Robards (1978), if the specimen is non-conductive, the charge will eventually become so great that imaging will be impaired and specimen damage will ensue. During SEM examination of the beaks, some damage was observed in those exposed to a longer period of examination. The chitinous composition of the beaks (Nixon 1985, Mangold and Bidder 1989) may be the cause of the poor visibility of the bands under SEM. However, because longitudinal increments can be detected by SEM, it may be possible that the orientation of the valleys along the *IRA* is essential to viewing such bands under SEM.

Analysis of beak microstructure data

The average increment width was calculated and plotted against the number of increments (Fig. 4). Increment width is quite constant along the *IRA*, although the first 50 bands seem to be slightly thicker.

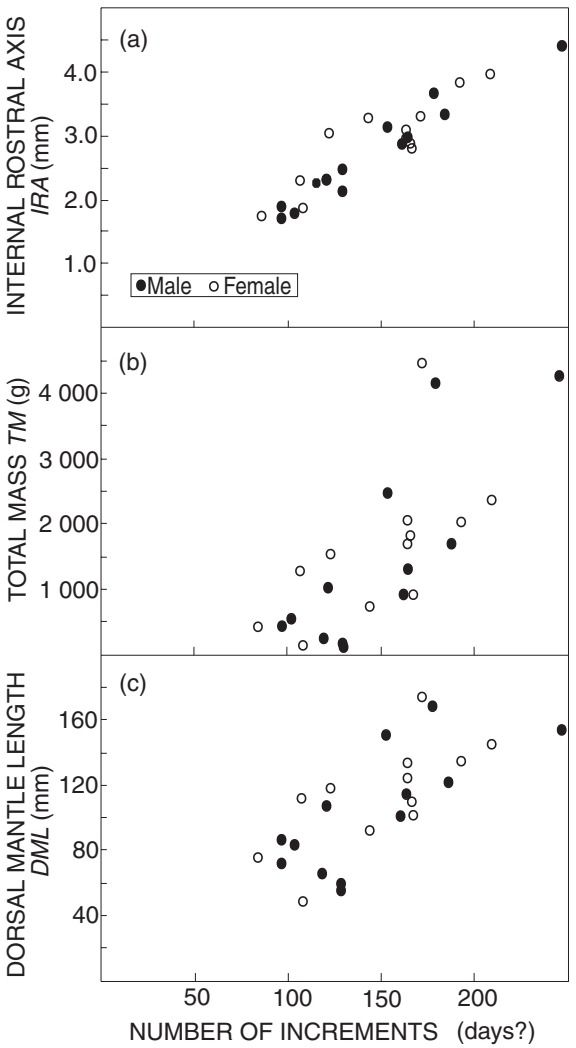

Fig. 5: Relationships between the number of increments counted in the beaks of *Octopus vulgaris* analysed and (a) internal rostral axis length, (b) total mass and (c) dorsal mantle length

There is a higher dispersion of the points from Increment 152, which is probably related to the relatively fewer beaks (<50%) with more than 152 bands. No age validation results are presented here, but the constant trend and appearance of the bands along the *IRA* may suggest a constant deposition, probably daily. In *Octopus vulgaris* and other shallow-water cephalopods, regular activity patterns and some evidence of endogenous rhythms induced by the light-dark cycle have been reported, from both field and laboratory animals (Cobb *et al.* 1995). This endogenous rhythm may be reflected on a chitinous structure such as the beaks.

In the sample analysed here, the octopus which revealed the fewest increments in the beak (84) was a 423.2 g *TM* female. A male of 4 302.3 g *TM* revealed the most bands (246). If these bands are laid down on a daily basis from hatching, and assuming an incubation time of two months for this species at a water temperature of 16°C (Nixon 1969), the results presented here are consistent with a lifespan of around 10–12 months, although no animals larger than 4 470.6 g *TM* were analysed. Smale and Buchan (1981) proposed a lifespan of 9–12 months in female and 12–15 months in male *Octopus vulgaris* from the South African coast, based on laboratory studies, although they found a large variation in individual growth rates in both males and females. Recent rearing of *O. vulgaris* from Senegal (Domain *et al.* 1997) has also shown large individual growth variation and exponential growth in mass, and suggests the same short lifespan of around 12–15 months for both males and females.

The relationship between the number of increments and the internal rostral axis for males and females separately is illustrated in Figure 5a. Taking into account the few animals analysed, it may be suggested that the *IRA* has positive allometric growth. Comparing the number of increments against total mass (Fig. 5b) and dorsal mantle length (Fig. 5c) revealed no sex-related differences. The trend also indicates a relationship between the number of increments and both animal mass and *DML*. Validation of deposition periodicity and the analysis of a larger sample, covering a larger size range, is needed to obtain the growth parameters of the species, but the positive relationships found here between age (number of increments) and both size and mass suggests that increments are laid down regularly during growth.

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