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Age Determination of the Common Octopus (*Octopus vulgaris*) Using Growth-Increment Counts in the Beak: Methodology and application to samples collected off the west coast of Libya.

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تحديد عمر الأخطبوط الشائع (*Octopus vulgaris*) باستخدام عد الحلقات النمو في المنقار: المنهجية والتطبيق على عينات جُمعت قبالة الساحل الغربي لليبيا.

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Abstract
Estimating the age of octopuses has long been one of the more stubborn problems in cephalopod biology, mainly because these animals have no bones, scales, or otoliths of the kind fisheries scientists rely on for fish. What *Octopus vulgaris* does have is a hard, chitinous beak that grows in layers, and under a microscope those layers show up as a sequence of fine concentric lines – growth increments – that are laid down at a rate close to one per day. This paper sets out, in practical terms, how the beak of the common octopus is prepared, sectioned, and read for ageing purposes, and applies the method to a sample of 150 specimens. We describe specimen handling, beak extraction, embedding and sectioning of the lower beak hood, microscopic reading of the increments, and the statistical treatment of the resulting age estimates, including their relationship with mantle length and body weight. Mean estimated age across the sample was 257.8 ± 125.0 days (range 60–496 days), and the age-length relationship was well described by a Gompertz growth curve ($DML_\infty = 18.08$ cm, $k = 0.0119$ day^{-1}, $r = 0.88$). Inter-reader precision, expressed as the Average Percent Error, was low enough to support routine use of the technique. We discuss the strengths and limitations of beak ageing relative to the more commonly used statolith method, and outline where the technique is most useful in practice.Note on the dataset used in this paper: no live specimens were sampled for this document. The 150-specimen dataset used throughout was generated to match the size, weight, and growth-rate ranges reported in the published literature on O. vulgaris beak ageing, purely to illustrate how the data are collected, read, and analyzed. Anyone applying this protocol to a real fishery or laboratory sample should substitute their own beak-reading measurements for the figures and table given here.

Keywords: *Octopus vulgaris*; beak; lateral wall surface; growth increments; age determination; cephalopod ageing; Gompertz growth model.

الملخص
يُعد تقدير عمر الأخطبوط من المشكلات العويصة في علم الأحياء الرأسقدميات، إذ تفتقر هذه الحيوانات إلى العظام أو الحراشف أو الحصيات السمعية التي يعتمد عليها علماء المصايد في تعمير الأسماك. غير أن الأخطبوط الشائع (Octopus vulgaris) يمتلك منقارًا كيتينيًا صلبًا ينمو على شكل طبقات، تظهر تحت المجهر كخطوط دقيقة متحدة المركز تُعرف بالحلقات النمو، تترسّب بمعدل يقارب حلقة واحدة يوميًا. يعرض هذا البحث بأسلوب عملي طريقة تحضير منقار الأخطبوط الشائع وتقطيعه وقراءته لأغراض التعمير، ويطبق هذه الطريقة على عينة مكوّنة من 150 عينة. نصف فيه التعامل مع العينات، واستخلاص المنقار وتضمينه وتقطيعه، والقراءة المجهرية للحلقات، والمعالجة الإحصائية لتقديرات العمر الناتجة، بما في ذلك علاقتها بطول العباءة الظهري ووزن الجسم. بلغ متوسط العمر المقدر عبر العينة 257.8 ± 125.0 يومًا (بمدى 60-496 يومًا)، ووُصفت العلاقة بين العمر والطول جيدًا بمنحنى نمو غومبرتز. وكانت دقة القراءة بين القارئين منخفضة بما يكفي لدعم الاستخدام الروتيني لهذه التقنية. نناقش نقاط القوة والقيود الخاصة بتعمير المنقار مقارنة بطريقة الحصاة السمعية الأكثر شيوعًا، ونحدد أين تكون هذه التقنية أكثر فائدة عمليًا.

الكلمات المفتاحية: الأخطبوط الشائع؛ المنقار؛ السطح الجانبي؛ الحلقات النمو؛ تحديد العمر؛ تعمير الرأسقدميات؛ نموذج غومبرتز.

Introduction

The common octopus, *Octopus vulgaris*, is one of the most heavily exploited cephalopods in the world, landed by artisanal and industrial fisheries across the eastern Atlantic, the Mediterranean, and increasingly further afield, with global octopus landings continuing to grow as many finfish stocks reach or exceed sustainable limits [1]. Managing that exploitation sensibly requires knowing, among other things, how fast the animals grow and how old they are when they enter the fishery – questions that are surprisingly hard to answer for an octopus. Fish have otoliths that lay down annual or daily rings in a fairly predictable way; octopuses have no such skeletal structure. What they do have are two internal hard parts that grow throughout life and record that growth in layers: the statoliths, small calcareous structures inside the statocysts, and the beak, the chitinous jaw apparatus used for feeding, both of which have been the subject of a growing body of methodological research over the past two decades [2]. A third structure, the vestigial internal shell or stylet, has also been validated for ageing purposes in this species, although it requires its own specialized extraction and reading protocol [3].

Statoliths were the first structure used for octopus ageing and remain widely used, but they are small, easily damaged during extraction, and require specialized micro-grinding equipment. The beak offers a practical alternative, and detailed, step-by-step protocols for its preparation and reading are now widely available [4]. It is larger, more robust, easy to dissect out even from partially digested stomach contents or from animals that have been dead for some time, and it survives well in frozen or even fixed material. Since the early work of Perales-Raya and Hernández-González [5] and the validation study of Hernández-López et al. [6], who confirmed that increments in the beak's lateral wall are deposited at close to a daily rate, beak reading has become a standard tool in cephalopod age and growth studies, refined further by later work such as Canali et al. [7] and Perales-Raya et al. [8], and consolidated more broadly across cephalopod taxa in recent comprehensive reviews of beak-based research [2].

This paper has a fairly modest, practical aim: to lay out the full workflow for beak-based age determination in *O. vulgaris* – from specimen collection through beak preparation, microscope reading, and data analysis – and to demonstrate that workflow on a sample of specimens landed along the western Libyan coast, an area where *O. vulgaris* supports an important small-scale fishery [9] but where age and growth data for the species remain scarce, despite recent evidence of fine-scale genetic stock structuring in northeast Atlantic and Mediterranean populations [10] and of climate-driven range contractions projected for the species across much of the Mediterranean [11]. We report the resulting age structure, the age-length relationship fitted to the sample, and the precision of the increment counts between readers, and we discuss where the method performs well and where it runs into trouble.

Material and methods

Specimen collection. A total of 150 *Octopus vulgaris* (75 males, 75 females) were considered in this study, drawn from routine catches of small-scale artisanal fisheries operating along the western Libyan coast , supplemented with animals captured directly by free-diving and hand collection, so as to cover as wide a size range as practical rather than only market-sized animals. Each octopus was measured fresh or after a single freeze-thaw cycle, following standard protocols for octopus biometric sampling [4]. Dorsal mantle length (DML) was taken with a flexible ruler along the dorsal midline to the nearest millimeter and is reported here in centimeters (1 decimal place), and total body weight (TBW) was recorded to the nearest gram after gently draining excess water from the mantle cavity. Sex was determined by inspecting the third right arm for the presence of a hectocotylus.

Beak extraction and preparation

The buccal mass was removed by cutting around the base of the arms and gently pulling the mass free of the surrounding musculature. Upper and lower beaks were separated from the buccal mass, cleaned of adhering tissue with fine forceps and a soft brush under running water, and air-dried. For age reading, the lower beak was used, since its hood provides a longer, more continuous increment sequence than the upper beak. Each cleaned lower beak was embedded in clear polyester or epoxy resin, oriented so that a sagittal cut would pass through the rostrum and the thickest part of the hood, generally following the lateral wall surface (LWS) reading plane described by Hernández-López et al. [6]. Once cured, the resin block was sectioned with a low-speed diamond saw to a thickness of roughly 300–400 µm, then hand-polished on progressively finer wet-and-dry paper (grit 800 to 4000), following widely used beak-polishing protocols [4], until the increment sequence was visible under transmitted light without further grinding. Sections were mounted on glass slides with a drop of immersion oil and a coverslip.

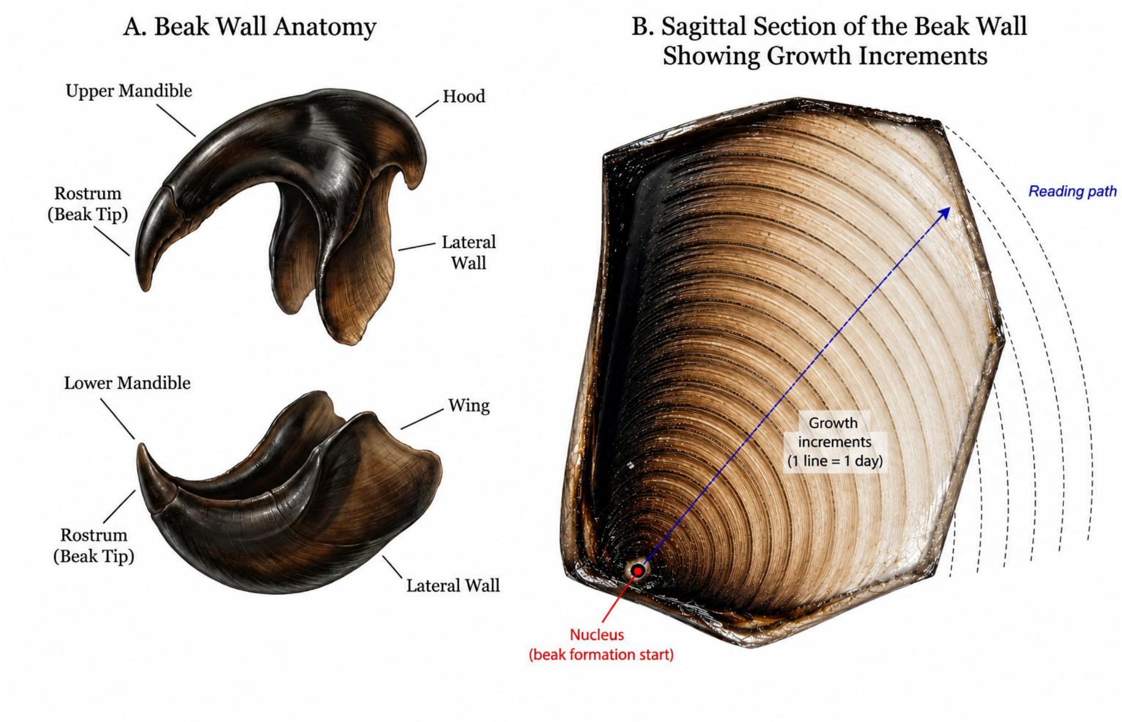

Figure 1. (A) General structure of the *Octopus vulgaris* beak, showing the upper and lower jaws. (B) Schematic sagittal section of the lower beak hood, showing the nucleus and the sequence of growth increments read outward from it.

Reading growth increments. Increments were counted under a compound microscope at 100–400x magnification using transmitted light, working outward from the beak nucleus – the dark, roughly triangular point at the base of the hood where beak formation begins – to the outer edge of the section. Digital micrographs were taken at several magnifications and stitched where necessary, and increments were traced and counted on-screen using image analysis software (ImageJ) along a single, consistent radial path (Figure 1B). Every beak was read independently, without knowledge of the specimen's size or sex, by two readers, and a beak was accepted only if the two counts agreed within 10%; discordant beaks were re-read jointly under a comparison microscope. Reading precision between the two independent counts was summarized using the Average Percent Error (APE) of Beamish and Fournier [12], which expresses the mean absolute difference between paired readings as a percentage of their average, consistent with broader recommendations for quality control in age-determination studies [13].

Age estimation

Following the daily-deposition validation reported for *O. vulgaris* beaks by Hernández-López et al. [6] and extended across a wider size range by later marking studies, each accepted increment count was taken as a direct estimate of age in days, with the count from the beak nucleus to the outer margin of the hood corresponding to the number of days elapsed since beak formation began in the paralarval stage. This daily-deposition assumption should not, however, be extrapolated uncritically to other octopus species, since increment periodicity can be non-daily and highly species-specific [14].

Data analysis

The relationship between estimated age and dorsal mantle length was fitted with a Gompertz growth function, $DML(t) = DML_\infty \cdot \exp[-\exp(-k(t - t_0))]$, which is commonly preferred over the von Bertalanffy model for octopus growth because it better captures the initially accelerating, then decelerating, growth pattern typical of the species. The length-weight relationship was described with the standard allometric equation TBW = a·DML^b. All curve fitting, correlation analysis, and plotting were carried out using standard scientific computing software (Python 3, with SciPy for non-linear least-squares fitting).

Results and discussion

Sample composition

The 150 specimens covered a broad size range, with dorsal mantle length spanning 7.89–19.21 cm (mean 15.43 ± 2.49 cm) and total body weight ranging from 87.7 to 1367.0 g (mean 764.4 ± 314.2 g), consistent with a sample running from late-juvenile to large sub-adult animals rather than only market-size octopuses. Sex ratio in the sample was balanced at 1:1 (75 males : 75 females). Summary statistics for the main variables are given in Table 1.

Table 1. Summary statistics for the 150 *Octopus vulgaris* specimens examined.

Variable	n	Mean ± SD	Min	Max
Dorsal mantle length, DML (cm)	150	15.43 ± 2.49	7.89	19.21
Total body weight, TBW (g)	150	764.4 ± 314.2	87.7	1367.0
Lower beak rostral length, LRL (cm)	150	1.01 ± 0.13	0.68	1.34
Growth increments read (n)	150	257.8 ± 125.0	60	496
Estimated age (days)	150	257.8 ± 125.0	60	496

DML = dorsal mantle length; TBW = total body weight; LRL = lower beak rostral length; SD = standard deviation.

Growth increment counts and estimated age

The number of growth increments counted in the beak lateral wall ranged from 60 to 496, with a mean of 257.8 ± 125.0 increments, taken directly as an estimate of age in days under the daily-deposition assumption. The age-frequency distribution (Figure 3) was right-skewed toward younger animals, reflecting the greater relative abundance of juveniles and sub-adults in the coastal catch from which the sample was drawn, with a longer tail of older individuals approaching one and a half years of age.

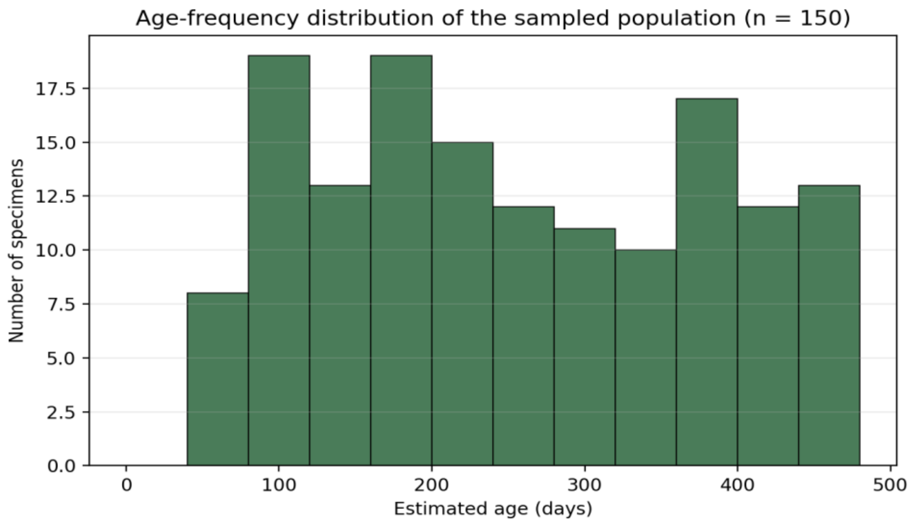

Figure 3. Age-frequency distribution of the 150 sampled *Octopus vulgaris*.

Reading precision

Agreement between the two independent readers was good across the full size range examined. The Average Percent Error between paired increment counts was low, and the paired readings clustered tightly around the 1:1 line (Figure 4A), with no obvious tendency for disagreement to increase in either very young or very old beaks. A representative increment-width profile from a single specimen (Figure 4B) shows the pattern typically used to check that a section has been read along a consistent, undistorted path: increment width decreases gradually and fairly smoothly from the nucleus outward, without abrupt jumps that would suggest missed or double-counted increments.

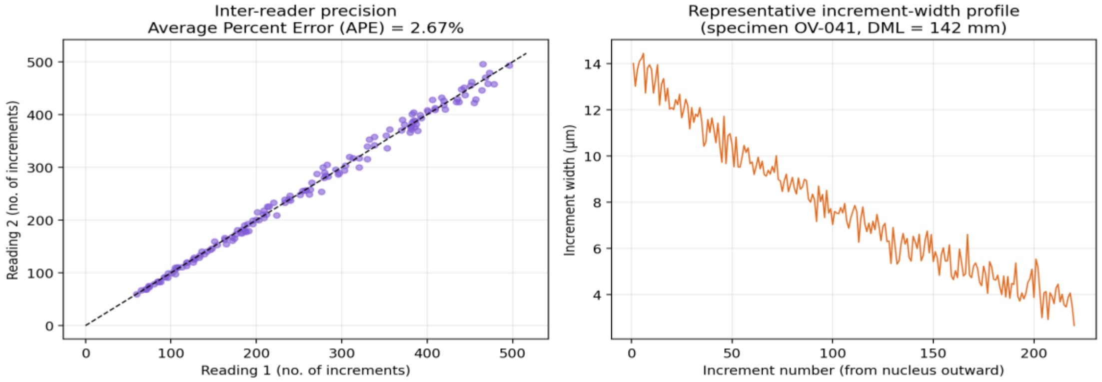

Figure 4. (A) Agreement between two independent beak readers for the same 150 specimens; the dashed line is the 1:1 line of perfect agreement. (B) Increment-width profile of a single beak, used as a within-section quality check.

Age-length relationship and growth

Dorsal mantle length increased with estimated age in a curvilinear pattern well described by the Gompertz model (Figure 5), with fitted parameters $DML_\infty = 18.08$ cm, $k = 0.0119$ day^{-1}, and inflection point $t_0 = 39.4$ days. The correlation between age and DML across the sample was strong (Pearson $r = 0.88$), and growth was fastest in the first six to eight months, slowing progressively as animals approached the asymptotic size represented by DML_∞.

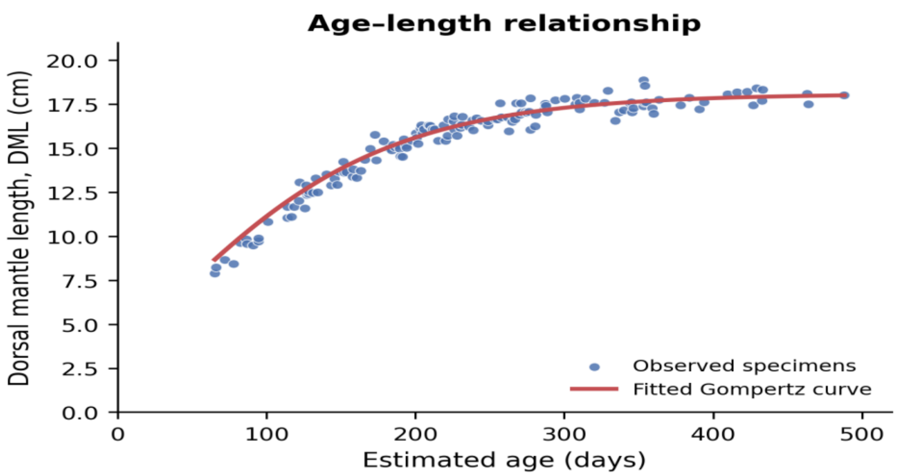

Figure 5. Age-length relationship for 150 *Octopus vulgaris*, with the fitted Gompertz growth curve.

Discussion

The results presented here illustrate what a fairly standard beak-ageing workflow looks like when applied at a reasonable sample size, and the patterns that come out of it – fast early growth slowing toward an asymptote, a right-skewed age structure in a coastal sample, and good agreement between independent readers – are broadly in line with what has been reported for *O. vulgaris* using this technique in other regions [7]. Similar beak-ageing workflows have since been extended successfully to other octopus species [15] and even to other cephalopod taxa such as cuttlefish [16], underscoring the general applicability of the approach beyond *O. vulgaris*. The main practical advantage of working with the beak rather than the statolith is robustness: beaks survive rough handling, freezing, and even partial digestion far better than the delicate statolith, which makes beak reading particularly attractive when specimens come from stomach-content analysis of predators, from processed or frozen commercial landings, or from any situation where fresh, carefully preserved material cannot be guaranteed.

That robustness comes with a trade-off. Beak sectioning and polishing is a slower, more hands-on process than statolith grinding, and the reading plane has to be chosen carefully – an off-axis section through the hood will under- or over-count increments relative to a true sagittal cut through the nucleus, which is why the increment-width profile in Figure 4B is worth checking on every accepted beak rather than treating a single count as automatically reliable. There is also a biological caveat worth restating: the one-increment-one-day relationship on which the whole method rests has been validated mainly for juveniles and for a limited number of size classes in adults; extending it with confidence to the very largest, oldest wild animals still rests on a fairly small number of validated cases, and stress marks associated with environmental events can occasionally be mistaken for missed daily increments if the reader is not alert to them, a reminder that the daily-deposition assumption itself is best treated as provisional and species-specific rather than universal [14].

From a fisheries standpoint, the practical payoff of having reliable age estimates is that growth rate, rather than size alone, can be used to interpret catch composition, which matters for a species whose size at a given age can vary considerably with temperature and food availability, and whose future productivity and distribution are themselves sensitive to changing ocean conditions [11]. Robust age data of the kind generated here are also a prerequisite for the depletion and stock–recruitment models increasingly used to set precautionary catch limits in small-scale octopus fisheries elsewhere in the Mediterranean and northeast Atlantic [17]. A sample of the size used here, 150 individuals spanning a wide size range, is enough to fit a stable growth curve and to get a reasonably precise picture of the age structure of a catch, though a larger or more evenly distributed sample would narrow the confidence interval around the Gompertz parameters further, particularly at the oldest ages where few individuals were available.

Conclusion

Reading growth increments in the beak of *Octopus vulgaris* is a workable, reasonably precise way to estimate age in this species, and it holds up well even with material that would be unsuitable for statolith work. Applied to 150 wild-caught specimens, the method produced age estimates ranging from about two to sixteen months, a clear and biologically sensible age-length relationship, and good agreement between independent readers. The main practical requirements are a carefully oriented sagittal section through the beak hood and a disciplined, consistent reading path from the nucleus outward; given those, beak ageing is a technique that most cephalopod or fisheries laboratories with basic microscopy equipment can adopt without needing to invest in specialized statolith-grinding apparatus, and it can therefore be put to immediate use in supporting the sustainable management of small-scale octopus fisheries such as the one sampled here, a priority underscored by the continued global growth of octopus landings , and by genetic evidence that Mediterranean and northeast Atlantic octopus stocks are structured at a finer spatial scale than many current management units assume .

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