Ageing octopods from stylets: development of a technique for permanent preparations

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Previous attempts at ageing octopods from stylets have relied on preparations that deteriorate with time. Some techniques require an immediate photographic record, others allow real-time enumeration but do not provide a permanent archive. A technique is described that produces permanent and archivable preparations of octopod stylets. Stylets were dehydrated in ethanol and infiltrated with a low-viscosity resin. Subsequent polymerization of the resin allowed the embedded stylet to be ground and polished to reveal the stylet microstructure. This comprised increments that are probably suitable for age estimation. The technique was developed using stylets of *Octopus vulgaris* and *Eledone cirrhosa*. Increments were composed of light and dark bands and were clearly defined at ×400 and at ×625 magnifications. The number of increments ranged from 189 to 399. The stylets of a deep-sea species (*Bathypolypus sponsalis*) and an Antarctic species (*Megaleledone setebos*) were also examined. Each appeared to have growth increments, despite the perception that the environments they inhabited may not provide daily cues. Using the technique developed, the pre-hatch nucleus was seldom well defined, as reported for *O. pallidus*, stylets of which were prepared using a non-permanent method. Reasons for this are discussed. The microstructure clarity revealed is probably associated with the ultra-low viscosity of the resin used.

Keywords: age, growth increments, octopus, statolith, stylet.

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Introduction

To understand the population dynamics, life history, and biology of a species, it is important to be able to determine the age of an animal accurately. In cephalopods, several methods have been used to estimate age and growth rates indirectly through length frequency analyses, or directly through laboratory culture, counting of periodic growth increments in hard structures, and tag and recapture studies (Sifner, 2008).

By far the most common and successful method for estimating age and growth in cephalopods is through the counting of periodic growth increments in hard structures (e.g. *Sepioteuthis lessoniana*; Jackson *et al.*, 1993). Almost all hard parts of cephalopods contain regular growth increments in their microstructure. Examples are the squid gladius or pen (e.g. *Moroteuthis robusta*; Bizikov and Arkhipkin, 1997), cuttlefish cuttlebone or sepion (e.g. *Sepia officinalis*; Bettencourt and Guerra, 2001), and octopus vestigial shell or stylet (e.g. *Octopus pallidus*; Doubleday *et al.*, 2006), beak (e.g. *Octopus vulgaris*; Raya and Hernández-González, 1998), eye lens (e.g. *Eledone cirrhosa*; Boyle, 1983), and statolith (e.g. *Idiosepius pygmaeus*; Jackson, 1989).

Statoliths are routinely used to estimate the age of squid and cuttlefish and are considered analogous in their function and microstructure composition to fish otoliths (Campana and Neilson, 1985). Growth increments laid down daily have now been validated in the statoliths of a range of species from temperate (e.g. *Sepia officinalis*; Bettencourt and Guerra, 2001), subtropical (e.g. *Lolliguncula brevis*; Jackson *et al.*, 1997), and tropical regions (e.g. *Idiosepius pygmaeus*; Jackson, 1989). Validation is achieved either by hatching and raising individuals in culture (known age) or by chemically marking the statoliths (e.g. with oxytetracycline) of wild-caught animals, which are subsequently held in captivity (e.g. *Alloteuthis subulata*; Lipiński, 1986) or released and recaptured after a known period (e.g. *Loligo reynaudii*; Lipiński *et al.*, 1998). The microstructure of squid statoliths may also provide a wealth of other ecological information, such as hatching date, ontogenetic shifts, lunar periodicity in activity (i.e. second-order bands), stressful events such as mating (i.e. stress- or checkmarks), and prevailing environmental conditions (e.g. the statolith elemental or chemical composition; Arkhipkin, 2005). Few studies have used the eye lens, gladius, or sepion, but growth increments in the gladius appear to be deposited subdaily in young and daily in older *Sepioteuthis lessoniana* (Jackson *et al.*, 1993), whereas the deposition rate of growth increments in the sepion appears to vary with temperature in *Sepia officinalis* (Bettencourt and Guerra, 2001).

Efforts to estimate the age of octopuses were initially largely unsuccessful. For example, the statoliths of octopuses are soft and chalk-like, and appear to lack growth increments (e.g. *Octopus dofleini*; Robinson and Hartwick, 1986). However, several recent studies have revealed growth increments with an assumed daily periodicity in the microstructure of the beak (Raya and Hernández-González, 1998; Hernández-López *et al.*,

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2001) and in the stylets (vestigial shell; Sousa Reis and Fernandes, 2002) of *Octopus vulgaris*. The daily periodicity of growth increments in the stylet microstructure of *Octopus pallidus* has now been validated (Doubleday *et al*., 2006), and stylet increment analysis has been shown to be a feasible method of estimating octopus age and growth (Leporati *et al*., 2007). The current methods require stylets to be ground, polished, viewed, and photographed within 5 min, because the sections dry out, crack, and become unreadable after that (Leporati *et al*., 2007). Although Sousa Reis and Fernandes (2002) prepared stylet sections that lasted at least several days, they were mounted in a non-permanent mounting medium, such as Aqua-Mount, and the techniques for preparing permanent and archivable preparations of stylets have not yet been developed. Techniques that produce sections that do not deteriorate with time would probably decrease the number of repeat sections required to obtain the age of an octopus and reduce the discard rate (see Leporati *et al*., 2007). Such permanent sections could also be archived and would facilitate recounting, allowing researchers to address pertinent questions of precision and bias. More importantly, detailed interpretation of growth increments relies heavily on the ability to change the focus and depth of field. This is not possible with a digital photographic archive. Permanent preparations would also allow researchers to use image analysis techniques with overlays to measure and count increments at various magnifications live on screen, probably leading to more accurate readings. Given that the information derived from age-determination studies is critical in developing fisheries management tools, and that octopod species are widely harvested, methodologies that yield permanent stylet preparations, rather than a two-dimensional photographic archive, are essential. We describe a method to prepare octopod stylets that allows them to be stored permanently on microscope slides.

Material and methods

Dissection and dehydration

Our specimens of four species of octopod, *Octopus vulgaris*, *Eledone cirrhosa*, *Bathypolypus sponsalis*, and *Megaleledone setebos*, had either been frozen and defrosted or were fixed in 4% formalin. Stylets were carefully dissected, then stored in 70% ethanol. A small section of stylet (~1–2 mm long) was cut from the postrostral region using a razor blade (see Doubleday *et al*., 2006). Stylet sections were dehydrated in 90% ethanol, then in absolute ethanol, for 1 h each. For very large stylets (e.g. from *M. setebos*), longer periods were used (several hours in 90% ethanol, and overnight in absolute ethanol).

Embedding

Dehydrated stylet sections were embedded in LR White™ (London Resin Company, Reading, UK), a low-viscosity acrylic resin, following the manufacturer's instructions. Briefly, ethanol was allowed to evaporate from the stylet pieces, then these were placed in glass vials containing LR White™ resin solution (~2–3 ml) for 24 h. For larger stylets, the solution was changed once after 24 h, and then left for another 24 h. Following infiltration, the stylet pieces were removed from the solution and placed upright on the inside-lid surface of a prepared conical capsule 10 mm in diameter (BEEM™, West Chester, USA; Figure 1). The capsules were prepared by removing the conical base using a scalpel to leave just the cylindrical part and lid as a base. Approximately 10 ml of fresh LR White™ resin was polymerized using a single drop of LR White™ UV accelerator solution. While still liquid, this was added to the capsule until the stylet piece was at least covered completely. The capsule was placed in a refrigerator for 1–2 h while the resin hardened. Cooling is essential to prevent an excessive rise in temperature attributable to the polymerization reaction: the temperature increase can be detrimental to stylet microstructure. Once polymerization was complete, the cylindrical block of resin containing the embedded portion of stylet was extruded from the capsule and affixed with a cyanoacrylate-based fast-acting adhesive to the centre of a clean microscope slide, with the cut surface of the stylet touching the slide (Figure 1).

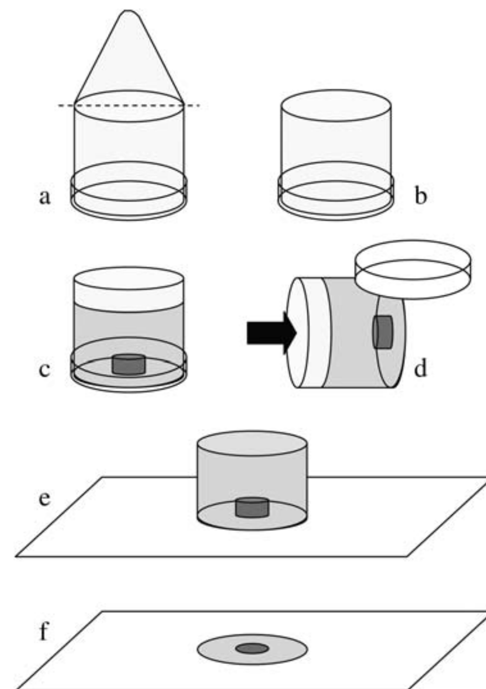

Figure 1. Stylet preparation. (a, b) The conical part of a 10 mm diameter BEEM™ capsule is cut-off (dashed line); (c) the stylet piece is placed in an upright position on the inside capsule lid and covered in liquid LR White™ resin and polymerized; (d) the hardened resin block is pushed out of the capsule; (e) the resin block is affixed to a microscope slide; (f) the resin block is ground until a thin stylet section remains, then the stylet surface is polished.

Grinding and polishing

The resin block was ground down using a Rotacraft™ (Shesto Ltd, Willesden, UK) RC12 variable speed hand tool with sandpaper grinder attachment, to remove most of the excess resin. Once the stylet surface emerged, the resin and the stylet were ground and polished by hand sequentially using 1200-grade carborundum fine sandpaper on a flat surface, 12 μm lapping film, and finally wet felt impregnated with 0.05 μm aluminium oxide (Al_2O_3) powder. Slides were intermittently rinsed and dried to facilitate visual checks under a light microscope (×100 and ×400 magnifications). These ensured that stylet sections were sufficiently transparent and that growth increments were visible and not being polished out.

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Visualization and counting of growth increments

Stylet microstructure and growth increments were observed at ×400 or ×625 magnifications, using a Leica Dialux 20 binocular transmitted-light microscope. Growth increments were best viewed under full light power, with the condenser adjusted to optimize brightness and contrast. Digital images of whole stylets and growth increments were captured with a Nikon Coolpix 4500 camera. At greater magnification, series of images were captured working outwards from the stylet centre. Digital images were processed using a desktop PC and the automatic contrast function in Adobe Photoshop Elements 4.0 software. Processed images were printed on a Minolta Magicolor desk laser jet printer. Growth increments were marked and counted by hand. Two non-consecutive counts were made using duplicate printouts, and the number of growth increments reported here is the mean of these two counts. If the two counts differed by more than 10%, the stylet was designated unreadable and discarded. The results from those stylets are not included in the results below, apart from a brief report on discard rate.

Mean growth increment width was calculated by dividing the number of growth increments across a short distance (~20 μm) by that distance (as measured with a calibrated eyepiece graticule). Growth increment width, whenever possible, was estimated close to the innermost as well as the mid- and outermost regions of the stylets.

Table 1. Species, mantle length (ML), and mean number of growth increments for all octopus specimens whose stylets were processed using LR White™ resin.

Species	ML (mm)	Mean number of growth increments after two counts	
		Left	Right
Bathypolypus sponsalis	29		68
	29		129
	36		106
	52	193	
	55	158	158
	58		171
	62	186	202
	68	207	
	38		141
	40		148
	52		184
	54		293
	56	278	
	57		211
	58		330
	65		220
Eledone cirrhosa	129	303	348
	152	385	399
Megaleledone setebos	55	167	
	102	351	383
	89	417	398
	190	1 077	
Octopus vulgaris		228	238
		189	191

Results

The discard rate was low. No stylets of either *O. vulgaris* or *E. cirrhosa* were discarded, and for both, four were read. Six stylets of *B. sponsalis* were discarded, 18 read, and 5 stylets of *M. setebos* were discarded, with 6 read. Only three octopods failed to yield at least one good stylet, and two of these cases pertained to *M. setebos*. Large specimens of this species have a very large number of increments in their stylets (see below and Table 1), making counting more challenging. Additionally, a few stylets of *B. sponsalis* were so small that it was not possible to orientate them vertically during the embedding process.

Stylet microstructure

Octopus vulgaris

Transverse sections of *O. vulgaris* stylets ($n = 2$) sometimes appeared to be cracked, although this did not prevent enumeration of the increments. Three reasonably well-defined regions were apparent, which possibly correspond to growth in (i) the embryo, (ii) the planktonic phase, and (iii) the benthic phase (Figure 2a). Growth increments were clearly defined even at ×625 magnification (Figure 2b). Some structure was apparent in the innermost region of the stylet (particularly at the periphery of the innermost region), but it was not possible to enumerate growth rings there. Growth increments (consisting of both a light and a dark band) were therefore counted in the outer two regions only. There was consistency between counts of left and right stylets: in the first specimen, these were 189 and 191, and in the second, 228 and 238. Darker growth increments, possibly representing stress checks, were also visible in the stylet microstructure (Figure 2b). In the outer region of one of these stylets, they appeared at fairly regular intervals, but they were less prevalent in the second stylet of this animal. The mean (±s.d.) width of the growth increments (in μm) in the innermost, mid-, and outermost regions was 2.13 ± 0.25, 1.91 ± 0.22, and 1.74 ± 0.09, respectively.

Eledone cirrhosa

Viewed at ×125 magnification, transverse sections of *E. cirrhosa* stylets ($n = 2$) were clearly divided into two regions. The inner region possibly represents the embryonic growth or all growth prior to the benthic phase (Figure 2c). At high magnification, a second faint check mark was sometimes visible (Figure 2d). Growth increments were less clear in the inner region, and were noticeably fainter towards the centre and periphery of the outer region (Figure 2d). Towards the stylet edge, growth increments became harder to discern. Dark bands covering several growth increments were also visible, perhaps indicating a change in stylet composition or ontogenetic development. Counts of growth increments were made in the outer region, because the rings were less discernible in the inner region. Counts of left and right stylets were reasonably consistent at 303 and 348 in the first specimen (mantle length, ML, 129 mm), and 385 and 399 in the second (ML 152 mm). Mean (±s.d.) widths of growth increments (in μm) towards the innermost, mid-, and outermost regions of the four stylets were 1.78 ± 0.15, 1.75 ± 0.01, and 1.69 ± 0.05, respectively.

Bathypolypus sponsalis

A single check mark was apparent in some stylets of *B. sponsalis* ($n = 16$; Figure 2e). There seemed to be no other distinctive regions or even stress checks. Growth increments were faint, but regularly spaced throughout the entire stylet microstructure, and

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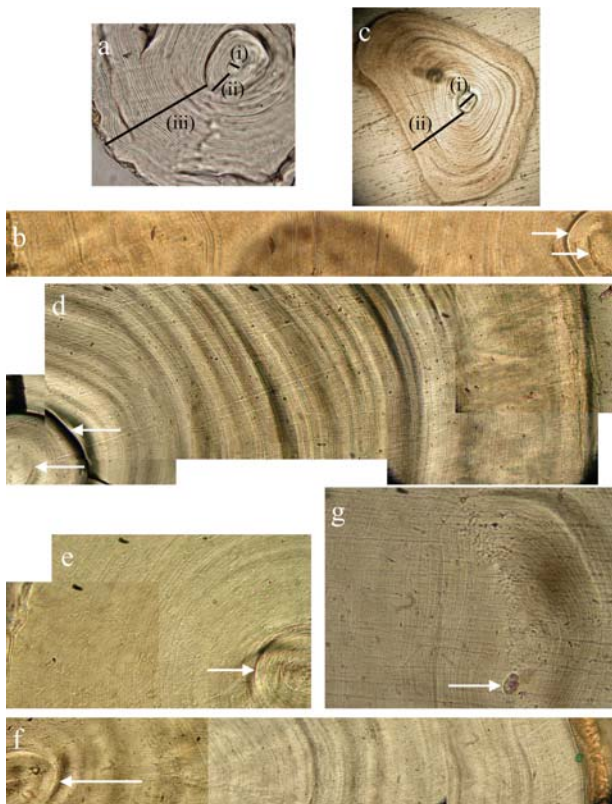

Figure 2. (a) Transverse section of an *Octopus vulgaris* stylet at ×125 magnification showing regions that may correspond to growth in (i) the embryo, (ii) the planktonic phase, and (iii) the benthic phase. (b) Growth increments of an *Octopus vulgaris* stylet at ×625 magnification. Arrows indicate checkmarks potentially referable to hatching and settling. The image is a collage of overlapping fields of view. (c) Transverse section of an *Eledone cirrhosa* stylet at ×125 magnification showing (i) an inner and (ii) an outer region. (d) Growth increments of an *Eledone cirrhosa* stylet as seen at ×400 magnification. Arrows indicate a potential additional inner checkmark as well as the more evident outer checkmark. The image is a collage of overlapping fields of view. (e) Transverse section of a *Bathypolypus sponsalis* stylet (ML 62 mm) showing growth increments at ×400 magnification. The arrow indicates a single checkmark. The image is a collage of overlapping fields of view. (f) Microstructure of a *Megaleledone setebos* (ML 102 mm) stylet at ×400 magnification. The image is a collage of overlapping fields of view, and the arrow points to an early checkmark. (g) Transverse section of a *Megaleledone setebos* stylet at ×400 magnification. The arrow indicates the darkened inner region.

the number of growth increments was enumerated from the centre in all cases. The number of growth increments (#GI) ranged from 68 to 330, and increased significantly with increasing ML (#GI = 3.8478 ML − 5.3691; $p = 0.003$; $r^2 = 0.4739$). In two specimens, counts of left and right stylet were compared, and revealed reasonable consistency (Table 1). Mean width (±s.d.) of the growth increments (in mm) towards the innermost, mid-, and outermost regions of 18 stylets was 1.80 ± 0.13, 1.80 ± 0.12, and 1.76 ± 0.15, respectively.

Megaleledone setebos

Stylets in this species ($n = 4$) were very large (up to 4.7 mm in diameter) in the biggest individuals. The growth increments were often faint, particularly towards the centre, but they continued to the very centre of the stylet. As in *B. sponsalis*, a single checkmark was apparent in some stylets (Figure 2f). Growth increments were enumerated from the centre. The number of growth increments ranged from 167 to 1077. Mean (±s.d.) growth increment widths (in mm) towards the mid- and outermost regions of the stylets were 1.71 ± 0.05 and 1.72 ± 0.15, respectively. A single stylet had a darkened inner region (Figure 2g), showing some resemblance to the pre-hatch nucleus described by other authors, but in that particular case, we believe it to have resulted from the failure of the resin to penetrate the entire stylet.

Discussion

The general appearance of the stylet microstructure, number of growth increments, and growth increment width in *O. vulgaris* and *E. cirrhosa* suggest that growth increments are laid down daily, as described previously for octopod stylets (e.g. Doubleday *et al.*, 2006), squid statoliths (e.g. Jackson *et al.*, 1993), and fish otoliths (e.g. Campana and Neilson, 1985). Moreover, the numbers of growth increments are congruent with a maximum estimated lifespan of 24 months in both *O. vulgaris* from the Atlantic (Mangold, 1983) and *E. cirrhosa* from the North Sea (Boyle *et al.*, 1988).

The *O. vulgaris* and *E. cirrhosa* were purchased from the local fishmarket, the former eviscerated and reported to be from Portugal and the latter from UK waters. All were frozen. Sousa Reis and Fernandes (2002) reported that freezing caused cracks between concentric growth increments. Cracking was a much greater problem with *O. vulgaris* and *E. cirrhosa* stylets in this study than with *B. sponsalis* and *M. setebos* stylets, both of which had been preserved in formalin, reinforcing previous conclusions about freezing.

Generally, the clarity of the increments using this technique was excellent. During development of the technique, other methodologies were trialled. We found two factors to be key in the production of clear, non-deteriorating, permanent preparations. The first was that temperature needs to be kept low during the curing process of the resin; higher temperature caused the stylet to crack and darken, rendering enumeration of growth increments impossible. The second was that resins with greater viscosity did not penetrate the stylet fully. This happened just once using the LR White^{TM} resin (Figure 2g), noticeably in the species with the largest stylets, *M. setebos*, where the distance the resin had to penetrate was greatest. We suggest that it is the ultra-low viscosity of the LR White^{TM} resin, facilitating its penetration to the very centre of the stylet, and its ability to polymerize at low temperatures, that makes the technique described here successful.

Using the technique described, the microstructure of the inner part of the stylet appeared to differ between species. Stylets of both *O. vulgaris* and *E. cirrhosa* appeared to have a clear checkmark close to the centre; other authors have interpreted checkmarks as marking the boundaries of life history stages. The innermost area has been taken generally to represent the embryonic stage (Sousa Reis and Fernandes, 2002; Doubleday *et al.*, 2006). However, the checkmarks in this study do not resemble those found by other authors, the most notable difference being that we discerned faint increments in the central region, whereas other studies found these checkmarks defining the outer edge of a region apparently lacking concentric structure.

Doubleday *et al.* (2006), who found no growth increments in the pre-hatch nucleus, validated the biological provenance of

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that structure by examining the stylets of day-old hatchlings. It seems unlikely that the large-egged species, *B. sponsalis* and *M. setebos*, would hatch without stylets. It was possible to enumerate growth increments from the centre in both these species, so it is likely that the inner region of their stylets corresponds to embryonic development. Either embryonic growth increments are clearer in these species, or our embedding technique better reveals them. We suspect that where checkmarks were visible in *B. sponsalis* and *M. setebos* (Figures 2e and f), they correspond to the edge of the pre-hatch nucleus seen in *O. pallidus* using the technique of Doubleday *et al*. (2006). The double checkmarks seen in stylets of *O. vulgaris* (which has a planktonic stage and therefore a potential change in growth; Figures 2a and b) support this notion. *E. cirrhosa* is more of an enigma. A second faint checkmark was sometimes discernible under high magnification (Figure 2d), but our understanding of the life history of this species is so poor that it is impossible to draw firm conclusions.

Naturally, it is necessary to validate that growth increments are laid down daily. This should be reasonably straightforward for *O. vulgaris* and *E. cirrhosa*, because they are regularly captured and can be kept in captivity. It will be much more difficult for deep-sea and Antarctic species, however, but it is still of note that growth increments are discernible in both these species despite the apparent absence of daily cues. The daily deposition of growth increments in statoliths and stylets has been shown in conditions of constant light (e.g. *Sepioteuthis lessoniana*; Jackson *et al*., 1993) and varying temperature (e.g. *Sepia officinalis*; Bettencourt and Guerra, 2001, and *O. pallidus*; Doubleday *et al*., 2006), suggesting deposition to be derived endogenously. Endogenously driven circadian rhythms of behavioural activity have also been observed in both *O. vulgaris* (Meisel *et al*., 2003) and *E. cirrhosa* (Cobb *et al*., 1995). Interestingly, daily growth increments in fish otoliths have also been validated for *Trematomus newnesi* from Antarctica (Radtke *et al*., 1989), and the deposition of growth increments in the microstructure of fish also appears to be under endogenous control (Campana and Neilson, 1985).

Although it might be premature to assume that the increments are daily in *B. sponsalis* and *M. setebos*, it is possible to speculate on the lifespan of these animals, should they be found to be so. Assuming daily deposition of growth increments, it would appear that all *B. sponsalis* examined were <1 year old and that the largest *M. setebos* was between 3 and 4 years old (potentially including embryonic development). Such results are incongruent with the predicted long embryonic periods of large eggs at low temperature (Nesis, 1999), and the slow growth rates observed in laboratory-held octopods from the deep sea (i.e. *B. arcticus*, 0.18% mean daily increase in body weight; O'Dor and Macalaster, 1983) and Antarctic (i.e. *Pareledone charcoti*, 0.11% mean daily increase in body weight; Daly and Peck, 2000). Moreover, the lifespan of *B. arcticus* (actually probably misidentified *B. bairdii*—see Muus, 2002) was predicted to be ~4 years (O'Dor and Macalaster, 1983). The large sizes reached by *M. setebos* (ML >200 mm) at low temperature suggest a long lifespan, but *O. dofleini* living in the cool waters of the North Pacific is estimated to have a lifespan of just 3–5 years, growing from a planktonic larva to typically more than 50 kg (Hartwick, 1983). Jackson and Lu (1994) also found that the number of growth increments in the statoliths of five squid species collected from the Southern Ocean was fairly low, ranging from just 131 to 277 (ML 95–355 mm).

Generally, growth increments in the stylets of deep-sea and Antarctic species were much less distinct than those of *O. vulgaris* and *E. cirrhosa* (except in the innermost regions, where they were clearer). The faintness of the growth increments could reflect the fact that the stylets had been stored in formalin, or could be attributable to differences in their elemental composition. Alternatively, it could relate to differences in the ambient environment; for example, growth increments in the statoliths of squid (e.g. *Sepioteuthis lessoniana*; Jackson *et al*., 1993, and *Lolliguncula brevis*; Durholtz and Lipiński, 2000) and the otoliths of fish (Campana and Neilson, 1985) kept under constant conditions of light and temperature in the laboratory were much less distinct than those collected from the wild. A feature of the deep-sea and Southern Ocean environments is the constant low temperature and either total absence or seasonal interruptions to the normal diel light cycle. Moreover, growth increments in the stylets of aquarium-held *O. pallidus* were less distinct than those collected from the wild (Doubleday *et al*., 2006). Conversely, the clarity of growth increments of *O. vulgaris* and *E. cirrhosa* may reflect the presence of additional zeitgebers to entrain endogenous rhythms such as changes in light and temperature, because both species are typically collected from relatively shallow water (0–150 m for *O. vulgaris*; Mangold, 1983; 0–200 m for *E. cirrhosa* in the North Sea; Boyle, 1983).

On the whole, this study has provided a novel methodology for examining growth increments in the stylets of octopods. The technique, which is based on dehydrating stylets in ethanol and embedding them in ultra-low-viscosity acrylic resin, so providing a permanent record of the growth increments, appears to be consistently useful in a variety of species. As the low-viscosity resin appears to penetrate the centre of the stylet allowing, in some species, growth increments to be enumerated throughout the entire stylet, the technique may have potential for estimating the duration of the embryonic and planktonic phases of life histories. Moreover, because the technique does not rely on a photographic record of the stylet, areas of the stylet that are difficult to read may be studied and re-studied with varying focal depth, to improve an accuracy of enumeration. Prior to use, validation of daily growth increments is required, as is validation of whether checkmarks that we suspect may mark major life history events (e.g. hatching and settling) actually relate to these biological processes.

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References

Arkhipkin, A. I. 2005. Statoliths as 'black boxes' (life recorders) in squid. Marine and Freshwater Research, 56: 573–583.

Bettencourt, V., and Guerra, A. 2001. Age studies based on daily growth increments in statoliths and growth lamellae in cuttlebone of cultured *Sepia officinalis*. Marine Biology, 139: 327–334.

Bizikov, V. A., and Arkhipkin, A. I. 1997. Morphology and microstructure of the gladius and statolith from the boreal Pacific giant squid *Moroteuthis robusta*. Journal of Zoology, 241: 475–492.

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Boyle, P. R. 1983. *Eledone cirrhosa*. *In* Cephalopod Life Cycles. 1. Species Accounts, pp. 365–386. Ed. by P. R. Boyle. Academic Press, London. 475 pp.

Boyle, P. R., Mangold, K., and Ngoile, M. 1988. Biological variation in *Eledone cirrhosa*: simultaneous comparison of North Sea and Mediterranean populations. Malacologia, 29: 77–87.

Campana, S. E., and Neilson, J. D. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences, 42: 1014–1032.

Cobb, C. S., Pope, S. K., and Williamson, R. 1995. Circadian rhythms to the light-dark cycles in the lesser octopus, *Eledone cirrhosa*. Marine and Freshwater Behaviour and Physiology, 26: 47–57.

Daly, H. I., and Peck, L. S. 2000. Energy balance and cold adaptation in the octopus *Pareledone charcoti*. Journal of Experimental Marine Biology and Ecology, 245: 197–214.

Doubleday, Z., Semmens, J. M., Pecl, G., and Jackson, G. 2006. Assessing the validity of stylets as ageing tools in *Octopus pallidus*. Journal of Experimental Marine Biology and Ecology, 338: 35–42.

Durholtz, M. D., and Lipiński, M. R. 2000. Influence of temperature on the microstructure of statoliths of the thumbstall squid *Lolliguncula brevis*. Marine Biology, 136: 1029–1037.

Hartwick, B. 1983. *Octopus dofleini*. *In* Cephalopod Life Cycles. 1. Species Accounts, pp. 277–291. Ed. by P. R. Boyle. Academic Press, London. 475 pp.

Hernández-López, J. L., Castro-Hernández, J. J., and Hernández-García, V. 2001. Age determined from the daily deposition of concentric rings on common octopus (*Octopus vulgaris*) beaks. Fishery Bulletin US, 99: 679–684.

Jackson, G. D. 1989. The use of statolith microstructures to analyze life-history events in the small tropical cephalopod *Idiosepius pygmaeus*. Fishery Bulletin US, 87: 265–272.

Jackson, G. D., Arkhipkin, A. I., Bizikov, V. A., and Hanlon, R. T. 1993. Laboratory and field corroboration of age and growth from statoliths and gladii of the loliginid squid *Sepioteuthis lessoniana*. *In* Recent Advances in Cephalopod Fisheries Biology, pp. 189–199. Ed. by T. Okutani, R. K. O'Dor and T. Kubodera. Tokai University Press, Tokyo. 752 pp.

Jackson, G. D., Forsythe, J. W., Hixon, R. F., and Hanlon, R. T. 1997. Age, growth, and maturation of *Lolliguncula brevis* in the northwestern Gulf of Mexico with a comparison of length-frequency versus statolith age analysis. Canadian Journal of Fisheries and Aquatic Sciences, 54: 2907–2919.

Jackson, G. D., and Lu, C. C. 1994. Statolith microstructure of seven species of Antarctic squid captured in Prydz Bay, Antarctica. Antarctic Science, 6: 195–200.

Leporati, S. C., Semmens, J. M., and Pecl, G. T. 2007. Cephalopod hatchling growth: the effects of initial size and seasonal temperatures. Marine Biology, 151: 1375–1383.

Lipiński, M. R. 1986. Methods for the validation of squid age from statoliths. Journal of the Marine Biological Association of the UK, 66: 505–526.

Lipiński, M. R., Durholtz, M. D., and Underhill, L. G. 1998. Field validation of age readings from the statoliths of chokka squid (*Loligo vulgaris reynaudii*) and an assessment of associated errors. ICES Journal of Marine Science, 55: 240–257.

Mangold, K. 1983. Octopus vulgaris. *In* Cephalopod Life Cycles. 1. Species Accounts, pp. 335–364. Ed. by P. R. Boyle. Academic Press, London. 475 pp.

Meisel, D. V., Byrne, R. A., Kuba, M., Griebel, U., and Mather, J. A. 2003. Circadian rhythms in *Octopus vulgaris*. Paläobiologische Abhandlungen, 3: 171–177.

Muus, B. 2002. The *Bathypolypus–Benthoctopus* problem of the North Atlantic. Malacologia, 44: 175–222.

Nesis, K. N. 1999. The duration of egg incubation in high-latitude and deep-sea cephalopods. Russian Journal of Marine Biology, 25: 499–506.

O'Dor, R. K., and Macalaster, E. G. 1983. *Bathypolypus arcticus*. *In* Cephalopod Life Cycles. 1. Species Accounts, pp. 401–410. Ed. by P. R. Boyle. Academic Press, London. 475 pp.

Radtke, R. L., Targett, T. E., Kellermann, A., Bell, J. L., and Hill, K. T. 1989. Antarctic fish growth: profile of *Trematomus newnesi*. Marine Ecology Progress Series, 57: 103–117.

Raya, C. P., and Hernández-González, C. L. 1998. Growth lines within the beak microstructure of the octopus *Octopus vulgaris*. South African Journal of Marine Science, 20: 135–142.

Robinson, S. M. C., and Hartwick, E. B. 1986. Analysis of growth based on tag-recapture of the giant Pacific *Octopus dofleini martini*. Journal of Zoology, 209: 559–572.

Sifner, S. K. 2008. Methods for age and growth determination in cephalopods. Ribarstvo, 66: 25–34.

Sousa Reis, C., and Fernandes, R. 2002. Growth observations on *Octopus vulgaris* from the Portuguese waters: growth lines in the vestigial shell as possible tools for age determination. Bulletin of Marine Science, 71: 1099–1103.

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