Cuttlebone Morphology Limits Habitat Depth in Eleven Species of *Sepia* (Cephalopoda: Sepiidae)

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Abstract. The cuttlebone is a rigid buoyancy tank that imposes a depth limit on *Sepia*, the only living speciose cephalopod genus with a chambered shell. Sections of 59 cuttlebones from a geographically diverse sample of 11 species were examined using confocal microscopy. *Sepia* species that live at greater depths had thicker septa and less space between pillars than did shallow species. A plate theory analysis of cuttlebone strength based on these two measures predicted maximum capture depths accurately in most species. Thus cuttlebone morphology confers differing degrees of strength against implosion from hydrostatic pressure, which increases with increasing habitat depth. Greater strength may come at the cost of increased cuttlebone density, which impinges on the cuttlebone's buoyancy function.

Introduction

The chambered shells of cephalopods serve as buoyancy devices despite being made of heavy calcium carbonate. Unlike the swim bladders of fish, they are unpressurized, so they do not change significantly in volume or buoyancy as the animal changes depth (Denton and Gilpin-Brown, 1961a), and no adjustments to the buoyancy system are necessary during vertical movements (Denton, 1974; Ward, 1987). However, the shells must be strong enough to resist implosion from hydrostatic pressure, which increases with depth (Denton and Gilpin-Brown, 1961a, b; Ward and Boletzky, 1984). Thus the demands of buoyancy and strength conflict: if a chambered shell is too dense, buoyancy decreases; if it is too weak, the danger of fractures at the animal's normal habitat depths increases.

The functional morphology of chambered shells, or phragmocones, in living and fossil cephalopods has been the subject of much research (*e.g.*, Westermann, 1975;

Ward, 1987; Hewitt and Westermann, 1986; Jacobs, 1990; Daniel *et al.*, 1997; for a review, see Jacobs, 1992). Shell strength is of interest in part because habitat depth is ultimately limited by resistance to implosion. Habitat depth has implications for the separation of populations, for speciation, and for evolution. The high diversity of one fossil group, the ammonoids, has fueled efforts to understand the functional morphology of their phragmocones.

There are two possible approaches to studying phragmocone strength against implosion. First, living cephalopods (or their fresh shells) can be subjected to increasing hydrostatic pressures until the shells break. This has been done for individuals and shells of *Nautilus* (Raup and Takahashi, 1966; Hewitt and Westermann, 1987; Ward, 1987), *Spirula* (Denton, 1971), and *Sepia* (Birchall and Thomas, 1983; Ward and Boletzky, 1984). It is problematic to use dried shells, however, because they are weaker than living shells to an uncertain degree (Jacobs, 1992). Although imploding fresh shells provides accurate data on strength against hydrostatic pressure, this method is impractical for rarely caught species and impossible to use for fossils.

Alternately, theoretical analyses may be used to estimate shell strength against implosion. This method allows inferences about the functional morphology and paleobiology of extinct animals, as well as of living shelled cephalopods, many of which are rarely observed in the wild. Theoretical approaches have been applied to fossil nautiloids (*e.g.*, Westermann, 1973) and ammonoids (Westermann, 1975; Hewitt and Westermann, 1986; Jacobs, 1990; Daniel *et al.*, 1997). A disadvantage of theoretical analyses is that it is difficult to test the accuracy of the estimates, which depend on a number of simplifying assumptions.

For example, breaking stress must be analyzed at the weakest part of the shell, but it is not always clear how to identify this region. Various studies of fossil cephalopods have assumed it to be the outer shell (Jacobs, 1990), the last

septum (Westermann, 1973; Daniel *et al.*, 1997), or the siphuncle (as reviewed in Jacobs, 1992). If the shell fails catastrophically during implosion, as is the case for *Nautilus* (Ward *et al.*, 1980), empirical tests do not establish the site of initial failure. Thus relative strengths of different parts of the shell must sometimes be calculated theoretically without recourse to direct verification, even in a living animal.

Constructing a model of shell strength requires that structural geometry, loading conditions, and mode of failure be defined to form the basis for an appropriate model. Even when models are carefully chosen, they are simplifications of complex structures and circumstances. Some simplifications have little effect on strength estimates; others have larger or nonlinear effects that make it difficult to evaluate the reliability of the model's output. Thus it would be informative to evaluate the robustness of a theoretical model on a living cephalopod of known habitat depth to gauge how reliable such estimates are for fossil forms.

Habitat depth and morphology vary little among species of *Nautilus*, and the internally shelled squid *Spirula* is monospecific. Only one living genus, *Sepia*, presents a wide range of habitat depths and shell morphologies among its nearly 100 species (Adam and Rees, 1966). Its phragmocone, or cuttlebone (Fig. 1), is internal and has a complex microscopic morphology. A method for estimating habitat depth from cuttlebone morphology would improve our
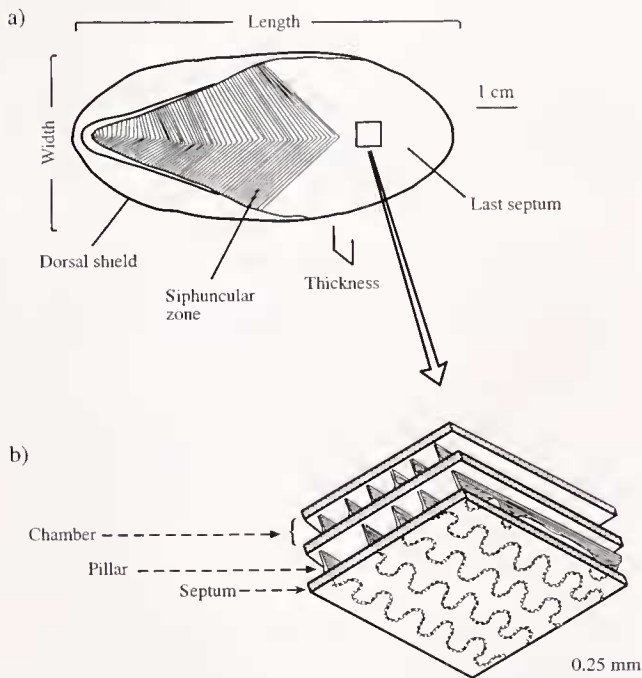

Figure 2. Confocal micrograph of *Sepia hieronis* cuttlebone. Measurements are septal thickness (*t*), pillar spacing (*r*), and chamber height (*h*).

knowledge of little known species of *Sepia*, some of which have been described only on the basis of cuttlebones washed up on beaches.

Sepia is a genus of dibranchiate cephalopod that lives in coastal waters of Europe, Africa, Asia, and the South Pacific. It may be the only shelled cephalopod that alters its buoyancy on a daily basis; individuals of *S. officinalis* are heavy and rest on the bottom during the day, becoming more buoyant at night when they swim and hunt (Denton and Gilpin-Brown, 1961d). Extensive data from fisheries provide reliable estimates of maximum habitat depth in many species. Most species have a shallow habitat, not ranging deeper than 100 to 150 m. A dozen species have maximum capture records of 400 m and more (Adam and Rees, 1966; Roeleveld, 1972; Ward and Boletzky, 1984). There are very few species with intermediate maximum capture depths (200 to 300 m), though specimens of deeper dwelling species are often found at intermediate depths. This natural gap in maximum habitat depths permits the division of species into "shallow" and "deep" categories.

The cuttlebone appears to be a highly efficient structure, using a minimum of materials to achieve required strength. The siphuncular region is flat and open, rather than being a tube as in most chambered cephalopods, so it is not subject to explosion under hydrostatic pressure (Jacobs, 1992). The pillars buttress adjacent septa (see Fig. 1b, Fig. 2). It has been claimed that the pillars in cuttlebones are homologous to structures found on the siphuncle in *Spirula, Nautilus*, and fossil cephalopods (Bandel and Boletzky, 1979), but the cuttlebone's pillars are unique in having spread over the entire septal surface and in playing a major role in structural support of the septa. Though not homologous to ammonite sutures, which have been claimed to have a buttressing function (see Jacobs, 1992), pillars are similar to them in

Figure 1. (a) Ventral view of the cuttlebone (anterior is to the right) of *Sepia papillata*. Chambers are emptied through the siphuncular zone, maintaining buoyancy. The last septum is the site of failure due to excessive hydrostatic pressure. (b) Microscopic structure of cuttlebone, showing chambers and the undulating, transverse pillars between the septa.
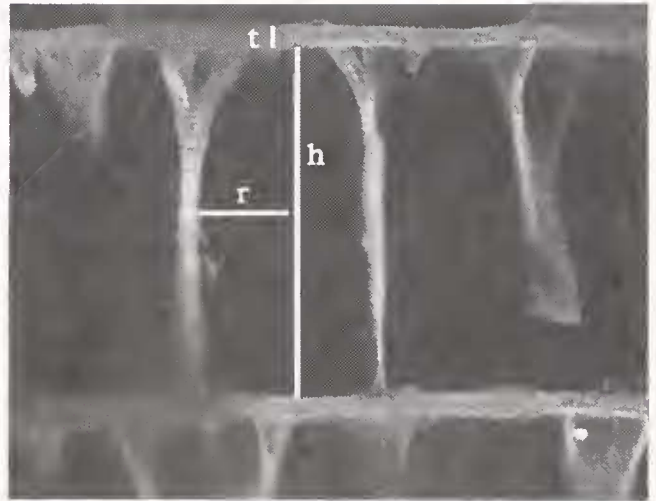
Table 1

Comparison of estimated and actual maximum capture depths for Sepia spp. *used in this study*

| Species | Number of specimens | Estimated max depth* | | Max capture depth (m)† |
		Species medians	Max specimen medians	
Deep				
S. australis	7	530 (460–611)	780 (708–840)	470 (a)
S. elegans	4	263 (204–301)	411 (310–479)	430 (b)
S. hieronis	6	332 (239–406)	855 (753–1087)	459 (a)
S. orbignyana	9	236 (209–248)	300 (212–335)	430 (b)
S. rex	7	216 (200–256)	377 (268–453)	400 (c)
Mean		306	545	438
Shallow				
S. apama	7	88 (80–104)	195 (106–263)	110 (c)
S. latimanus	4	61 (56–68)	99 (84–109)	100 (c)
S. mestus	4	42 (37–55)	73 (62–114)	160 (c)
S. officinalis	3	51 (44–65)	189 (64–227)	150 (c)
S. papillata	5	76 (68–84)	94 (62–142)	125 (a)
S. vercoi	3	109 (92–127)	130 (125–155)	100 (d)
Mean		67	130	124

* Estimated depths based on species medians and maximal specimen medians for r/t, assuming a safety factor of 1.33 (see text for details); 95% confidence intervals in parentheses.

† Maximum capture data from (a) Roeleveld, 1972, (b) Adam and Rees, 1966, (c) Ward and Boletsky, 1984, and (d) Nesis, 1987.

appearance (Figs. 1b, 2). In distributing hydrostatic pressure loads from the last septum to internal septa, pillars constitute a major innovation in cephalopod shell architecture.

The site of failure in an imploding cuttlebone has been shown empirically to be the last septum (Ward and Boletzky, 1984). The effect of hydrostatic pressure on the ventral surface of the septum is thus the proper subject in a theoretical strength analysis of cuttlebones.

Given the critical role of the septum for supporting loads and of the pillars for transmitting loads, microscopic morphology is likely to be significant in determining cuttlebone strength. Engineering plate models show that both a thicker septum and a smaller distance between pillars produce a stronger cuttlebone. Measurements of septal thickness and pillar spacing were together expected to distinguish the cuttlebones of deep and shallow species. Here, I compare cuttlebone morphologies of 11 species of *Sepia* from a variety of geographical locales and habitat depths (Table 1). Maximum depths of specimens and species predicted from theoretical models are compared with species' maximum capture depths reported in the literature to test the reliability and sensitivity of the models. The results have implications for the precision of theoretical strength models in inferring the habitat depth of extinct cephalopods.

Materials and Methods

The 59 dried cuttlebones used in this study (Table 2) were from two collections housed at the University of Washington (Burke Museum and P. Ward, Dept. of Geological Sciences). Specimens had been collected from beaches in Australia, South Africa, and the Mediterranean. Like most cephalopods, cuttlefish die after reproducing, and their cuttlebones subsequently wash up on beaches. Specimens used were within the size range of adults for each species, so it is reasonable to assume that they were from mature animals. Species chosen for the study are frequently caught by fishing and research vessels, so their maximum capture depths are likely to be adequately sampled.

Confocal microscopy

A razor blade was used to cut 5-mm-wide cross-sections from the midventral region, at right angles to the septal surface. This procedure sampled the most recently grown 10 to 20 chambers.

Images were direct-scanned at slow speed with 10×, 20×, and 40× lenses on a Bio-rad confocal microscope (MRC 600), using neutral density 1 and number 1 filters and a BHS filter block. The cuttlebone's chitinous component autofluoresces in the laser, and because this component coats all internal surfaces, it accurately represents their dimensions. Micrographs (see Fig. 2), with image scale bars, were stored digitally. Chamber height (h), septal thickness (t), and half-distance between adjacent pillars (r) were measured using NIH Image 1.61. Scale bars were used to check the accuracy and precision of the measuring technique.

Inter-pillar radius (r) was measured at the middle of pillars rather than at their tops, since in most specimens it

Table 2

Specimen identification numbers for Sepia *cuttlebones used in this study*

	Sepia australis	S. elegans	S. hieronis	S. orbignyana	S. rex	S. apama	S. latimanus	S. mestus	S. officinalis	S. papillata	S. vercoi
Burke Museum Collection											
	95961c	95959	95968a	95955	95958a	95909a		95970a		95951	
	95961f		95968b	95960.8	95958b	95909b		95970b		95952	
	95961o		95968c	95960	95958d	95907b		95970c		95953	
	95961q			95960.9	95958e	95954		95970d		95954	
	95906			95960b	95958f	95910				95925b	
	95912			95960c	95972						
	95915			95960e	95973						
				95960g							
Ward Collection											
		KS0002	S27	K57		K2002	S21		S9		K1100
		KS0003	97-01			K1001	S31		S11		K1101
		KS0004	97-02				S32		S15		K1102
							S33				

was difficult to see the location of pillars as they contacted the septum. Only regions in sharp focus were measured. An average of 19 chambers were measured in each specimen, for a total of 1093 chambers. Septal thickness, chamber height, and multiple examples of inter-pillar spacing were measured for each chamber. Measurements within a chamber formed the basis for analyses at the specimen, species, and depth-group levels.

Maximum capture data

Data on species' maximum habitat depths come from fisheries and research captures of *Sepia* (Adam and Rees, 1966; Roeleveld, 1972; Ward and Boletzky, 1984; Nesis, 1987). Individual variation may cause the typical maximum depth to be overestimated for a species. However, maximum capture depth proves adequate to divide species into two depth groups— "deep" and "shallow" (see Table 1). Species with a shallow maximum capture depth (less than 200 m) live on the continental shelves, whereas those that have been captured at depths of 350 m and more live on the outer continental slope and bathyal regions (Roeleveld, 1972; Nesis, 1987), indicating that the division into deep and shallow categories reflects a difference in natural histories.

Macroscopic measurements

In the only previous study of cuttlebone strength, Ward and Boletzky (1984) suggested that a high radius of septal curvature and a small, narrow cuttlebone were characteristic of a deep habitat. To test this interpretation, I measured cuttlebone length, width, and thickness to the nearest millimeter with digital calipers. Length, relative width (width/length), and relative thickness (thickness/width; a proxy for septal radius of curvature) of deep and shallow species were compared using Kolmogorov-Smirnov tests. The same specimens were used for confocal microscopy and macroscopic measurements, except in *S. australis, S. orbignyana,* and *S. papillata,* where different but size-comparable specimens were used.

Sampling and statistics

The data were not normally distributed and could not be normalized using logarithmic or other transformations; thus medians were used as the measure of central tendency. Bootstrap resampling (1000 replicates) was used to obtain 95% confidence intervals, using the program Resampling Statistics (Sells Software Service). Specimen confidence intervals for the r/t ratio were similarly computed, for use in depth predictions.

The Kolmogorov-Smirnov test and the Mann-Whitney test were applied using StatView 5.0 (Abacus Concepts) to determine whether deep and shallow groups were significantly different in the variables measured. Because there were unequal numbers of observations, a random subsample of measurements was taken, corresponding to the smallest number of measurements obtained in a species. To obtain equal numbers of samples in the deep and shallow groups, more measurements were used from each of the five deep species than from the six shallow species.

Because the predicted depths were based on ratios taken from non-normal distributions, the nonparametric Kendall rank correlation coefficient was used to relate predicted depths of specimens and species to maximum capture data. This test is a conservative estimate of the degree of correlation in the data.

Modeling

Two models, a plate theory model (Timoshenko, 1940) and a square grid model (Hewitt and Westermann, 1986), were applied to estimate maximum depth from measured values of septal thickness (t) and pillar spacing (r), under different sets of assumptions.

Plate theory model (Fig. 3A)

The plate theory model assumes a rigid, thin, circular plate with fixed edges (zero-displacement, zero-moment, positive shear). The plate is assumed to be uniformly loaded perpendicular to its surface, which is equivalent to hydrostatic loading provided the plate does not deform significantly. Under these conditions, the highest stress occurs at the plate edge, where deformation is least because of intersection with the pillar below. The maximum stress, or pressure P_{max} in newtons per square meter, that can be withstood by the structure is expressed as:

$$P_{max} = 4/3 \; s \; (r/t)^2 \qquad (1)$$

where s = tensile material strength in N/m^2
        r = plate radius in m
        t = plate thickness in m

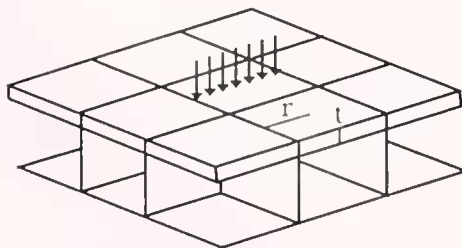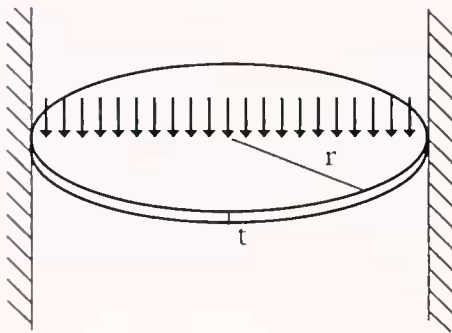Because s is unknown for cuttlebone, a measured value for

Nautilus septum under tensile stress was used (78 MN/m^2; Currey, 1980). This may be an overestimate of strength because Nautilus septa are nacreous aragonite whereas cuttlebone septa are prismatic aragonite (Bandel and Boletzky, 1979). However, the two reported prismatic values (for the bivalves *Pinna muricata* and *Atrina vexillum*) were 62 and 60 MN/m^2, respectively (Currey, 1980). Thus using the values for nacreous cephalopod septa should overestimate the depth of cuttlebone implosion only slightly, if at all.

Equation 1 can be rewritten to express P_{max} in terms of depth (each meter of depth underwater is equivalent to 1.015×10^4 N/m^2), using the value of 78 MN/m^2 for material strength.

$$\text{Meters depth}_{max} = 10246 \; / \; (r/t)^2 \qquad (2)$$

Equation (2) was used to estimate maximum depth (*i.e.*, estimated implosion depth) using median values of r and t for each specimen and species.

Square grid model (Fig. 3B)

A model used by Hewitt and Westermann (1986, p. 58) to estimate the strength of ammonite septa is readily applicable to cuttlebones. Both the geometry of the model and the boundary conditions of support differ from the circular plate model. The square grid model assumes a flat square of shell simply supported on a rectangular suture grid: the flat square corresponds to the septum and the grid to the pillars. The plate is loaded uniformly. Because the plate's edges are not fixed, the maximum bending stress occurs in the center of the plate, and is given by:

$$b_{max} = 0.22 \; P \; (2r/t)^2 \qquad (3)$$

where b_{max} = maximum bending stress in N/m^2
        P = pressure in N/m^2
        r = half-distance between grid members in m
        t = plate thickness in m

This equation can be simplified, as for Equation (1), to express maximum implosion depth in meters underwater. The value of bending strength (b_{max}) given by Currey (1980) for *Nautilus* nacre is 193 MN/m^2; the equation simplifies to:

$$\text{Meters depth}_{max} = 21900 \; / \; (r/t)^2 \qquad (4)$$

An important difference between the square grid model and the circular plate model is that the former assumes simply supported edges, while the latter assumes the septum to be fused to the pillars. Values reported in Table 1 and Figure 5 are from the circular plate model, which more accurately represents cuttlebone morphology.

Figure 3. Illustration of the circular plate model (A) and square grid model (B), the latter after Hewitt and Westermann (1986).

Safety margin

Implosion depth divided by maximum habitat depth gives the safety margin, an index of how close a structure comes to its ultimate strength limits during normal use. A safety margin can justifiably be applied to the cuttlebone because (1) it is static, in that it cannot be remodeled, and (2) the consequences of failure are sudden and usually fatal (Ward and Boletzky, 1984). Additionally, the stress on the cuttlebone is predictable, being simply a linear function of depth.

The concept of safety margins has been explored in living and extinct cephalopod shells (Denton, 1974; Ward *et al.*, 1980; Jacobs, 1992; Daniel *et al.*, 1997). Denton (1974) concludes that the safety margin is about 1.3 to 1.4 for the shells of living cephalopods. I assumed a safety factor of 1.33 for cuttlebones. Data reported as "predicted implosion depth" come directly from Equation 2; data reported as "predicted depth" are predicted implosion depths divided by 1.33.

Results

Microscopic measurements

Results of the Kolmogorov-Smirnov tests indicate that deep species had significantly thicker septa ($P < 0.0001$, $n = 276$, $\chi^2 = 99.8$), more closely spaced pillars ($P < 0.0001$, $n = 960$, $\chi^2 = 36.8$), and shorter chambers ($P < 0.0001$, $n = 252$, $\chi^2 = 10.7$) than did the shallow species, although there was some overlap. The ratio r/t (used in the plate theory analyses) was also significantly different between deep and shallow species ($P < 0.0001$, $n = 252$, $\chi^2 = 134.3$), as was septal density, t/h ($P < 0.0001$, $n = 252$, $\chi^2 = 77.8$). Mann-Whitney tests gave the same results, except for chamber height, which was not significantly different ($P > 0.2$, $U = 7254$). Table 3 presents species median values for these measurements with 95% confidence intervals.

Distributions of microscopic measurements were nearly all right-skewed, but differed qualitatively between deep and shallow species (Fig. 4). In shallow species, septal thickness (t) and septal density (t/h) measurements were smaller and had lower variance compared with deep species. In contrast, shallow species' chamber heights (h) and plate ratio (r/t) values had larger values and much larger variance. The plate ratio is minimized in deep species primarily by increased septal thickness (t), but deep species also showed a slight reduction in variance of pillar spacing (r).

Maximum depth calculations

Table 1 and Figure 5 show the estimated maximum depths of the 11 species and 59 specimens, along with actual maximum capture depths. For all species, maximum depths predicted by Equation (2) (divided by a safety factor of 1.33) were within an order of magnitude of known maximum capture depths, and the estimated values correlated significantly with the recorded data (Kendall rank correlation test, $P = 0.024$). In each species, the specimen with the

Table 3

Morphological measurements for Sepia *cuttlebones (species medians and bootstrapped 95% confidence intervals)*

Species	t (μm)	r (μm)	h (μm)	r/t	t/h %
Deep					
S. australis	5.5 ± 0.5	20.6 ± 0.9	144 ± 14	3.81 ± 0.28	3.8 ± 0.2
S. elegans	5.6 ± 1.23	34.2 ± 1.5	174 ± 14	5.41 ± 0.62	3.0 ± 0.2
S. hieronis	8.7 ± 0.6	42.2 ± 2.2	242 ± 17	4.81 ± 0.36	3.4 ± 0.3
S. orbignyana	7.4 ± 0.5	40.9 ± 1.9	246 ± 13	5.70 ± 0.27	3.0 ± 0.1
S. rex	5.1 ± 0.1	30.3 ± 1.6	138 ± 13	5.96 ± 0.43	3.7 ± 0.3
Mean*	6.88	34.2	192	5.43	3.85
Shallow					
S. apama	4.5 ± 0.4	40.3 ± 2.2	321 ± 13	9.33 ± 0.68	1.4 ± 0.1
S. latimanus	3.6 ± 0.3	38.0 ± 2.4	184 ± 29	11.23 ± 0.61	2.0 ± 0.4
S. mestus	2.7 ± 0.1	34.5 ± 2.9	91 ± 7	13.47 ± 1.53	3.0 ± 0.2
S. officinalis	4.2 ± 0.9	45.5 ± 12.3	259 ± 53	12.26 ± 1.24	1.5 ± 0.6
S. papillata	4.8 ± 0.2	43.1 ± 3.8	211 ± 17	10.09 ± 0.59	2.1 ± 0.3
S. vercoi	3.6 ± 0.2	30.8 ± 2.1	150 ± 32	8.41 ± 0.27	2.3 ± 0.3
Mean*	3.93	40.5	212	11.18	2.34
P values†	<0.0001	<0.0001	0.0094	<0.0001	<0.0001

Note that there is no overlap between deep and shallow species medians of septal thickness (t) and plate ratio (r/t), which are important factors in strength against implosion. Septal density (% t/h) shows almost no overlap, with deep species having higher values. Deep species also tend to have shorter chambers (h), although they overlap with shallow species. Medians for pillar spacing (r) are similar among species, but the shallow group has a strongly right-skewed distribution (see Fig. 3) at the level of chamber measurements, which leads to a significant difference from the deep species in this measurement as well.

* Means based on specimen measurements.

† P values from Kolmogorov-Smirnov tests of deep vs. shallow chamber measurements.
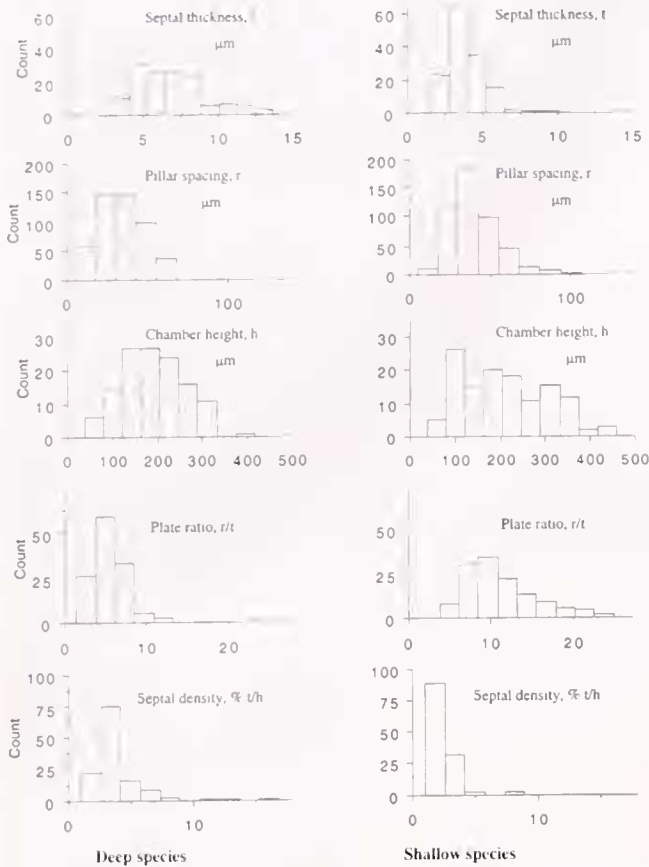
Figure 4. Histograms of deep and shallow groups for *t*, *r*, *h*, *r/t*, and % *t/h*. Compared to deep species, shallow species have thinner septa (*t*), a higher plate ratio (*r/t*), lower density of septal spacing (% *t/h*), and more right-skewed distributions for pillar spacing (*r*), chamber height (*h*), and plate ratio (*r/t*). Distributions for *r/t* differ more than those for *r* alone and *t* alone; this is because cuttlebone strength can be increased either by increasing *t* or by decreasing *r*, and deep species have used both strategies.

maximum predicted depth was also significantly correlated with the maximum capture depths for the species ($P = 0.016$).

For most species, variability in predicted depths among specimens was high (see Fig. 5). For example, predicted depths for specimens of *S. rex* ranged from 145 to 377 m, for *S. hieronis* from 130 to 855 m, and for *S. apama* from 68 to 195 m. However, no specimen of a shallow species had a predicted depth larger than 195 m, and within deep species, no specimen had a predicted depth shallower than 130 m. Deep species were more variable than shallow species, particularly the two with the deepest capture records (*S. australis* and *S. hieronis*).

Macroscopic measurements

Relative cuttlebone width (width/length) was greater in specimens of shallow species; the difference was significant at $P = 0.0047$ in a Kolmogorov-Smirnov test ($n = 40$

specimens). Relative thickness (thickness/width), an estimator of septal curvature, did not differ significantly ($P > 0.90$). Total length differed significantly among deep and shallow species ($P < 0.0001$), with deep specimens being uniformly small (45 to 102 mm) and shallow specimens either small or large (32 to 290 mm). Species averages and significance levels are summarized in Table 4.

When deep and shallow species were analyzed separately using the Kendall rank correlation test, neither specimen lengths nor relative widths were significantly correlated with *r/t* (length: for deep species, $P > 0.069$, $n = 18$, $Z = 1.82$; for shallow species, $P > 1.0$, $n = 18$, $Z = 1.0$; relative width: for deep species, $P = 0.45$, $n = 18$, $Z = -0.76$; for shallow species, $P > 1.0$, $n = 18$, $Z = 0.038$).

Discussion

The cuttlebones of deep and shallow species were readily distinguishable on the basis of microscopic measurements. In deep species, plate ratio (*r/t*) was consistently lower, and
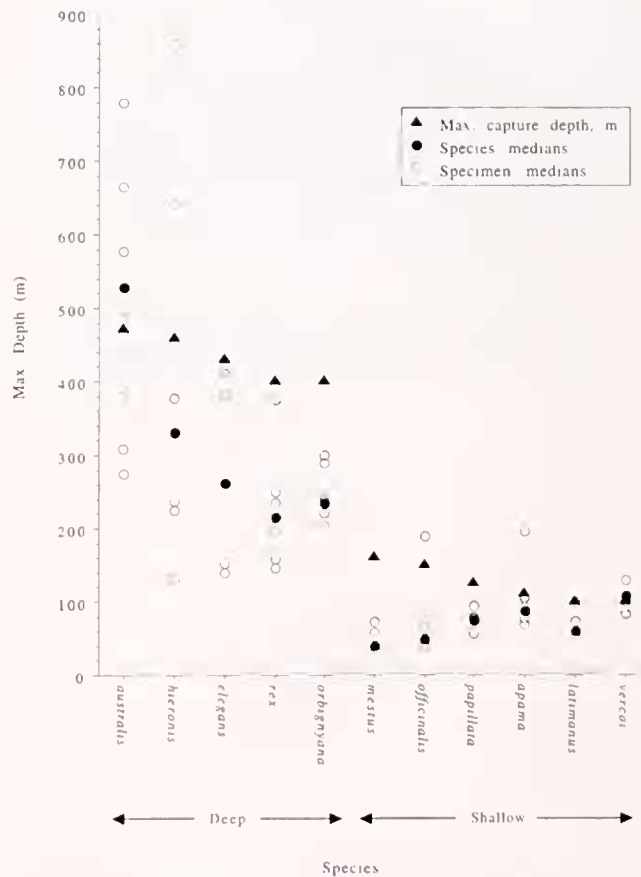

Figure 5. Estimated maximum capture depths of species and specimens (from *r/t* medians applied to Equation (2) and divided by a safety factor of 1.33), and species maximum capture depths (see Table 1). Deep species are more variable in estimated depths than are shallow species. In most species, maximal specimen estimates are close to maximum capture depths reported in the literature.

Table 4

Macroscopic cuttlebone measurements of Sepia cuttlebones (species and depth category means from a total of 40 measured specimens)

Species	Average length (mm)	Relative width (width/length)	Relative thickness (thickness/width)
Deep			
S. australis	50.6	0.32	0.24
S. elegans	53.1	0.27	0.31
S. hieronis	68.3	0.40	0.30
S. orbignyana	77.0	0.29	0.36
S. rex	86.8	0.34	0.27
Mean	69.6	0.32	0.30
Shallow			
S. apama	170.5	0.45	0.25
S. latimanus	254.5	0.33	0.38
S. mestus	80.4	0.44	0.18
S. officinalis	146.0	0.36	0.34
S. papillata	107.3	0.45	0.28
S. vercoi	32.0	0.25	0.36
Mean	158.5	0.41	0.28
P values*	<0.0001	0.0047	0.9000

Deep species were smaller than shallow species, and relatively narrower, although there was a good deal of overlap in the latter. Relative thickness (thickness/width), a proxy for septal radius of curvature, did not differ between deep and shallow species.

* P values from Kolmogorov-Smirnov tests of individual specimens.

septal thickness (t) and septal density (t/h) were consistently higher (see Table 3). Chamber height (h) was less characteristic of habitat depth, but tended to be lower in deep species. Note that septal thickness alone was not sufficient to account for strength differences: for example, *S. australis* had septa barely thicker than those found in several shallow species, yet lives at much greater depths.

Two of the measurements in combination, septal thickness and pillar spacing, were causally linked to cuttlebone strength on the basis of plate theory. Maximum predicted depths based on Equation (2) were, in most cases, well within the ranges reported for each species. Microscopic cuttlebone morphology was thus a reliable predictor of species' habitat depth in the 11 species studied.

Deep and shallow species were also distinct in the distribution of these measurements (see Fig. 4). Deep species had narrower distributions for plate ratio and chamber height, and shallow species had narrower distributions for septal thickness and septal density. This implies selective pressure for thinner septa and less dense cuttlebones in shallow species, and thicker septa and minimization of the plate ratio (r/t) in deep species.

Strength and depth estimates

Implosion depth, as opposed to maximum capture depth, is known within 50 m (Ward and Boletzky, 1984) for the three Mediterranean species: *S. officinalis* (200 m), *S. orbignyana* (550 m), and *S. elegans* (600 m). The largest predicted implosion depths (calculated directly from Equation 2) for specimens of these species were 252 m, 400 m, and 548 m, respectively; the 95% bootstrapped confidence intervals encompassed Ward and Boletzky's data.

In most species, estimated maximum depths were close to maximum capture depths (see Fig. 5). However, *S. mestus* and *S. orbignyana* had lower estimated implosion depths than their maximum capture depths would suggest. It may be that the maximum capture data for these species came from anomalous individuals. *S. hieronis* and *S. australis* present the opposite problem: estimates of strength in several specimens are much higher than capture records would imply. This may be because small changes in r/t lead to large changes in strength in the region of morphospace occupied by deep species (see Fig. 6). Given the problems of increased chamber flooding at greater depths in other cephalopods (Ward and Chamberlain, 1983), it would be surprising if these individuals actually lived as deep as their cuttlebone strength would allow. In any case, these discrepancies demonstrate the need for empirical data on intraspecific variation in capture depths and implosion depths.

Significance of within-species variation

Intraspecific variability in strength estimates was high, particularly in the deep species (Fig. 5). This may be largely due to the quadratic order of Equations (2) and (4) (Fig. 6), which means that for deep species slight changes in r/t cause large changes in strength.

The fact that the distribution of r/t was much narrower for deep species than shallow species (Fig. 4) suggests that selective pressure controls r/t in a region of morphospace where slight variations in r/t lead to large changes in strength. It may be that cuttlefish are depth-limited in an evolutionary sense not by an inability to grow stronger cuttlebones (for as Fig. 6 shows, very slight decreases in r/t lead to large increases in strength in deep species' morphospace), but by their inability to control variations in r/t tightly enough. The pillars of deep species tend to be aligned in long rows, which may reduce variation in r, whereas those of many shallow species are more random in orientation.

It is possible that cuttlebone growth is adaptively plastic with regard to strength. Chamber height is known to be influenced by aspects of the environment. Starvation causes cuttlefish to grow shorter chambers (Boletzky, 1974), and such cuttlebones are more resistant to implosion (Ward and Boletzky, 1984). Many species undertake seasonal depth migrations associated with growth and reproduction (*e.g.*, *S. australis*, Roeleveld, 1972; *S. officinalis*, Boletzky, 1983). In *S. officinalis*, more closely spaced chambers are produced in the deeper winter habitat (Denton and Gilpin-Brown,
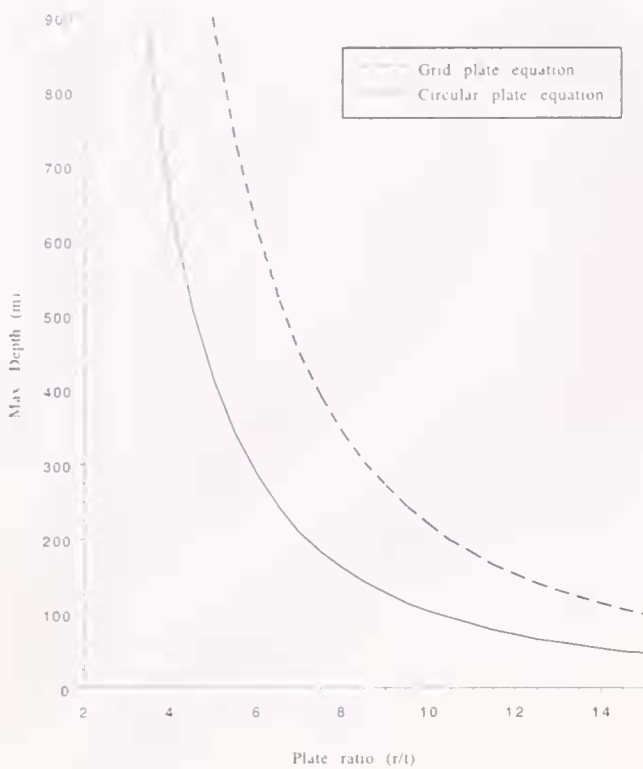
Figure 6. Comparison of predicted maximum depths based on r/t values for two models of cuttlebone strength (see Fig. 3). The curves could also be considered depth estimates based on different values of material strength. Predicted depths do not change greatly in the region of morphospace where shallow species are found ($r/t > 8$) but increase drastically in the morphospace of deep species ($r/t < 6$).

1961d, Hewitt and Stait, 1988). It is uncertain whether these changes are due to depth itself, or to slower growth associated with colder temperatures and reduced feeding, but it is likely to be a combination of both factors.

Other morphologies related to habitat depth

Cuttlebone strength imposes an absolute depth limit, but other aspects of cuttlefish functional morphology are likely to limit habitat depth as well. The cuttlebone faces an osmotic pumping limit: below about 240 m, the siphuncle probably cannot maintain an osmotic gradient to prevent cameral flooding. Reports by Denton and Gilpin-Brown (1961c) and Jacobs (1992) notwithstanding, this argument would appear to apply to *Sepia*, which has a very large siphuncular region. This limit can, however, be surpassed for short periods of time. Even the deep *Sepia* species seem to spend most of their time at the shallower part of their depth range (Roeleveld, 1972), perhaps due to the increasing difficulty of preventing flooding of the cuttlebone at greater depths.

Ward and Boletzky (1984) suggested that a short, narrow cuttlebone, a high radius of septal curvature, and a high density of septal spacing were key features associated with a deeper habitat. In the current study, shallow species had longer and relatively wider cuttlebones than deep species (Table 4). The ratio of cuttlebone thickness to width (a proxy for radius of septal curvature) did not differ significantly. Septal density was significantly higher among deep species. However, this metric combines two morphologies, septal thickness and chamber height, which influence strength in different ways. A thicker septum increases strength, whereas a taller chamber lessens it by affecting the pillars' buckling load.

Why might morphologies not directly related to strength, such as cuttlebone length and relative width, nevertheless correlate with maximum habitat depth? One possibility is that growth rate is limited by depth. Shell volume must increase at the same rate as body volume to maintain neutral buoyancy. In *Nautilus*, growth rate is limited by the rate at which chambers can be emptied, which is reduced at greater habitat depths (Ward and Chamberlain, 1983). Depth-based limitations to growth rate may account for the observations that deep species of *Sepia* generally have small cuttlebones, whereas shallow species in the genus can have either small or large ones (P. Ward, University of Washington, unpubl. data).

Limitations to modeling structural strength from morphology

Sources of error in strength estimates include (1) error in assignment of morphological parameters, (2) simplification of morphology, (3) error in material strength estimate, and (4) disregard for the effects of pillar strength. Nevertheless, the model is fairly robust to error in the range of r/t values observed in cuttlebones (Fig. 6). The fact that two models with different boundary conditions nevertheless give similar and biologically reasonable results also suggests that the estimates for r and t were adequate.

Median values of inter-pillar distance (r) and septal thickness (t) were used to characterize specimens and species, thus obscuring individual and intraspecific variability. Modeling the septum as a flat circular or square plate neglected the fact that regions of unsupported septum are irregular in shape. The septum thickens where it contacts the pillars, which should increase strength beyond the current estimates.

In both models the material strength term is uncertain. Although cuttlebones are composed of prismatic aragonite, a measured value for *Nautilus* nacre was used because it, too, functions in resisting implosion. The value reported for prismatic bivalve shell was only 20% lower (Currey, 1980), so it would not have greatly changed strength estimates. Cuttlebone material strength and implosion pressures were not measured directly because specimens were dry and of varying history. Microcracks and other damage occurs to an

unknown degree, and bending and crushing tests do not provide reliable data under these conditions (Jacobs, 1992). The only fresh specimens readily available are aquacultured *S. officinalis*, and cuttlebones of captive-raised *Sepia* tend to grow abnormally (pers. obs.). Finally, because individual components of the cuttlebone are minute and cutting a sample would probably create microcracks, assessing material strength using a three-point bending test is impractical even with a fresh specimen.

The complex shapes of pillars make them intractable for theoretical analysis, but some qualitative points may be made. Pillar buckling becomes increasingly likely with greater chamber height. In shallow species (which tend to have taller chambers), the pillars are both thinner and more extensively folded than in the deeper species; the latter feature increases structural rigidity. In the current analysis, pillar strength is not addressed: I simply assumed that pillars and septa are equally strong. This is likely to be the case, since the cost incurred for including excess material in a buoyancy device is significant, and strength is limited by the weaker member in the structure.

Conclusion

The cuttlebone is an impressive feat of evolutionary design. Even cuttlebones of shallow species are strong enough to withstand 10 or more atmospheres of pressure. The deep species are caught between the Scylla and Charybdis of strength and buoyancy requirements, and hampered by the major consequences for strength of slight variations in plate ratio morphology. The cuttlebones of *S. australis* and *S. hieronis* may have become as strong as minor tinkering with morphology will permit. Even *Spirula* is limited to depths of 1200 to 1800 m, and its small size may be a result of depth-related limits to chamber emptying rate and hence growth rate. Ironically, only by eschewing the chambered shell altogether, as octopuses and squids have done, can cephalopods invade truly bathyal depths.

The success of the modeling approach at predicting maximum depths in these 11 species of *Sepia* bodes well for its use with extinct forms. Nevertheless, the degree of resolution such a model can achieve needs to be established. Does the variability in the specimen data correspond to variability in strength, or does it reflect the limitations of the modeling method? The current study demonstrates that theoretical strength models can distinguish shallow-water species from those that live several hundred meters deep.

The phragmocone of cephalopods is a buoyancy system that works best, in terms of both strength and emptying capability, in shallow water. This perception has been obscured by the fact that *Nautilus*, the best-known chambered cephalopod, lives at depths of 200 m and greater, essentially in a refugium. *Sepia* may well be a modern-day analog of ammonites in ecology and evolutionary pattern as well as in mode of buoyancy control.

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