



Springer

Dear Author:

Please find attached the final pdf file of your contribution, which can be viewed using the Acrobat Reader, version 3.0 or higher. We would kindly like to draw your attention to the fact that copyright law is also valid for electronic products. This means especially that:

- You may print the file and distribute it amongst your colleagues in the scientific community for scientific and/or personal use.
- You may make your article published by Springer-Verlag available on your personal home page provided the source of the published article is cited and Springer-Verlag and/or other owner is mentioned as copyright holder. You are requested to create a link to the published article in Springer's internet service. The link must be accompanied by the following text: "The original publication is available at [springerlink.com](http://springerlink.com)". Please use the appropriate DOI for the article. Articles disseminated via SpringerLink are indexed, abstracted and referenced by many abstracting and information services, bibliographic networks, subscription agencies, library networks and consortia.
- Without having asked Springer-Verlag for a separate permission your institute/your company is not allowed to place this file on its homepage.
- You may not alter the pdf file, as changes to the published contribution are prohibited by copyright law.
- Please address any queries to the production editor of the journal in question, giving your name, the journal title, volume and first page number.

Yours sincerely,

Springer-Verlag

N. H. Landman · J. K. Cochran · R. Cerrato · J. Mak  
C. F. E. Roper · C. C. Lu

## Habitat and age of the giant squid (*Architeuthis sanctipauli*) inferred from isotopic analyses

Received: 5 August 2002 / Accepted: 3 October 2003 / Published online: 28 November 2003  
© Springer-Verlag 2003

**Abstract** The age and habitat of the giant squid, *Architeuthis sanctipauli* Velain, 1877, were determined based on isotopic analyses of the statoliths of three female specimens captured off Tasmania, Australia, between January and March 1996. Assuming that the aragonite of the statoliths formed in equilibrium with seawater,  $\delta^{18}\text{O}$  analyses indicated that the squid lived at temperatures of 10.5–12.9°C, corresponding to average depths of 125–250 m and maximum depths of 500 m. The capture records indicated that these squid may have occasionally ranged still deeper, to as much as 1000 m. All the statoliths were labeled with bomb  $^{14}\text{C}$  ( $\Delta^{14}\text{C} = +22.9\text{‰}$  to  $+44.6\text{‰}$ ), consistent with the depths inferred from  $\delta^{18}\text{O}$ . A thin section through one of the statoliths revealed 351 growth increments grouped into check-ring structures every 10–16 increments. A model for statolith growth and the pattern of temporal change in  $\Delta^{14}\text{C}$  in the water column was used to estimate the ages of the three specimens. These estimates were very sensitive to the choice of depth range over which  $\Delta^{14}\text{C}$  values were integrated. Assuming that the capture depths represented the maximum habitat depths of these individuals, the calculations suggested an age of 14 years or less. More refined age estimates require a better

understanding of the variation of  $\Delta^{14}\text{C}$  and temperature with depth in the areas in which the squids live.

### Introduction

The giant squid, *Architeuthis sanctipauli*, is a reclusive yet widespread cephalopod about which very little is known. It is increasingly caught in association with deep-sea fisheries around New Zealand and Australia (Zeidler and Gowlett-Holmes 1996; Norman and Lu 1997), although recent efforts to observe this squid in its natural habitat have been unsuccessful (Landman and Ellis 1998; Roper 1998). We report the first isotopic analysis of statoliths of three individuals in order to determine their habitat and age. Statoliths of cephalopods are analogous to the otoliths of fishes and have been used to determine growth rate (Lipinski 1993; Jackson 1994). Statoliths are composed of calcium carbonate in the form of aragonite (Lipinski 1993). Isotopes of carbon ( $^{12}\text{C}$ ,  $^{13}\text{C}$ ,  $^{14}\text{C}$ ) and oxygen ( $^{16}\text{O}$ ,  $^{18}\text{O}$ ) that are incorporated into the statolith can be used as tracers to determine the water temperature and, hence, habitat depth, at which precipitation occurred, and to estimate growth rate and age.

Communicated by J.P. Grassle, New Brunswick

N. H. Landman (✉)  
American Museum of Natural History, 79th Street and Central  
Park West, New York, NY 10024, USA  
E-mail: landman@amnh.org  
Fax: +1-212-7695783

J. K. Cochran · R. Cerrato · J. Mak  
Marine Sciences Research Center, State University of New York,  
Stony Brook, NY 11794, USA

C. F. E. Roper  
National Museum of Natural History, Smithsonian Institution,  
Washington, DC 20560-0118, USA

C. C. Lu  
National Chung Hsing University, 250 Kuo Kuang Road, 402  
Taichung, Taiwan, China

### Materials and methods

We analyzed statoliths from two subadult and one adult female of the giant squid *Architeuthis sanctipauli* Velain, 1877 (Table 1). Two of the squid were collected near one another off the west coast of Tasmania (specimens 1 and 3), as reported by Norman and Lu (1997), and another (specimen 2) was collected off King Island, northwest of Tasmania, as reported by Zeidler and Gowlett-Holmes (1996). The specimens were caught in non-closing trawls by fishing vessels at depths of 460–1000 m. Each statolith was ~2 mm long (range: 2.12–2.15 mm) and ~1.75 mg in mass, comparable in size to statoliths of other *Architeuthis* spp. (Jackson et al. 1991; Roeleveld and Lipinski 1991; Gaudie et al. 1994; Lipinski 1997; Brunetti et al. 1998; see Lipinski 1993 for a discussion of the terminology used to describe statoliths).

**Table 1** *Architeuthis sanctipauli*. Collection dates, sites, and depths, and isotopic data. Squids and statoliths have been deposited in the Museum of Victoria (MV), Melbourne, Australia with the accession numbers shown. Approximate capture location was the location of the deep-sea fishing vessel when squid was caught. Precision is 0.2‰ for  $\delta^{18}\text{O}$  and 0.1‰ for  $\delta^{13}\text{C}$ ; errors are  $\pm 1$  standard deviation for  $\Delta^{14}\text{C}$ .  $\delta^{18}\text{O}$  temperature was calculated using the molluscan aragonite temperature equation (Grossman and Ku 1986) with  $\delta^{18}\text{O}_{\text{water}} = -0.2\text{‰}$  (Bigg and Rohling 2000)

Specimen	Capture date	Stage	Approximate capture location	Dorsal mantle length (cm)	Fishing depth (m)	$\delta^{18}\text{O}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\Delta^{14}\text{C}$ (‰)	$\delta^{18}\text{O}$ temperature (°C)
1 (MV F74482)	22 Mar 1996	Adult	42°54'S; 145°0'E	240	460	2.1	-1.2	+44.6 $\pm$ 9.0	11.0
2 (MV 78297)	1 Oct 1996	Subadult	40°S; 143°9'E-40°15'S; 143°15'E	159 (without fins)	980-1000	1.7	-1.5	+34.0 $\pm$ 9.2	12.9
3 (MV 74479)	14 Mar 1996	Subadult	42°15'S; 144°41'E-42°00'S; 144°35'E	191	840-1000	2.2	-1.1	+22.9 $\pm$ 9.3	10.5

One statolith of each pair was reserved for sectioning and archiving, and the remaining statolith was analyzed for carbon and oxygen isotopes (Fig. 1). Because of their small size, we could not subsample the statoliths and had to analyze them whole. Consequently, the isotopic results integrate over the lifetime of the individual. One statolith from specimen 3 was embedded in epoxy and ground on both sides into a thin section to examine growth increments.

Statoliths analyzed for carbon and oxygen were rinsed in distilled water and then dissolved in 95% orthophosphoric acid for 24 h at 25°C. The resulting  $\text{CO}_2$  was cryogenically transferred to a sample bottle. Stable C and O isotopes were determined using a Finnigan MAT 252;  $^{14}\text{C}$  was determined using accelerator mass spectrometry.  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values are relative to VPDB;  $\Delta^{14}\text{C}$  is calculated taking into account the measured  $\delta^{13}\text{C}$  of the sample (Stuiver and Polach 1977). Precision is  $\pm 0.2\text{‰}$  for  $\delta^{18}\text{O}$  and  $\pm 0.1\text{‰}$  for  $\delta^{13}\text{C}$ . Precision for the  $\Delta^{14}\text{C}$  measurements is ca.  $\pm 9\text{‰}$  (Table 1). Stable isotopic analyses were carried out at the Max Planck Institute for Chemistry (Mainz, Germany), and radiocarbon analyses were performed at Lawrence Livermore Laboratory.

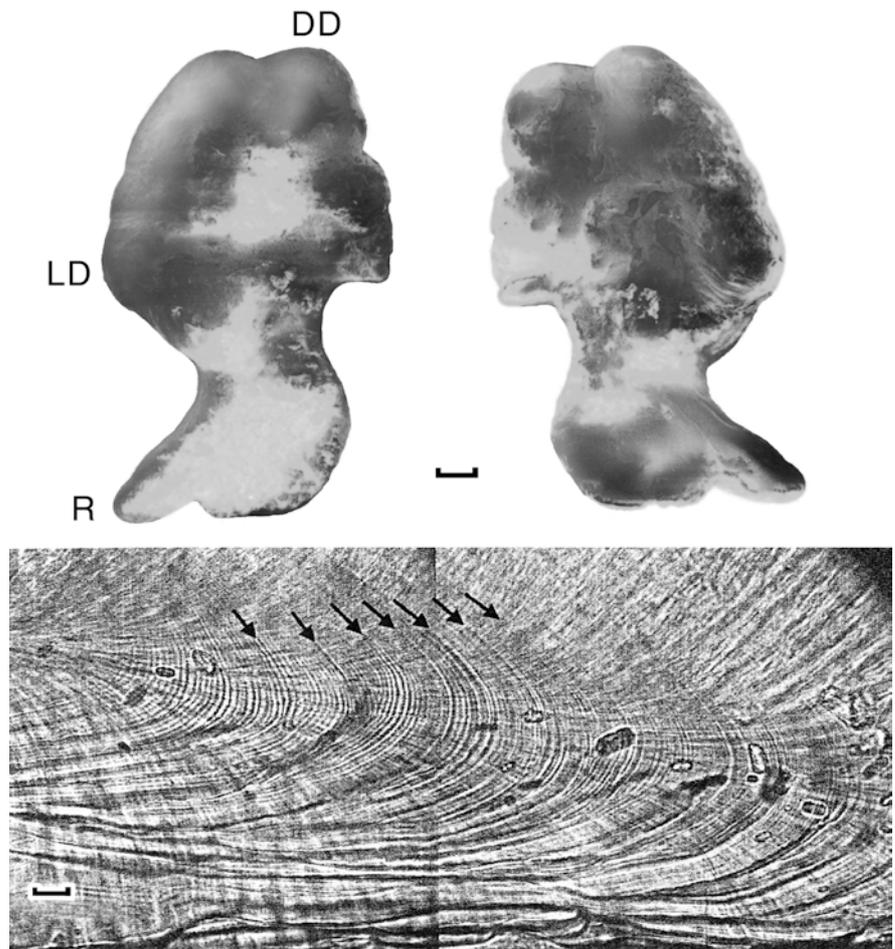
## Results and discussion

### Habitat

The values of  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  for the three specimens are reported in Table 1. The fractionation of oxygen isotopes in calcium carbonate secreted in equilibrium with seawater is temperature dependent (Urey 1947; O'Neil et al. 1969; Grossman and Ku 1986), and the isotopic ratio of  $^{18}\text{O}$  to  $^{16}\text{O}$  can be used to estimate temperatures of formation of the carbonate provided that the  $\delta^{18}\text{O}$  of the water is known. The cephalopod, *Nautilus belauensis*, secretes its shell in isotopic equilibrium with the water in which it lives (Landman et al. 1994). We presumed that the aragonite in the statoliths of these squids was also deposited in equilibrium with seawater and assumed a  $\delta^{18}\text{O}$  value of the water of  $-0.2\text{‰}$  based on the database of seawater  $\delta^{18}\text{O}$  values compiled by Bigg and Rohling (2000). Using the equation pertaining to oxygen isotopic equilibrium between aragonite and water in molluscs (Grossman and Ku 1986), we calculated temperatures of 10.5–12.9°C for the three specimens (Table 1). The uncertainty of these temperatures, based on the precision of the  $\delta^{18}\text{O}$  measurements, is ca.  $\pm 1^\circ\text{C}$ .

These temperatures can be converted to habitat depths, provided that the water column structure in which the squid lived is known (Fig. 2). The best available data are temperature profiles collected in January 1991 from shelf-slope areas off Tasmania and King Island, SE Australia, near the capture localities (Gardiner-Garden et al. 1991). We used the data from the Tasman Shelf to calculate the depths for squids 1 and 3 and the data from the Victoria Shelf to calculate the depth for squid 2. The calculated depths were 220 m for specimen 1, 125 m for specimen 2, and 260 m for specimen 3 (Table 2). These estimates represent averages over the lifetime of the squid and assume that they continuously secreted the statoliths throughout their lives. Interpretation of these estimates is complicated,

**Fig. 1** *Architeuthis sanctipauli*.  
**Upper panel:** anterior (left) and posterior (right) views of statolith from specimen 3 showing dorsal dome (DD), lateral dome (LD), and rostrum (R). Scale bar: 200  $\mu\text{m}$ .  
**Lower panel:** thin section through part of dorsal dome region of statolith from specimen 3. Direction of growth is to the right. Section runs through statolith focus to outer edge of dorsal dome, perpendicular to increments, including maximum growth axis of dorsal dome. Increment width averaged  $\sim 3.2 \mu\text{m}$  and showed no systematic variation over the dorsal dome, yielding an estimate of  $a_1 = 0.80$  (Eq. 3, ASE = 0.01,  $r^2 = 0.96$ ) similar to the exponent (0.53) describing the relationship between total statolith length and increment number in *Ancistrocheirus lesueurii* (Arkhipkin 1997). Arrows indicate location of seven check-rings. Nonlinear regression of statolith weight versus dorsal dome length yielded an allometric exponent of  $a_2 = 1.16$  (Eq. 4, ASE = 0.27,  $r^2 = 0.95$ ). Scale bar: 20  $\mu\text{m}$

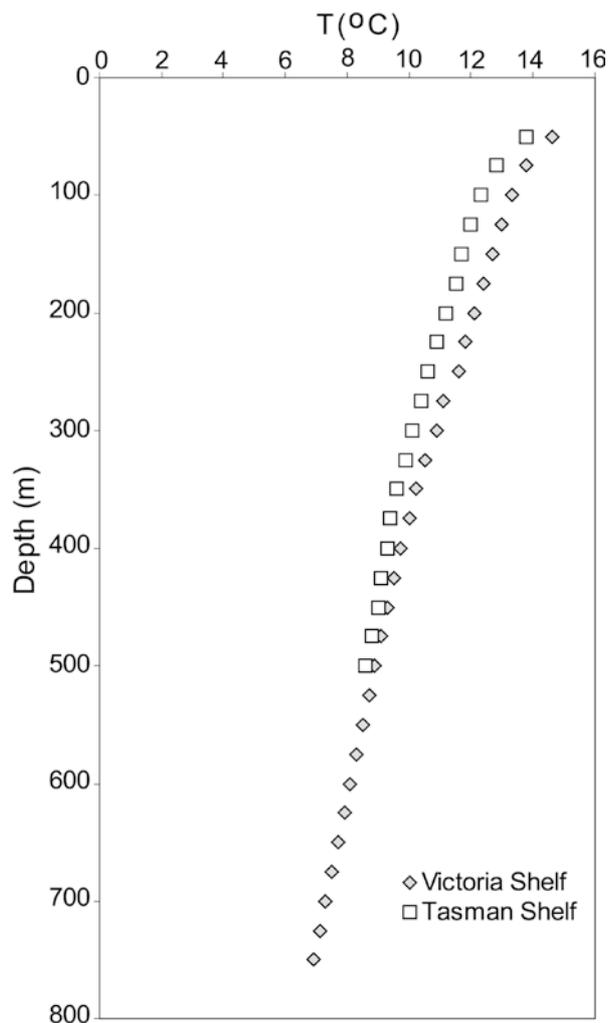


because the squid may have migrated vertically and horizontally or have spent the early part of their lives in shallow water, only descending to depth later on. If we integrate the temperature–depth profile over the water column (with 50 m as the shallowest depth), the calculated temperatures translate into maximum habitat depths of as much as 500 m (Table 2). This is consistent with reported capture depths of 30 *Architeuthis* spp. specimens from New Zealand, which averaged 450–500 m (Förch 1998). The capture records for our specimens suggest that the squids may have occasionally ranged to depths as great as 1000 m (Table 1).

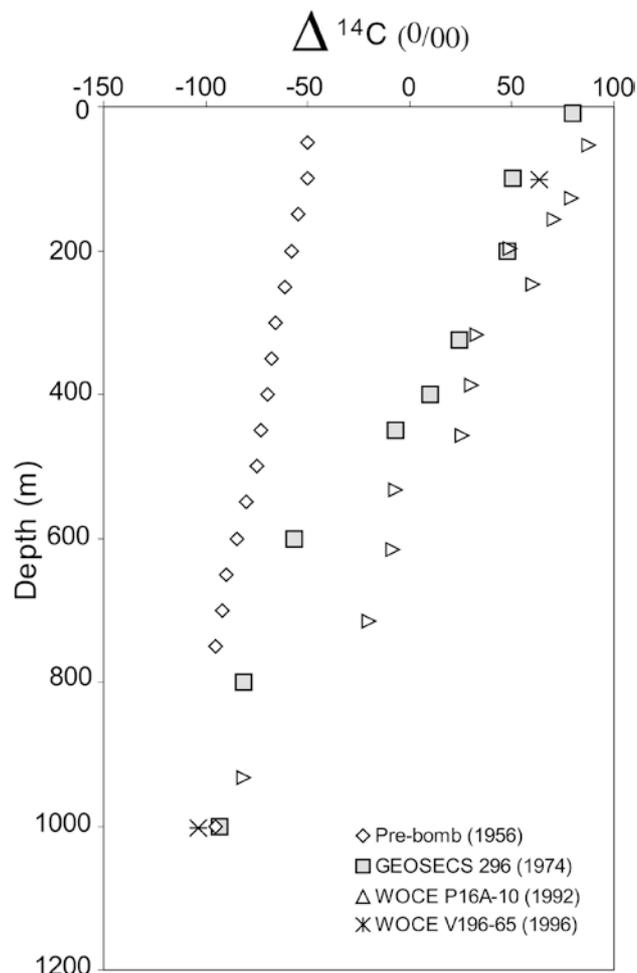
The carbon isotope composition of molluscan carbonate reflects both the  $\delta^{13}\text{C}$  of the dissolved inorganic carbon (DIC), from which the carbonate is precipitated, and any contribution from metabolic carbon (derived from food). Because metabolic processes are temperature dependent, the metabolic contribution may vary with temperature, causing a slight temperature dependence of the  $\delta^{13}\text{C}$  of the precipitated carbonate, with lighter values at higher temperatures (Grossman and Ku 1986; Kalish 1991a; Thorrold et al. 1997). The  $\delta^{13}\text{C}$  values of the specimens range from  $-1.5\text{‰}$  to  $-1.1\text{‰}$  and show a correlation with the  $\delta^{18}\text{O}$ -calculated tempera-

tures (Table 1). The fraction of metabolic carbon incorporated into biogenic carbonates has been estimated at  $\sim 50\%$  in the shells of marine gastropods, bivalves, and barnacles (Tanaka et al. 1986) and  $\sim 30\%$  in the otoliths of Australian salmon (Kalish 1991b). Such calculations require knowledge of the  $\delta^{13}\text{C}$  of the DIC in the water in which the organism lived and the  $\delta^{13}\text{C}$  of the metabolic carbon (usually assumed as equal to the  $\delta^{13}\text{C}$  of the tissue of the organism). We do not know the  $\delta^{13}\text{C}$  of the DIC of the water in which the squid lived, but open-ocean values range from ca.  $+1.5\text{‰}$  to  $+0.5\text{‰}$  in the upper 1000 m of the water column. If the  $\delta^{13}\text{C}_{\text{DIC}}$  of the water in which the squid lived averaged ca.  $+0.7\text{‰}$  and the  $\delta^{13}\text{C}$  of their food was typical of marine carbon, ca.  $-20\text{‰}$ , then using the model of Tanaka et al. (1986) and Kalish (1991b), we calculated that the fraction of metabolic carbon incorporated into the statoliths was  $\sim 35\%$ .

The upper  $\sim 1000$  m of the water column has been strongly affected by the introduction of radiocarbon to the oceans as a consequence of atmospheric testing of atomic weapons (Broecker and Peng 1982). Bomb radiocarbon first became evident in surface waters of the South Pacific after 1956 and increased progressively thereafter until about 1980, when values began to



**Fig. 2** Temperature ( $^{\circ}\text{C}$ ) versus depth (m), January 1991, on the Victoria and Tasman shelves based on Gardiner-Garden et al. (1991)



**Fig. 3**  $\Delta^{14}\text{C}$  ( $\text{‰}$ ) versus depth (m) from Geochemical Ocean Sections Study (*GEOSECS*, 1974) and World Ocean Circulation Experiment (*WOCE*, 1992, 1996) stations closest to squid capture localities

decline (Landman et al. 1988; Druffel and Griffin 1995). Data on depth profiles of  $\Delta^{14}\text{C}$  in the dissolved inorganic carbon reservoir of seawater following the introduction of bomb radiocarbon mainly come from the Geochemical Ocean Sections Study (*GEOSECS*) (Williams et al. 1976) and World Ocean Circulation Experiment

(*WOCE*) expeditions and indicate that the bomb  $^{14}\text{C}$  pulse was mixed progressively deeper with time (Fig. 3). All three squid specimens have positive  $\Delta^{14}\text{C}$  values ( $+22.9 \pm 9.3\text{‰}$  to  $+44.6 \pm 9.0\text{‰}$ , Table 1), indicating that they are labeled with bomb radiocarbon. If the fraction of metabolic carbon in the statoliths is  $\sim 35\%$ ,

**Table 2** *Architeuthis sanctipauli*. Habitat depths,  $\Delta^{14}\text{C}$  parameters, and calculated ages of three specimens. Fishing depth was estimated from  $\delta^{18}\text{O}$ -derived temperatures and temperature–depth

relationships near squid capture sites. Depth range corresponds to depth in column 2;  $b_1$ – $b_4$  are parameters describing temporal trend of  $\Delta^{14}\text{C}_{\text{DIC}}$  over depth range analyzed, see Fig. 4

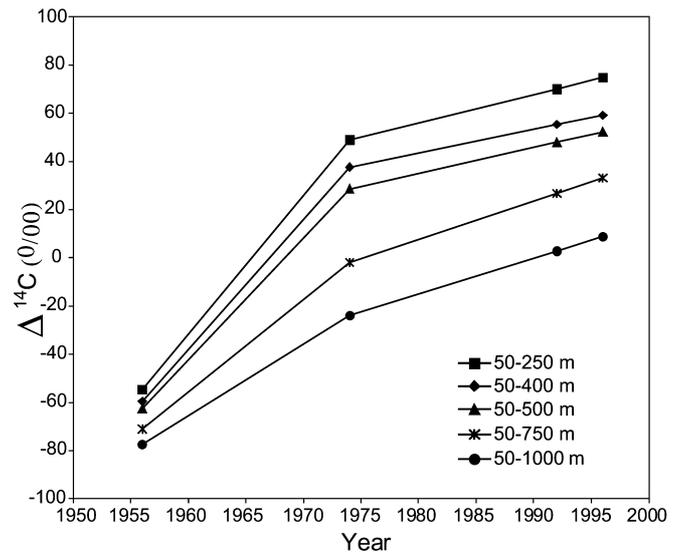
Specimen	Depth (m)	Depth range (m)	$b_1$ ( $\text{‰ year}^{-1}$ )	$b_2$ ( $\text{‰}$ )	$b_3$ ( $\text{‰ year}^{-1}$ )	$b_4$ ( $\text{‰}$ )	Age estimate (years)	95% confidence interval (years)
Scenario 1: depth ranges based on $\delta^{18}\text{O}$ data								
1	$220 \pm 90$	50–400	0.98	37.6	5.41	–59.7	26	0–37
2	$125 \pm 50$	50–250	1.17	49.0	5.76	–54.6	38	27–51
3	$260 \pm 90$	50–500	1.07	28.6	5.07	–62.6	35	20–46
Scenario 2: depth ranges based on capture records								
1	460	50–500	1.07	28.6	5.07	–62.6	14	0–33
2	980–1000	50–750	1.59	–1.9	3.84	–71.1	0	0–21
3	840–1000	50–750	1.59	–1.9	3.84	–71.1	13	0–31

then the  $\Delta^{14}\text{C}$  values of the statoliths dominantly reflect the  $\Delta^{14}\text{C}$  of the dissolved inorganic carbon reservoir. We note that WOCE  $\Delta^{14}\text{C}$  data collected in 1996 (the date the squid were captured), although sparse, show that values of  $\Delta^{14}\text{C}_{\text{DIC}}$  at 1000 m were ca.  $-100\text{‰}$  (Fig. 3). If the squid lived permanently at this depth, were short-lived (i.e. approximately 1 year old), consumed prey with  $\Delta^{14}\text{C}$  values typical of 1996 surface water (ca.  $+75\text{‰}$ , Fig. 3), and incorporated  $\sim 35\%$  metabolic carbon, then the  $\Delta^{14}\text{C}$  values of the statoliths would be ca.  $-34\text{‰}$ . These values represent a maximum because if the squid were longer-lived, they would have consumed prey with lower values of  $\Delta^{14}\text{C}$  reflecting the temporal history of bomb  $\Delta^{14}\text{C}$  in the water column (Fig. 3). The positive  $\Delta^{14}\text{C}$  values of the statoliths thus reinforce the depths inferred from the  $\delta^{18}\text{O}$ -calculated temperatures, suggesting that the squid could not have been living permanently at a depth of 1000 m, but instead lived shallower or experienced a range of depths.

### Age

We can determine the age of each specimen using the  $\Delta^{14}\text{C}$  of the whole statolith, provided that the  $\Delta^{14}\text{C}$  of the statolith is dominated by the  $\Delta^{14}\text{C}$  of the dissolved inorganic carbon reservoir, the temporal change in  $\Delta^{14}\text{C}_{\text{DIC}}$  in the water column is known, and a model of statolith growth is specified. The most detailed data available on temporal changes in  $\Delta^{14}\text{C}$  in the upper water column near the localities where the squid were caught are from stations at the same latitude in the South Pacific. Comparison of the 1974 GEOSECS station 296 ( $44^{\circ}59'\text{S}$ ;  $166^{\circ}42'\text{W}$ ) with the 1992 WOCE station P16A-10 ( $41^{\circ}0'28''\text{S}$ ;  $150^{\circ}30'5''\text{W}$ ) shows increases in  $\Delta^{14}\text{C}$ , as the bomb signal was mixed in the upper  $\sim 1$  km of the water column (Fig. 3). Data on radiocarbon distributions in the Indian Ocean are sparse, especially with respect to temporal variation. However, WOCE station V196-65 ( $44^{\circ}42'\text{S}$ ;  $144^{\circ}6'\text{E}$ ) is closest to the squid capture localities, and, although  $\Delta^{14}\text{C}$  measurements were made only at 100 and 1000 m, the values are comparable to those at similar depths at station P16A-10. For estimating the ages of our specimens, we used the temporal variation in  $\Delta^{14}\text{C}$  recorded at GEOSECS station 296 and WOCE station P16A-10 (Fig. 3; Table 2). The depth profiles of  $\Delta^{14}\text{C}$  were integrated (using trapezoidal integration) over the estimated depth ranges of the squid to obtain the temporal variation of  $\Delta^{14}\text{C}$  in the water in which the squid lived (Fig. 4; Table 2).

The thin section of the statolith from specimen 3 revealed growth increments (Fig. 1) similar to those observed in the statoliths of other *Architeuthis* spp. (Jackson et al. 1991; Lipinski 1993, 1997; Gauldie et al. 1994; Brunetti et al. 1998; Lordan et al. 1998). We counted 351 very regular growth increments averaging  $3.2\ \mu\text{m}$  in width from the nucleus to the edge of the dorsal dome, counting each couplet of dark and light



**Fig. 4** Change in  $\Delta^{14}\text{C}$  versus time over depths of 50–250, 50–400, 50–500, 50–750, and 50–1000 m (Table 2). Depth-integrated WOCE station P16A-10 (1994) and GEOSECS station 296 (1974) data (see Fig. 3) were used to model temporal variation of  $\Delta^{14}\text{C}$  in a piecewise linear function of the form:

$$\Delta^{14}\text{C}(t) = \begin{cases} b_1(t - 1974) + b_2 & 1974 \leq t \leq 1996 \\ b_3(t - 1956) + b_4 & 1956 \leq t \leq 1974 \\ b_4 & t \leq 1956 \end{cases}$$

Year 1956 was taken as the last year for which pre-bomb radiocarbon values were clearly evident at the habitat depth of the squid. Constants  $b_{1-4}$  were derived from (assumed) linear trends of  $\Delta^{14}\text{C}$  variation between time points

bands as a single increment. The growth increments were bounded by 27 or 28 conspicuous check-ring structures similar to those described by Gauldie et al. (1994) for *A. kirki* and by Arkhipkin (1997) for *Ancistrocheirus lesueurii*. (The exact number of check-ring structures is unknown, because it became difficult to detect them near the margin due to the thinness of the thin section.) The mean number of growth increments between successive check-ring structures was 12.8 and ranged from 10 to 16.

To determine an appropriate model for statolith growth, we modeled the relationship between dorsal dome length and increment number as a power function of the form:

$$d = k_1 n^{a_1} \quad (1)$$

where  $d$  is dorsal dome length (not increment width),  $n$  is increment number, and  $k_1$  and  $a_1$  are constants that were estimated by nonlinear least squares regression for the statolith of specimen 3. Except for the substitution of dorsal dome length for total length, Eq. 1 is identical in form to that used for the mesopelagic squid *A. lesueurii* (Arkhipkin 1997). Assuming that growth increments were deposited at periodic intervals, increment number was related to age by a simple linear function of the form:

$$n = k_2(t - t_b) \quad (2)$$

where  $t$  is date,  $t_b$  is birth date, and  $k_2$  is a constant. By utilizing both equations, dorsal dome length was described as a function of age:

$$d = k_3(t - t_b)^{a_1} \quad (3)$$

The final step in developing a statolith growth model was to relate statolith weight to dorsal dome length. An allometric relationship between weight and dorsal dome length was assumed:

$$w = k_4 d^{a_2} \quad (4)$$

where  $w$  is statolith weight, and  $k_4$  and  $a_2$  are constants. Estimates for these constants were obtained by nonlinear least squares regression using statolith weight versus dorsal dome length measurements for all three specimens. The growth model was then obtained by combining Eqs. 3 and 4:

$$w(t - t_b) = k_5(t - t_b)^c \quad (5)$$

where  $c = a_1 a_2$ .

We used whole statolith  $\Delta^{14}\text{C}$  measurements to estimate age by combining the model for statolith growth with estimates of the temporal variation in  $\Delta^{14}\text{C}$  over the range of depths at which the squids lived, assuming that  $\Delta^{14}\text{C}$  was incorporated throughout the lifetime of the organism (Fig. 4). The overall relationship was written in finite difference form:

$$\Delta^{14}\text{C}(t_f - t_b) = \sum_{t=t_b}^{t_f} \frac{\Delta w(t - t_b)}{w(t_f - t_b)} \Delta^{14}\text{C}(t) \quad (6)$$

where  $t_f$  is the time of collection,  $\Delta^{14}\text{C}(t_f - t_b)$  is the measured  $\Delta^{14}\text{C}$  value in the whole statolith,  $w(t_f - t_b)$  is the weight of the statolith,  $\Delta w(t - t_b)$  is the growth increment of the statolith between time  $t$  and time  $t + \Delta t$ , and  $\Delta^{14}\text{C}(t)$  is the estimate of  $\Delta^{14}\text{C}$  in the water at time  $t$ . Using Eq. 6, the fraction of the statolith weight added between time  $t$  and time  $t + \Delta t$  can be expressed as:

$$\begin{aligned} \frac{\Delta w(t - t_b)}{w(t_f - t_b)} &= \frac{[w(t + \Delta t - t_b) - w(t - t_b)]}{w(t_f - t_b)} \\ &= \frac{[(t + \Delta t - t_b)^c - (t - t_b)^c]}{(t_f - t_b)^c} \end{aligned} \quad (7)$$

Substituting Eq. 7 into Eq. 6 yields the final expression:

$$\Delta^{14}\text{C}(t_f - t_b) = \sum_{t=t_b}^{t_f - \Delta t} \frac{[(t + \Delta t - t_b)^c - (t - t_b)^c]}{(t_f - t_b)^c} \Delta^{14}\text{C}(t) \quad (8)$$

Given  $\Delta^{14}\text{C}(t_f - t_b)$ , the measured  $\Delta^{14}\text{C}$  value in the whole statolith, an estimate of the exponent  $c$  obtained from nonlinear regressions of dorsal dome length versus increment number and statolith weight versus dorsal dome length ( $c = a_1 a_2$ ), and the temporal variation in the value of  $\Delta^{14}\text{C}$  in the water, Eq. 8 was solved iteratively for the date of hatching of each specimen. We note that

one component of  $c$  (i.e.  $a_1$ ) is based on an analysis of growth increments from the statolith from specimen 3, and both the age and errors estimated for specimens 1 and 2 assume that the estimate for  $a_1$  can also be applied to these two other specimens. This is a reasonable assumption because variation in age is more sensitive to measurement error than to variability in the value of the exponent  $c$ .

Determination of the age of the specimens is very dependant on the choice of depth range over which we integrate  $\Delta^{14}\text{C}$ . We present two scenarios. In the first scenario, we used the depth range inferred from the  $\delta^{18}\text{O}$  data. The calculated age estimates range from 26 to 38 years (Table 2). We performed a Monte Carlo analysis of error, taking into account the analytical error for the  $\Delta^{14}\text{C}$  values as well as the estimated errors for the parameters in  $c$  in Eq. 8. This yielded 95% confidence intervals of 0–37 years for specimen 1, 27–51 years for specimen 2, and 20–46 years for specimen 3 (Table 2). The range of overlap of the confidence intervals for all three specimens is 27–37 years. These ages suggest that the growth increments in the statolith of specimen 3 could be lunar monthly in origin, perhaps related to increased feeding in shallow water during the new or full moon. This would, therefore, imply that the check-ring structures are annual, perhaps related to migratory activity, and that the age of this squid is approximately 29 years. However, a lunar monthly periodicity of growth increment formation has not been documented in any other squid.

In the second scenario, we recalculated the ages of the squids using a depth range more consistent with the reported depth of capture of the specimens, with 50 m as the shallowest depth. We selected a maximum depth of 750 m for specimens 2 and 3, because integrating to 1000 m produced values of  $\Delta^{14}\text{C}$  less than those observed in the specimens. The recalculated ages are 14 years for specimen 1, 0 for specimen 2, and 13 years for specimen 3, implying an overall age of 14 years or less, if the specimens are considered together (Table 2). A Monte Carlo analysis of error yielded 95% confidence intervals of 0–33 years for specimen 1, 0–21 years for specimen 2, and 0–31 years for specimen 3. The range of overlap of the confidence intervals for all three specimens is 0–21 years. This large range of variation could easily accommodate the possibility that the growth increments in the statolith of specimen 3 were secreted daily and, therefore, that the age of this squid is approximately 1 year.

The two scenarios yield a two- to threefold difference in age estimate (Table 2). The results from the second scenario are more consistent with the ages of most other squid (Forsythe and Van Heukelem 1987). Published hypotheses about the age of the giant squid range from 1 to 3 years, based on the assumption that the growth increments in the statoliths are deposited daily (Jackson et al. 1991; Gauldie et al. 1994; Lipinski 1997; Brunetti et al. 1998; Lordan et al. 1998). On the other hand, Wood and O'Dor (2000) determined the relationship

between age and size in cephalopods based on a sample of 18 different species, and concluded that larger cephalopods take longer to reach maturity. Their sample of cephalopods was restricted to species < 17 kg in mature weight. However, using their equation (excluding *Nautilus* spp.) and assuming an average weight of 150 kg for a female giant squid comparable in size to those studied here, yields an age of about 13 years, consistent with the age estimates of specimens 1 and 3 in scenario 2.

Selecting among these various estimates is difficult. Our approach requires assumptions about the temporal change in  $\Delta^{14}\text{C}$  in the water column, the range of depths over which the squid live, and the mode of growth of the statoliths. In many ways, our results provide a blueprint for further studies. However, these studies require a better understanding of the physical and chemical properties of the water column in which the squids live, especially the variation in  $\Delta^{14}\text{C}$  and temperature with depth, and more specimens covering a wider range of ontogenetic stages. Such investigations will provide more information on the age and habitat of the giant squid and improve the chances of finding live specimens.

**Acknowledgements** WOCE data for station P16A-10 were provided by Dr. E. Druffel, University of California, Irvine. WOCE data from the Indian Ocean stations were provided by Dr. B. Tilbrook, CSIRO Marine Research, Australia. We thank S. O'Shea (Auckland University of Technology, New Zealand) for helpful comments on an earlier version of this manuscript.

## References

- Arkhipkin AI (1997) Age and growth of the mesopelagic squid *Ancistrocheirus lesueurii* (Oegopsida: Ancistrocheiridae) from the central-east Atlantic based on statolith microstructure. *Mar Biol* 129:103–111
- Bigg GR, Rohling EJ (2000) An oxygen isotope data set for marine water. *J Geophys Res* 105:8527–8535
- Broecker WS, Peng TH (1982) Tracers in the sea. Eldigio Press, Lamont Doherty Geological Observatory, Palisades, N.Y., USA
- Brunetti NE, Elena B Rossi GR, Sakai M, Pineda SE, Ivanovic ML (1998) Description of an *Architeuthis* from Argentine waters. In: Payne AIL, Lipinski MR, Clark MR, Roeleveld MAC (eds) Cephalopod biodiversity ecology and evolution. *S Afr J Mar Sci* 20:355–362
- Druffel ERM, Griffin S (1995) Regional variability of surface ocean radiocarbon from southern Great Barrier Reef corals. In: Cook GT, Harkness DD, Miller BF, Scott EM (eds) Proc 15th Int  $^{14}\text{C}$  Conf. *Radiocarbon* 37:517–524
- Förch EC (1998) The marine fauna of New Zealand: Cephalopoda: Oegopsida: Architeuthidae (giant squid). NIWA Biodiversity Mem 110:1–113
- Forsythe JW, Van Heukelem WF (1987) Growth. In: Boyle PK (ed) Cephalopod life cycles, vol 2. Academic, London
- Gardiner-Garden RS, Evans S, Middleton JF, Craig P (1991) A description of oceanographic data from Bass Strait, Jan–May 1991. University of New South Wales, Kensington, Australia
- Gauldie RW, West IF, Förch EC (1994) Statocyst, statolith, and age estimation of the giant squid *Architeuthis kirki*. *Veliger* 37:93–109
- Grossman EL, Ku T-L (1986) Oxygen and carbon isotope fractionation in biogenic aragonite: temperature effects. *Chem Geol* 59:59–74
- Jackson GD (1994) Application and further potential of statolith increment analysis in squids and sepioids. *Can J Fish Aquat Sci* 51:2612–2625
- Jackson GD, Lu CC, Dunning M (1991) Growth rings within the statolith microstructure of the giant squid *Architeuthis*. *Veliger* 34:331–334
- Kalish JM (1991a)  $^{13}\text{C}$  and  $^{18}\text{O}$  isotopic disequilibrium in fish otoliths: metabolic and kinetic effects. *Mar Ecol Prog Ser* 75:191–203
- Kalish JM (1991b) Oxygen and carbon isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). *Mar Biol* 110:37–47
- Landman NH, Ellis R (1998) *Architeuthis*—at last. *Curator* 41:150–152
- Landman NH, Druffel ERM, Cochran JK, Donahue DJ, Jull AJT (1988) Bomb-produced radiocarbon in the shell of the chambered nautilus: rate of growth and age at maturity. *Earth Planet Sci Lett* 89:28–34
- Landman NH, Cochran JK, Rye DM, Tanabe K, Arnold JM (1994) Early life history of *Nautilus*: evidence from isotopic analyses of aquarium-reared specimens. *Paleobiology* 20:40–51
- Lipinski MR (1993) The deposition of statoliths: a working hypothesis. In: Okutani T, O'Dor RK, Kubodera TT (eds) Recent advances in fisheries biology. Tokai University Press, Tokyo, pp 241–262
- Lipinski MR (1997) Morphology of giant squid *Architeuthis* statoliths. *S Afr J Mar Sci* 18:299–303
- Lordan C, Collins MA, Perales-Raya C (1998) Observations on morphology, age and diet of three *Architeuthis* caught off the west coast of Ireland in 1995. *J Mar Biol Assoc UK* 78:903–917
- Norman MD, Lu CC (1997) Sex in giant squid. *Nature* 389:683–684
- O'Neil JR, Clayton RN, Mayeda K (1969) Oxygen isotope fractionation in divalent metal carbonates. *J Chem Phys* 51:5547–5558
- Roeleveld MAC, Lipinski MR (1991) The giant squid *Architeuthis* in southern African waters. *J Zool (Lond)* 224:431–477
- Roper CFE (1998) Tracking the giant squid: mythology and science meet beneath the sea. *Wings* 21:12–17
- Stuiver M, Polach HA (1977) Discussion: reporting of  $^{14}\text{C}$  data. *Radiocarbon* 19:355–363
- Tanaka N, Monaghan MC, Rye DM (1986) Contribution of metabolic carbon to mollusc and barnacle shell carbonate. *Nature* 320:520–523
- Thorrold SR, Campana SE, Jones CM, Swart PK (1997) Factors determining  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  fractionation in aragonitic otoliths of marine fish. *Geochim Cosmochim Acta* 61:2909–2919
- Urey HC (1947) The thermodynamic properties of isotopic substances. *J Chem Soc* 1947:562–581
- Williams R, Mantyla A, Sanborn KM, Wylie JG (1976) GEOSECS Pacific Expedition: sections and profiles, vol 4. National Science Foundation, Washington, D.C., USA
- Wood JB, O'Dor RK (2000) Do larger cephalopods live longer? Effects of temperature and phylogeny on interspecific comparisons of age and size at maturity. *Mar Biol* 136:91–99
- Zeidler W, Gowlett-Holmes KL (1996) A specimen of giant squid, *Architeuthis* sp. from South Australian waters. *Rec S Aust Mus* 29:85–91