

# Statolith microchemistry traces the environmental history of the boreoatlantic armhook squid *Gonatus fabricii*

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**ABSTRACT:** Statoliths of the gonatid squid *Gonatus fabricii* from Disko Bay, West Greenland, were analysed by laser ablation inductively coupled plasma mass-spectrometry (LA-ICP-MS) to determine the concentrations and spatial distributions of 9 minor and trace elements (Sr, Ba, Mg, U, Zn, Mn, Y, Zr, Na). Element composition was assigned *in situ* to distinct statolith regions, corresponding to ontogenetic stages of an individual squid's life. Significant variations in concentrations of all measured elements except Na were found between different regions of the statolith. Variations in Ba/Ca ratios suggest that juveniles inhabit surface waters, while larger specimens move to deeper waters. U/Ca and Sr/Ca ratios increased towards the outer statolith region, suggesting migration of adult squid into colder waters. Mg/Ca ratios decreased progressively from the core to the edge, most likely related to changes in protein concentrations in their microstructure during ontogenesis. This study is one of the first to apply LA-ICP-MS to cephalopod statoliths. Our results emphasise the strong potential of spatially resolved statolith analyses to gather information on life history, migrations and habitat use of cephalopods.

**KEY WORDS:** Statolith · Trace elements · Laser ablation ICP-MS · Cephalopods · Squid · *Gonatus fabricii* · Greenland

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## INTRODUCTION

The boreoatlantic armhook squid *Gonatus fabricii* (Lichtenstein, 1818) is the most abundant squid of the arctic and subarctic waters of the North Atlantic and is considered to have some commercial potential (Nesis 1965, Kristensen 1984, Zumholz & Frandsen 2006). This species is frequently found in the stomachs of marine mammals and fishes (Lick & Piatkowski 1998, Bjørke 2001) and is likely to play an important role in the ecosystem off West Greenland. *G. fabricii* is caught frequently as by-catch in the fishery of deep-water prawn *Pandalus borealis*, and locally it is kept and used as bait. The life cycle of the squid is characterised by well-defined ontogenetic vertical migrations as described by Arkhipkin & Bjørke (1999). Hatching is pro-

posed to take place at great depths (Arkhipkin & Bjørke 1999). Its early life stages are found in large numbers in west Greenland epipelagic waters (Piatkowski & Wieland 1993), while larger squids occur mainly in mesopelagic and bathypelagic layers (Kristensen 1984).

Trace element analysis of biomineralised tissues has become a frequently used method for determining environmental histories of many aquatic organisms. Although more commonly applied to fish otoliths (Campana 1999, 2005) and corals (e.g. Smith et al. 1979), many other marine organisms have calcified structures that may be similarly useful as natural tags (Zacherl et al. 2003a, Becker et al. 2005). Cephalopod statoliths share many structural similarities with fish otoliths (Clarke 1978). They are composed primarily of

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aragonite crystals associated with an organic matrix (Radtke 1983). Alternating layers of organic-rich and organic-poor material are deposited periodically. The 'one increment per day' hypothesis has been validated for several species (Jackson 1994, Bettencourt & Guerra 2001), though not for *G. fabricii*. A comprehensive review of recent developments in statolith studies is given by Arkhipkin (2005).

Several studies have shown that statoliths contain a number of elements at minor and trace levels (e.g. Hurley et al. 1985, Durholtz et al. 1997, Lipinski et al. 1997, Yatsu et al. 1998, Ikeda et al. 2002a,b, Arkhipkin et al. 2004). Fundamental results on the biomineralisation process have been presented by Bettencourt & Guerra (2000), but the exact principles of elemental uptake into statoliths are still unknown. Strontium has been shown to be essential for normal statolith development (Hanlon et al. 1989), while magnesium may also be a key factor in the calcification process within the statocyst (Morris 1991, Bettencourt & Guerra 2000). Inspired by an inverse relationship between Sr/Ca and temperature in scleractinian corals (Beck et al. 1992), several studies have attempted to use Sr/Ca ratios in statoliths as a temperature proxy (e.g. Rodhouse et al. 1994, Ikeda et al. 2003). Arkhipkin et al. (2004) showed a good relationship between Sr/Ca and ambient temperature in statoliths of the Patagonian longfin squid *Loligo gahi* collected in the field. However, no experimental studies have shown evidence that Sr/Ca ratios in squid statoliths are temperature dependent.



Fig. 1. Location of Disko Bay, West Greenland (box), where specimens of *Gonatus fabricii* were sampled from bottom trawl hauls in 2003. Map plotted with Ocean DataView (Schlitzer 2007)

The analysis of elemental signatures of whole dissolved statoliths ('elemental fingerprints') makes it possible to distinguish between populations and cohorts. This has been demonstrated convincingly for *Loligo gahi* (Arkhipkin et al. 2004). Furthermore, the same authors highlighted the potential of spatially resolved elemental analyses to determine stage-specific migrations and habitat use.

Based on optical increment analysis, Kristensen (1980) and Arkhipkin & Bjørke (2000) defined 4 distinct growth zones (Z1, Z2, Z3, Z4) in the statoliths of *Gonatus fabricii* corresponding to different ontogenetic stages and thereby different habitat use, separated from each other by distinct checks. Z1 represents the embryo phase before hatching; Z2 and Z3 develop during the juvenile phase (up to 60 mm pen length). Z4 is formed during the subadult and adult periods.

No published information on the chemical composition of *Gonatus fabricii* statoliths has been available until now. In this study, transects on ground *G. fabricii* statoliths from the core to the edge were analysed, thereby determining the chemical composition of the different growth zones. The aim of this study was (1) to quantify the elemental composition and spatial distribution of elements within *G. fabricii* statoliths and (2) to find possible relations between statolith elemental composition and the habitat use and life history of individual squids.

## MATERIALS AND METHODS

**Sample collection.** Specimens were sampled on board the RV 'Paamiut' as by-catch during a bottom trawl survey targeting deep-water prawn *Pandalus borealis* from July to August 2003 in Disko Bay, West Greenland (Fig. 1). Cephalopods were frozen on board immediately after capture and defrosted for further analyses at IFM-GEOMAR, Kiel. Sex was determined, and maturity stages (I–II, immature; III–IV, maturing; V, mature) were estimated according to the appearance and relative size of the reproductive organs (Lipinski 1979). Pen length (PL) was measured to the nearest 1 mm; total body wet mass (BM) was measured to the nearest g. The sample used for this study consisted of 16 males (all specimens were assigned to maturity stage III) and 10 females (all specimens were assigned to maturity stage II). All specimens were caught within 3 hauls on the same day in the same area (stations: 68° 57.51' N, 52° 07.35' W; 69° 09.46' N, 53° 20.15' W; 69° 09.46' N, 53° 02.85' W). The statoliths were dissected from the cephalic cartilage, washed in distilled water, stored in Eppendorf caps and kept dry.

**Statolith processing and reading.** In total, 26 statoliths were prepared and analysed. One statolith from

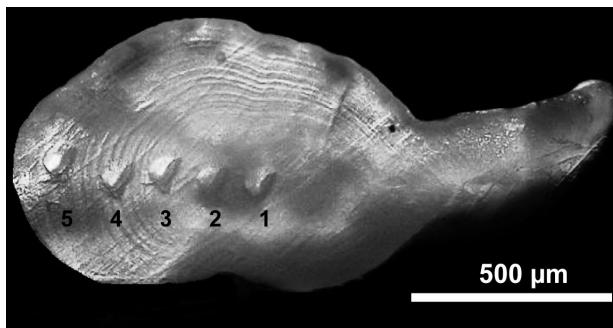


Fig. 2. *Gonatus fabricii*. Ground statolith showing the location of analytical points as laser ablation craters. Points are numbered from the core to the edge

each pair was attached concave side up onto a microscopic glass slide with thermoplastic resin (Buehler) and was ground on one side with wet waterprooflapping film (3M®; 30 µm, 15 µm, 3 µm) until the ground surface extended to the edge (i.e. the most extended point) of the dorsal dome, and the core (corresponding to the embryonic phase) could be seen just beneath the ground surface. Ground statoliths were finally polished with Buehler micropolish II (0.3 µm and 0.05 µm) and observed under a Leitz Laborlux K microscope (250× magnification) to locate the growth zones as defined by Kristensen (1980).

**Elemental analyses.** The polished statoliths used for element analysis were triple-rinsed in MilliQ water (>18 MΩ cm). Five analytical points were located along a transect from the core to the edge of each statolith (Fig. 2). The first point was placed in the core, i.e. the region laid down before hatching, which was designated zone Z1 according to Kristensen (1980). Points 2 and 3 were placed in the post-nuclear zones Z2 and Z3, while Points 4 and 5 both were located in Z4, representing the subadult and adult phase in a squid's life (Kristensen 1980, Arkhipkin & Bjørke 1999). In some cases, especially at the core, the measured element ratios changed after some time when the laser drilled into a different zone. Here only the first part of the time series was integrated for data quantification to obtain a true 2-dimensional profile along the sample surface.

The chemical analyses of statoliths were carried out at the Institute of Geosciences, University of Bremen, using a 266 nm frequency-quadrupled Nd:YAG laser (Finnigan UV LaserProbe) coupled to a ThermoFinnigan Element2 ICP-MS. Helium was used as sample gas in the ablation cell ( $0.4 \text{ l min}^{-1}$ ) and argon was subsequently added ( $0.8 \text{ l min}^{-1}$ ) to the gas flow. Ablation parameters included a laser energy of 2 to 2.5 mJ (Q-switched mode) and a pulse rate of 5 Hz resulting in crater sizes of 60 to 80 µm. Eleven isotopes were

recorded during 90 s with 5 samples at each peak's flat top and a total dwell time of 50 ms per isotope. Owing to the lack of matrix-matched standards, the glass reference materials NIST610 (for  $^{23}\text{Na}$ ,  $^{43}\text{Ca}$ ,  $^{88}\text{Sr}$ ) and NIST612 (for  $^{23}\text{Na}$ ,  $^{25}\text{Mg}$ ,  $^{43}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{64}\text{Zn}$ ,  $^{89}\text{Y}$ ,  $^{90}\text{Zr}$ ,  $^{93}\text{Nb}$ ,  $^{138}\text{Ba}$ ,  $^{238}\text{U}$ ) were used for external calibration taking the accepted concentrations of Pearce et al. (1997). Both standards were analysed and a new calibration line established after every 5 to 11 data points (standard bracketing) to compensate for any instrumental drift. GeoPro software was used to define a blank of 8–20 s duration and a stable signal period of 15–50 s duration following a pre-ablation period of 5–13 s for each measured point. For quantification, the blank was subtracted and Ca was used as internal standard element with an assumed stoichiometric concentration of 40 wt%. Single-point calibration was preferred over 2-point calibration, because the 2 differently coloured NIST standards show distinct ablation behaviour and element fractionation at a laser wavelength of 266 nm (Guillong et al. 2003).

The external analytical precision was determined through replicate analyses of the homogeneous standard reference materials NIST612 and GOR128G (basaltic glass with low concentrations of some trace elements; Jochum et al. 2000). The data indicate a precision of better than 3% for concentrations above  $1 \mu\text{g g}^{-1}$  (Na, Sr, Mg, Ba, Mn and Zn) and better than 13% for concentrations between 0.01 and  $1 \mu\text{g g}^{-1}$  (Y, Zr and Nb). Assessing the accuracy is difficult due to the lack of a homogeneous matrix-matched reference material. Analyses of GOR128G yielded a good to moderate accuracy (Table 1a), which was limited by concentrations far outside the calibration range for some elements (Na, Mn) as well as by different element fractionation of the translucent calibration standard and the opaque basaltic glass (Guillong et al. 2003).

We also analysed a powdered *Arctica islandica* bivalve shell both by laser ablation inductively coupled plasma mass-spectrometry (LA-ICP-MS), using the same analytical method and parameters as for statolith analyses, and by solution ICP-MS calibrated with concentration-matched pure element standards. Even though the measured concentrations covered a wide range of  $10^{-3}$  to  $10^3 \mu\text{g g}^{-1}$ , the laser ablation data showed excellent to moderate accuracy (Table 1b).

**Statistical analysis.** Two-way analysis of variance (ANOVA) was used to test for significant differences in element/Ca ratios among specimens and between the statolith growth zones (location of measurements). Specimens were treated as random variables, locations of measurements were treated as categorical variables and element/Ca ratios were treated as dependent variables in the analyses.

Table 1. (a) Accuracy and reproducibility (as expressed by the relative standard deviation, RSD) of 10 consecutive LA-ICP-MS analyses of basaltic glass reference material GOR128G (Jochum et al. 2000) using the same analytical method and parameters as for statolith analyses. (b) Averaged results of 10 replicate LA-ICP-MS analyses of a pressed pellet made from a powdered *Arctica islandica* bivalve shell, which has trace element concentrations similar to statoliths, as compared to 2 analyses of the same powder by solution ICP-MS calibrated at appropriate concentrations with pure element standards. Despite a wide range of measured concentrations, there is good agreement between both methods for most elements

(a) Element	Average ( $\mu\text{g g}^{-1}$ )	Ref. value ( $\mu\text{g g}^{-1}$ )	RSD (%)
Sodium	3906	4130	1.5
Strontium	28.17	31	1.4
Barium	0.954	1.09	1.7
Zinc	84.52	74	3.6
Manganese	1247	1390	1.1
Yttrium	9.91	11.3	1.4
Zirconium	8.08	10.2	1.4
Niobium	0.085	0.11	4.5
Uranium	0.011	0.013	13.0

(b) Element	LA-ICP-MS ( $\mu\text{g g}^{-1}$ )	Solution 1 ( $\mu\text{g g}^{-1}$ )	Solution 2 ( $\mu\text{g g}^{-1}$ )
Strontium	1049	1186	1195
Magnesium	126.1	109	102
Barium	11.06	11.89	11.83
Zinc	0.847	1.38	1.09
Yttrium	0.023	0.020	0.020
Zirconium	0.046	0.017	0.027
Niobium	0.007	0.0020	0.0019
Uranium	0.010	0.0109	0.0107

Table 2. *Gonatus fabricii*. Ranges of concentrations and element/Ca ratios in statoliths measured by LA-ICP-MS. In the right column, data from Arkhipkin et al. (2004) for *Loligo gahi* statoliths, measured by solution-based ICP-MS, are listed for comparison

Element	Concentration range ( $\mu\text{g g}^{-1}$ )	Element/Ca ratio range	
		( $\mu\text{mol mol}^{-1}$ ) <i>Gonatus fabricii</i>	( $\mu\text{mol mol}^{-1}$ ) <i>Loligo gahi</i>
Sodium	3200 to 5050	15 000 to 22 000	
Strontium	5500 to 7100	6300 to 8100	~8000 to 8500
Magnesium	25 to 140	110 to 590	~70 to 350
Barium	7.8 to 11.3	5.7 to 8.2	~3 to 8
Zinc	0.6 to 2.9	0.9 to 4.5	
Manganese	1.8 to 3.7	3.2 to 6.8	~1 to 3
Yttrium	0.02 to 0.14	0.056 to 0.081	
Zirconium	0.01 to 0.026	0.012 to 0.029	
Uranium	0.005 to 0.022	0.002 to 0.009	
Lead	0.013 to 0.076	0.006 to 0.037	~0.002 to 0.030

Table 3. *Gonatus fabricii*. Analysis of variance (ANOVA) table summarising the effect of statolith measurement location on element/Ca ratios. df: degrees of freedom; SS: sum of squares; MS: mean squares

Element	df	SS	MS	F	p
Strontium	4	8.19	2.05	16.65	0.0001
Barium	4	4.17	1.04	4.90	0.0009
Magnesium	4	173540	43385	2.88	0.026
Sodium	4	2.51	0.63	0.20	0.936
Manganese	4	41.98	10.50	15.16	0.0001
Zinc	4	27.24	6.8	3.54	0.009
Yttrium	4	919.0	229.70	3.79	0.0059
Zirconium	4	267.62	66.90	5.99	0.0002
Uranium	4	124.21	31.10	32.23	0.0001

## RESULTS

The pen length of analysed squid ranged from 177 to 310 mm, corresponding to a weight range of 84 to 470 g. Total statolith length as defined by Arkhipkin & Bjørke (2000) ranged from 1.24 to 1.48 mm. Nine minor and trace elements (Sr, Ba, Mg, Mn, U, Zr, Y, Zn and Na) were analysed in the statoliths by LA-ICP-MS. The ranges of element/Ca ratios are given in Table 2. The lateral resolution achieved by laser ablation in this study, approximately 70  $\mu\text{m}$ , was adequate to sample single ontogenetic stages on the polished statolith surface.

The 2-way ANOVA test found no significant effect of specimens on the statolith elemental composition ( $p > 0.5$ ) and no interaction between specimens and location of measurements. The location of measurements, however, showed significant effects for all analysed elements except sodium (Table 3). Post-hoc Tukey multiple comparisons revealed significant differences in the elemental composition between distinct regions in the statoliths (Fig. 3) as described below.

Sr/Ca ratios were highest in the core region, decreasing towards the middle and slightly increasing towards the edge. Core values were significantly higher than all other points ( $p < 0.0003$ ). A significant difference was also found between Points 3 and 5 ( $p < 0.03$ ).

Ba/Ca ratios showed considerable variation within the post-hatch Zones Z2 to Z4. Concentrations in the outer regions (Points 4 and 5) were significantly higher than Points 2 and 3 ( $p < 0.007$ ). However, variations between the core and the rest were not significant.

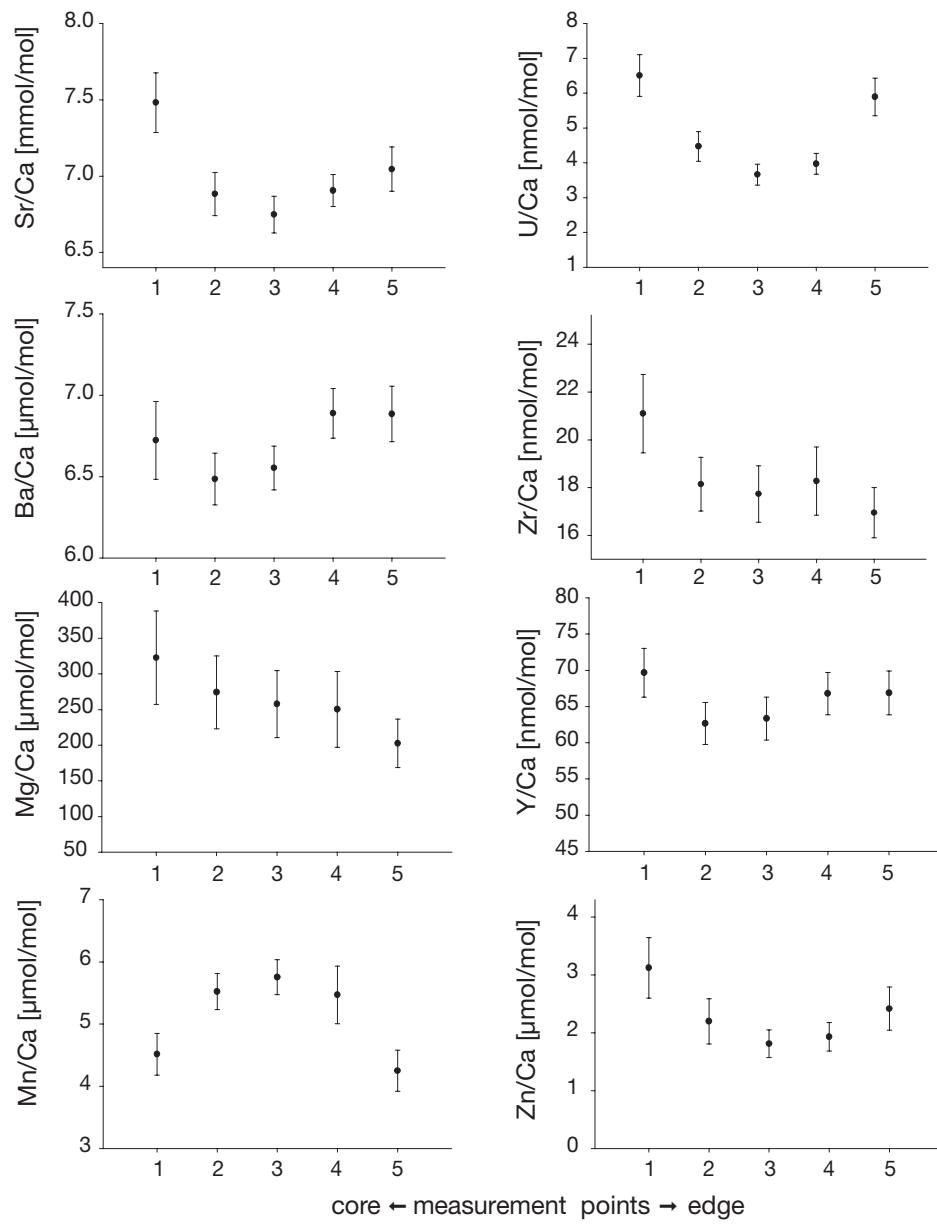


Fig. 3. *Gonatus fabricii*. Element/Ca ratios measured by LA-ICP-MS in different regions of statoliths from the core to the edge. Point numbers refer to numbers given in Fig. 2. Point 1 = Growth Zone Z1; Point 2 = Growth Zone Z2; Point 3 = Growth Zone Z3; Points 4 and 5 = Growth Zone Z4. Each point represents the mean of measurements in 26 statoliths, error bars indicate 95% confidence intervals

Mg/Ca ratios were generally high in the core, with the lowest values in the outermost region. Significant differences were observed only between Points 1 and 5 ( $p = 0.01$ ).

Mn/Ca ratios showed a dome-shaped pattern along the transect, with low values in the core and the outer region and the highest values in the middle. This pattern was negatively related to the Sr distribution. Values for Points 1 and 5 were significantly different from Points 2, 3 and 4 ( $p < 0.003$ ).

U/Ca ratios followed a U-shaped pattern along the transect, with high values in the core and the outer region and lower values in the middle. This pattern was very similar to the Sr/Ca distribution. Values for Points 1 and 5 were significantly different from Points 2, 3 and 4 ( $p < 0.0002$ ).

Zr/Ca ratios showed the highest values in the core, with lower values in the outer regions. Core values were significantly higher than all other points ( $p < 0.002$ ).

Y/Ca ratios in the core were significantly higher than in Growth Zones 2 and 3 ( $p < 0.008$ ), but did not differ from Growth Zone 4.

Zn/Ca ratios showed a similar pattern to Sr/Ca. The highest values were in the core region, decreasing towards the middle and slightly increasing towards the edge. Core values were significantly higher than Points 3 and 4 ( $p < 0.03$ ).

## DISCUSSION

### Pre-hatch zone

Analyses revealed that the elemental composition differed considerably between the core region (representing time before hatching) and the outer parts. Concentrations of 7 elements were significantly different between the pre-hatch zone and the post-hatch zones. This change could be due to the difference in the elemental compositions within the egg-capsules compared to seawater. As Yatsu et al. (1998) pointed out, Sr/Ca ratios in cephalopod statoliths are probably affected in a different way during the embryonic period compared to other ontogenetic stages, because embryos have no direct contact with ambient water but rely on their yolk. Since yolk composition may well be influenced by genetic factors, the elemental signature of the statolith core could be representative for separate populations. In cuttlefish eggs, the capsule was shown to protect embryos against trace metals like Zn and Cd (Bustamante et al. 2002). However, at least for Zn, this protection cannot be supported by our results. Further, there is also evidence that environmental conditions, specifically temperature, can affect Sr contents in statoliths of encapsulated marine neogastropod larvae (Zacherl et al. 2003b). In this context, it would be desirable to analyse the elemental composition of the liquid inside the eggs and the yolk to learn about relations between statolith composition and these factors.

### Strontium

*Gonatus fabricii* statoliths, similar to other cephalopods, corals and sclero-sponges, contain more Sr (Sr/Ca ratio  $\sim 6.3\text{--}8.1 \text{ mmol mol}^{-1}$ ) than fish otoliths ( $\sim 2\text{--}2.5 \text{ mmol mol}^{-1}$ , Campana 1999) or bivalve shells ( $\sim 0.8\text{--}1.6 \text{ mmol mol}^{-1}$ , e.g. Vander Putten et al. 2000). Among cephalopods, statolith Sr/Ca ratios in *G. fabricii* are relatively low compared with those of the oceanic ommastrephid squid *Todarodes pacificus* ( $8.5\text{--}10 \text{ mmol mol}^{-1}$ ; Ikeda et al. 2003) and a species of benthic octopus, *Octopus dofleini* ( $10.6\text{--}14.3 \text{ mmol mol}^{-1}$ ; Ikeda et al. 1999), but quite high compared with

the common cuttlefish *Sepia officinalis* ( $3.5\text{--}6.5 \text{ mmol mol}^{-1}$ ; Zumholz et al. 2006). However, statolith Sr/Ca data in the current study are very similar to those of loliginid squid species (Ikeda et al. 1997, Biemann & Piatkowski 2001, Arkhipkin et al. 2004).

Applying the generally suggested negative correlation between Sr/Ca and temperature (Arkhipkin et al. 2004), the higher Sr/Ca in the outer region compared to Growth Zone 3 may indicate a migration into colder waters. This scenario is supported by Ba/Ca and U/Ca patterns and complies with the proposed life-cycle of *Gonatus fabricii* (Arkhipkin & Bjørke 1999). However, no data for Sr/Ca incorporation into cephalopod statoliths from laboratory experiments exist for calibration.

The considerably higher core values may not be due to ambient water but rather to the yolk and the within-egg fluid surrounding the embryo during pre-hatch development. For example, Sr/Ca ratios in the primordium (the initial complex structure within fish otolith nuclei) of salmonids are considered to be directly influenced by the Sr content of the individual's yolk (Kalish 1990).

### Barium

Ba/Ca ratios from the current study are in the same range as the ratios for *Loligo gahi* (Arkhipkin et al. 2004). Ba is known to show nutrient-like distributions, i.e. very low concentrations in surface waters and considerably higher values in deeper waters (Chan et al. 1977). In corals, Ba is therefore used as an indicator for upwelling events (Lea et al. 1989). Experimental data indicate a positive correlation of Ba/Ca in gastropod larvae protoconchs (Zacherl et al. 2003b) and fish otoliths (Bath et al. 2000, Elsdon & Gillanders 2003, Bath-Martin et al. 2004) with Ba/Ca in the seawater. The Ba/Ca distribution found in our study corresponds to the previously stated ontogenetic migrations from surface waters during the juvenile stage to mesobathy-pelagic waters in the subadult and adult stages in *Gonatus fabricii* (Kristensen, 1984). Therefore, Ba/Ca in statoliths appears to be a very useful indicator for vertical movements of cephalopods as suggested by Arkhipkin et al. (2004) for *L. gahi*. However, seasonal or persistent upwelling conditions can complicate the interpretation.

### Magnesium

Magnesium has been assumed to be important for biomineralisation in squid statoliths (Morris 1991). Statolith core regions are characterised by relatively high

Mg/Ca ratios that decline progressively towards the edge. This trend probably reflects the growth rate, which is rather high in Growth Zones 2 and 3 and decreases considerably in Growth Zone 4 (Kristensen 1980, Arkhipkin & Bjørke 2000). Bettencourt & Guerra (2000) showed that Mg<sup>2+</sup> concentration in the statocyst endolymph is associated with the deposition of organic components into the statolith. Since the proportion of organic components in statoliths decreases with the size of the squid (Bettencourt & Guerra 2000), concentrations of Mg would be expected to decrease correspondingly, which is in accordance with the data presented in this study.

Mg/Ca ratios are positively correlated with temperature in coral skeletons (Mitsuguchi et al. 1996), but a corresponding relation has not been shown for cephalopods or fish. A relation of statolith Mg/Ca to Mg/Ca ratios or Mg concentrations in the seawater seems even more unlikely because this element is physiologically highly regulated and nearly uniformly distributed in ocean waters.

The Mg/Ca values in the core were slightly higher than the values reported for *Loligo gahi* statoliths (Arkhipkin et al. 2004), which is not surprising since these authors obtained the elemental composition integrated over the whole statolith, pooling high core values with lower outer values. However, by comparing statoliths from juveniles and adults, they found a similar ontogenetic pattern with declining Mg/Ca values in bigger specimens.

### Manganese

Mn/Ca values in this study were similar to values in *Loligo gahi* statoliths ( $\sim 1\text{--}3 \mu\text{mol mol}^{-1}$ ; Arkhipkin et al. 2004) determined by ICP-MS. However, the values of both data sets are substantially lower than in *Todarodes pacificus* ( $22\text{--}35 \mu\text{mol mol}^{-1}$ ) and *Octopus dofleini* ( $22\text{--}40 \mu\text{mol mol}^{-1}$ ) determined by proton-induced X-ray emission (Ikeda et al. 1995, 1999). The distribution of Mn in the world's oceans, however, is rather uniform (Donat & Bruland 1995). Therefore, as stated by Arkhipkin et al. (2004), these differences in statolith composition are probably due to taxon-specific uptake mechanisms or to instrumental artefacts rather than to environmental influences. In statoliths of *L. gahi* (Arkhipkin et al. 2004) a decrease of Mn/Ca with increasing squid size was observed. This is in accordance with the current study, considering that Arkhipkin et al. (2004) analysed the whole statolith of specimens  $>7.5$  cm mantle length. These statoliths already contain zones Z1 to Z3 as constant factors; therefore, a Mn/Ca ratio decreasing from Z3 towards the edge results in a decrease of Mn/Ca with increasing squid size.

### Uranium

Uranium has never before been determined in statoliths. Spatial and vertical distributions of dissolved U in the world's oceans have been reported to be rather uniform, at  $3.4\pm 0.3 \text{ ng g}^{-1}$  (Hossain & Ohde 2002). Reef-building corals incorporate U in the form of uranyl ions ( $\text{UO}_2^{2+}$ ) as impurities by replacing Ca<sup>2+</sup> (Ohde et al. 2003) in a concentration range of 2 to  $3.5 \mu\text{g g}^{-1}$  (Shen & Dunbar 1995). U concentrations in statoliths from this study were 2 orders of magnitude lower, but they showed considerable spatial variation.

Shen & Dunbar (1995) proposed a possible U/Ca temperature dependence of + 3 to 4 % per  $1^\circ\text{C}$  cooling for coral aragonite. Moreover, the same authors identified a probably positive salinity influence on coral U/Ca, which suggests that U uptake depends on the total U concentration in seawater and not on the U/Ca ratio. Applying these relations to results for *Gonatus fabricii* statoliths, low U values in Z2, Z3 and early Z4 (juvenile and subadult phases) correspond to the warm, low-saline surface water in Disko Bay. The higher U values in Z1 (time before hatching) and late Z4 (adult phase) indicate habitation in deeper, more saline waters. A significant correlation ( $r = 0.56$ ,  $p < 0.01$ ) was found between U/Ca and Sr/Ca, which agrees with previous studies on corals (Min et al. 1995). The combination of Sr and U, which are incorporated into corals at different ratios with respect to temperature, is used regularly for sea surface temperature reconstruction (Correge et al. 2001). Experiments on these relations have yet to be performed on cephalopods, but the data presented here suggest a strong potential of U/Ca in statoliths as a temperature proxy for future studies. Our results further showed significant variations within Z4, a region that is optically homogenous. This outlines the ability of spatially resolved chemical analyses to provide far more information than optical analyses alone.

### Other elements

Zinc, yttrium and zirconium concentrations in the statoliths did not vary significantly along the post-hatch ontogenetic stages. However, core values for all of these elements were significantly higher than in the outer regions. Zn has previously been measured in statoliths (Ikeda et al. 1996), but convincing relations to environmental factors have not been proposed yet. Y and Zr have never before been analysed in cephalopod statoliths, but spatial variations found in this study suggest some potential for future use as environmental

proxies. These elements should be further investigated in biogenic aragonite to evaluate their incorporation process.

Lead could not reliably be measured in *Gonatus fabricii* statoliths due to low concentrations and apparent surface contamination in some samples. Preliminary data showed Pb/Ca of 6.3 to 37 nmol mol<sup>-1</sup> for 2 specimens, which is comparable to Pb/Ca ratios found in *Loligo gahi* statoliths with solution-based ICP-MS (~2–30 nmol mol<sup>-1</sup>; Arkhipkin et al. 2004).

### Suitability of laser ablation ICP-MS

LA-ICP-MS as used in this study proved to be a well-suited technique for the micro-analysis of trace elements in statoliths over a range of concentrations from <0.01 µg g<sup>-1</sup> to several thousand µg g<sup>-1</sup>. A main factor limiting the accuracy of the data is the lack of suitable matrix-matched homogeneous calibration standards. The NIST610 glass often used for calibration differs strongly from the analyte by both colour and bulk composition, which results in different element fractionation during ablation (Guillong et al. 2003) and hence in limited accuracy for some elements. Additional errors arise by measured concentrations being far outside the calibration range, since NIST610 has much higher Y, Zr, Nb and U and much lower Sr contents than the samples. Despite these limitations, the good analytical precision combined with the spatial resolution enabled significant zonations to be recognised.

We chose a spatial resolution of about 70 µm to be able to differentiate between the squid's main ontogenetic stages. A higher spatial resolution down to about 10 µm is possible but would result in poorer signal quality, significantly lower intensities and hence in higher detection limits, making detection of trace elements such as U, Y and Zr impossible. Increased laser energy, in turn, would increase the deepening rate of the ablation crater and reduce the spatial resolution especially at the statolith core. Therefore, the analytical setup used here represented a reasonable compromise between spatial resolution and detection limits. In order to obtain true 2-dimensional profiles, the laser beam should ideally ablate along a raster in the core and along lines parallel to the growth lines. However, this was not possible with the laser system used in this study.

In comparison to solution-based ICP-MS analyses of *Loligo gahi* statoliths (Arkhipkin et al. 2004), we obtained the same magnitudes of concentrations by LA-ICP-MS (Table 2). The main advantage of laser ablation is the high spatial resolution, which counterbalances the slightly poorer accuracy and detection limits as compared to solution ICP-MS analyses. LA-

ICP-MS is therefore suitable to assign element composition *in situ* to distinct statolith regions, corresponding to ontogenetic stages of individual squids.

**Acknowledgements.** We are grateful to K. Wieland and the crew of RV 'Paamiut' for collecting the cephalopods, and R. P. Frandsen for assistance with dissecting specimens. H. Anders helped with solution ICP-MS analyses, and V. Epplé provided the *Arctica islandica* sample. G. Kraus and D. Schnack provided valuable comments, which considerably improved this manuscript. J. Schmidt is acknowledged for assistance with Fig. 1. This study was supported by grants from the Deutsche Forschungsgemeinschaft (Pi 203/11-1, Pi 203/11-2 and Ha 2100/9-1).

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*Editorial responsibility:* Otto Kinne (Editor-in-Chief),  
Oldendorf/Luhe, Germany

*Submitted:* January 9, 2006; *Accepted:* August 17, 2006  
*Proofs received from author(s):* March 1, 2007