Mar Biol (2007) 151:1321–1330
DOI 10.1007/s00227-006-0564-1

RESEARCH ARTICLE

Influence of temperature and salinity on the trace element incorporation into statoliths of the common cuttlefish (*Sepia officinalis*)

Karsten Zumholz · Thor H. Hansteen ·
Uwe Piatkowski · Peter L. Croot

Received: 28 June 2006 / Accepted: 17 November 2006 / Published online: 5 January 2007

Abstract The use of statolith chemistry to trace migration pathways and distinguish populations of cephalopods is based on the assumption that the elemental composition of statoliths is influenced by physicochemical properties of the ambient environment. However, such influences have not been investigated experimentally up until now. This study presents the first microchemical analyses of cephalopod statoliths obtained from laboratory experiments under different controlled temperature and salinity conditions. Our results show that statolith chemical composition is strongly related to both salinity and temperature in ambient waters. The Ba/Ca ratio is negatively related to temperature and shows no relation to salinity. The I/Ca ratio is positively related to temperature and negatively to salinity. No Sr/Ca relation was found to either salinity or temperature, suggesting that the well-established proxy strontium is not as useful in cephalopod statoliths as in other biomineralized aragonites. Microanalysis of trace elements, however, shows an enormous potential for field studies on distribution, migration and stock separation of cephalopods. Furthermore, Synchrotron X-ray Fluorescence Analysis is introduced as a promising novel method for statolith analysis, providing a spatial resolution of typically 10–15 µm combined with detection limits down to 0.5 ppm.

Communicated by O. Kinne, Oldendorf/Luhe.

K. Zumholz (✉) · T. H. Hansteen · U. Piatkowski ·
P. L. Croot
Leibniz - Institute of Marine Sciences,
IFM-GEOMAR, Düsternbrooker Weg 20,
24105 Kiel, Germany
e-mail: kzumholz@ifm-geomar.de

Introduction

Cephalopod statoliths share many geochemical and microstructural properties with the much better studied otoliths of teleost fishes (Radtke 1983). Both structures are composed of calcium carbonate and high molecular proteins. Visible increments are formed of alternating layers of protein and aragonite (Clarke 1978; Kristensen 1980; Jackson 1994). These growth rings are frequently used for age determination since a daily deposition of increments has been validated for several cephalopod species including *Sepia officinalis* (Bettencourt and Guerra 2001).

The trace element composition of mineralised tissues in many marine species is dependent on the composition and temperature of the surrounding seawater. This fact has been demonstrated experimentally for fish (e.g. Thorrold et al 1997; Bath-Martin et al. 2004; Elsdon and Gillanders 2003), corals (e.g. Shen et al. 1996) and gastropods (Zacherl et al. 2003). For cephalopod statoliths, however, environmental influences have been proposed on the basis of field studies only (Ikeda et al. 1996a, 2003; Yatsu et al. 1998). The only study so far that analysed statoliths from reared cephalopods found considerable variations in the Sr/Ca ratio in spite of constant conditions during rearing (Ikeda et al. 2002a, b).

Microchemical analyses have become a standard method in fish otoliths to reconstruct environmental histories of individuals and as biological tracers of stocks (e.g. Arai et al. 2004; Limburg 1995, 1996, 1998; Campana et al. 2000; for a comprehensive review see: Campana 1999). Compared to fish otoliths, there have been only few studies examining the chemical composition of cephalopod statoliths (e.g. Durholtz et al.

1997; Lipinski et al. 1997; Ikeda et al. 2002a, b; Bettencourt and Guerra 2000). Chemical signatures of statoliths have been used as a tool to determine environmental life-histories of the neon flying squid *Ommastrephes bartramii* (Yatsu et al. 1998) and the Japanese common squid *Todarodes pacificus* (Ikeda et al. 2003) as well as to distinguish stocks of the Patagonian longfin squid *Loligo gahi* (Arkhipkin et al. 2004), yet the method remains largely unverified. It is not documented whether the Sr/Ca ratio or any other trace element signature is primarily related to ambient temperature or whether it is simultaneously influenced by salinity or other factors. Diet has been shown to influence the statolith composition as well, albeit to a comparably small extent (Zumholz et al. 2006). Predictive relationships between statolith composition and temperature or salinity have not been determined so far. Ikeda et al. (1996a) proposed a relation of strontium, iron and zinc concentrations to temperature in statoliths of *T. pacificus* from the field. Nevertheless the same authors pointed out the necessity of rearing experiments under controlled environmental conditions to verify their observations.

Analyses of statoliths from the field can give only limited information about how and to what extent environmental factors contribute to observed differences. However, precise information is essential to reconstruct the environmental history and migratory pathways of cephalopods. Before statoliths can be used to reconstruct life histories from wild caught specimens, a calibration is required for as many elements and as many factors as possible. To provide first quantitative relations, we performed rearing experiments under controlled conditions to estimate the individual influence of temperature and salinity on the elemental composition of cuttlefish statoliths.

Cuttlefish are among the most important cephalopod resources in European waters (Denis and Robin 2001). Official statistics for *S. officinalis* indicate an average (1993–2003) production of 41,000 tons/year distributed almost equally between the Atlantic and the Mediterranean (FAO 2005).

Microchemical studies on statoliths so far were based on various techniques, including particle induced X-ray emission (PIXE) (Ikeda et al. 1996a, b; Lipinski et al. 1997; Durholtz et al. 1997), electron probe microanalysis (EPMA) (Bettencourt and Guerra 2000; Ikeda et al. 2002a, b; Ikeda et al. 2003), laser-ablation inductively coupled plasma mass spectrometry LA-ICP-MS (Zumholz et al. 2007a), and solution based ICP-MS (Arkhipkin et al. 2004). In this study, we applied Synchrotron X-ray Fluorescence Analysis (SYXRF) to cephalopod statoliths for the first time, an advanced method which provides spatially resolved determination of trace element concentrations down to 0.5 ppm. Synchrotron radiation (SR) has been used in recent years as an excellent excitation source for X-ray fluorescence spectrometry. SR offers, in contrast to conventional X-ray sources, highly brilliant polychromatic, polarized radiation with which micro-domain X-ray fluorescence spectrometry becomes possible. Recent SYXRF applications include mainly geological samples (e.g. Lowenstern 1993; Hansteen et al. 2000), but also biological material like human bones (Zoeger et al. 2005), tree stems (Martin et al. 2006) and human teeth (Barrea et al. 2003). The SYXRF-analyses of fish otoliths (Tsukamoto et al. 1998; Nakai et al. 1999), bivalve shells (Thorn et al. 1995) and corals (Allison et al. 2001) highlighted its suitability for bio-mineralized aragonite.

The aim of this study was to (1) find potential elemental proxies for temperature and salinity, (2) estimate first quantitative relations between statolith element concentrations, temperature and salinity of the surrounding water and (3) evaluate the suitability of SYXRF for this kind of analysis.

Materials and methods

Rearing experiments

Cuttlefish (*S. officinalis*) eggs were collected in Brittany, France in August 2003. Hatching took place at the Leibniz-Institut für Meereswissenschaften, Kiel in September 2003. Hatchlings were reared in a common tank in artificial seawater of salinity 32 for 10 days, at which time they were randomly distributed among 15 acid-washed 240 l glass tanks at a density of one cuttlefish per 20 l (12 animals per tank). Light was provided from fluorescent tubes on a 12:12 h day–night cycle. Ad libitum feedings were conducted daily with live mysid shrimp (Mysidaceae). Experimental tanks were randomly assigned three salinities (25, 32 and 39 with a constant temperature of 19°C) and three temperature treatments (19, 25 and 28°C with a constant salinity of 32). Three replicate tanks were used for each treatment. Constant salinities and temperatures were maintained throughout the whole experiments. Temperature was controlled permanently in climate chambers. Salinity was controlled daily. Artificial seawater (Instant-Ocean®) mixed with Baltic Sea water was used to regulate salinity. Treatments were chosen to represent the ranges of temperature and salinity that are potentially encountered by juveniles during the growing season. Experiments were terminated after 60 days. Surviving cuttlefish were killed; weight and dorsal mantle length of each specimen was measured. Due to high mortalities during experiments, replicates were pooled and only five specimens per treatment were analysed.

Preparation of samples

Statoliths were extracted from freshly killed animals, rinsed with distilled water and stored dry in eppendorf-caps until further preparation. Statoliths were ground to the focus in the lateral dome as doubly polished, 40 µm thick sections. The term lateral dome is used as defined by Clarke (1978), focus is defined as the starting point of crystallization (Kristensen 1980). Statolith sections were mounted on a 3.5 µm Mylar foil and analysed at Beamline L of the Hamburger Synchrotronstrahlungslabor HASYLAB at DESY in Hamburg. The surface of polished statoliths was cleaned with deionised water.

Analytical methods

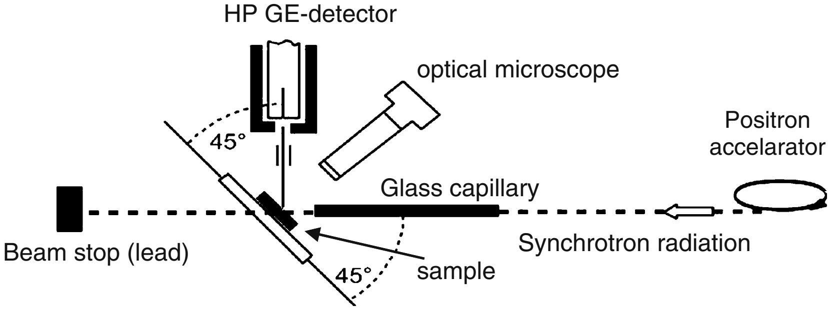
Water samples from each tank were collected in 20-day intervals, including the start and end of the experiment. Samples from each tank were averaged and the mean values were then used in subsequent analyses. Water analyses were carried out by solution ICP-MS at the Institute of Geosciences, University of Bremen, using a ThermoFinnigan Element2™. Prior to analysis, water samples from each experiment tank were diluted 1:200 for Ca, Mg and Sr, and 1:20 for all other elements. For sample introduction a PEEK cyclonic spray chamber with a micro-flow nebulizer operating in self-aspirating mode was used. In order to avoid mass interferences, Mg, Mn and Zn were measured at medium resolution (4,000) and all other elements at low (300) resolution. Internal precision as expressed by the relative standard deviation of nine analytical passes was typically 1–7% for most elements and increased for concentrations below 0.01 ng ml^{-1}.

Synchrotron X-ray Fluorescence microprobe (SYXRF) was used to determine the distribution of trace elements in statoliths. Doubly polished, 40 µm thick sections were analysed at the SYXRF set-up at HASYLAB Beamline L (Fig. 1), using white SR and a glass capillary producing a beam diameter of about 12 µm. The primary beam was passed through a 200 µm thick ultra-pure Cu foil; acquisition times were typically 1,000 s. The photon beam reaches the sample surface at a 45° angle, and is attenuated very little on passing through the sample. The analysed volume is thus tubular with a circular outline, and has a length = $(\sin 45°)^{-1} \times$ sample thickness. The characteristic radiation was detected by a High Performance Germanium (HPGe) solid-state detector (Detector Systems GmbH, Germany). Spectrum evaluation was performed with the AXIL software package (Espen et al. 1977; Grieken and Markowicz 1993). A fundamental-parameter approach was used to compute element concentrations (Vincze et al. 1993). The sample-specific variables thickness, density and major element concentration were used as input for the simulation procedure. Detection limits for the set-up at beamline L at HASYLAB using 1,000 s aquisition time are typically between 0.5 and 10 ppm for elements between $Z = 27$ (Co) and $Z = 64$ (Gd) using the Kα and Kβ lines for quantification (Lechtenberg et al. 1996; Hansteen et al. 2000). Detection limits were estimated at between 0.5 and 8 ppm for the elements of interest and the specific specimen composition and thickness, based on the attained counting statistics. Errors due to counting statistics when analysing the same point twice were found to be much less than 1% relative. Thus intra-sample variations of up to several % are clearly attributed to minor chemical heterogeneities. As the hard X-rays pass through the thin samples, the influence of a contaminated surface is minimized when using SYXRF.

Location of measuring points

Measuring points were focussed on the outer part of the lateral dome (Fig. 2) to represent the time of rearing experiments and a region of regular growth rates. Six points per specimen were measured, i.e.

Fig. 1 Schematic SYXRF set-up at HASYLAB, Hamburg



altogether 30 points on five specimens were analysed per treatment.

Statistical analysis

Analysis of variance (ANOVA) was used to test for significant element/Ca ratio differences (1) in water between tanks, (2) in statoliths between specimens and (3) between treatments. The assumptions of ANOVA were met: data were normally distributed and variances were homogeneous among factor levels.

Results

Water analyses

Elemental concentrations of calcium, barium and strontium in the tank water differed significantly between salinity treatments ($P < 0.001$), but not among temperatures ($P > 0.05$). Element/Ca ratios were neither significantly different between salinity treatments ($P > 0.05$) nor temperature treatments ($P > 0.05$). Iodine was not detectable in water samples.

Statolith analyses

SYXRF provides simultaneous multi-element-analyses of the sample. The following elements were quantifiable in the investigated statoliths: Sr, Ba, I, Y, Zr, Nb, Ni. Concentrations of Sr, Ba and Iodine were used for further analyses. No significant chemical variations between specimens from the same treatment were found ($P > 0.05$). Variation coefficients v (%) for concentration variations within each statolith ranged between 1 and 8.

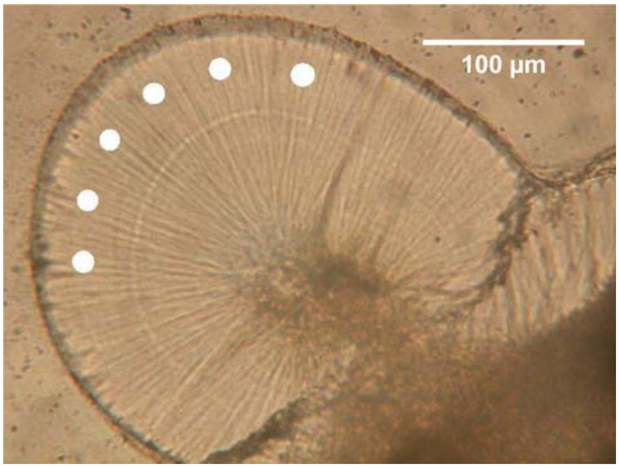


Fig. 2 *Sepia officinalis*. Ground statolith showing the location of analytical points in the outer region of the lateral dome as *white dots*. Location of measurements is displayed artificially, since SYXRF leaves no visible marks in or on the statolith

Salinity effect

Iodine/Calcium ratios differ significantly between salinity treatments (Table 1). Post-hoc Tukey multiple comparisons revealed that the I/Ca ratio for the salinity 25 treatment differed significantly from those corresponding to salinities 32 and 39 (Fig. 3). Differences between salinities 32 and 39, however, were not significant. Both barium and strontium did not show any relation to salinity.

Temperature effect

Ba/Ca is negatively related to temperature while I/Ca shows a positive relationship to temperature (Table 1). For both elements, post-hoc Tukey multiple comparisons revealed that determined element/Ca ratios for the 19°C treatment varied significantly from 25 to 28°C (Fig. 4). Differences between 25 and 28°C, however, were not significant. Strontium did not show any clear relationship to temperature.

The following linear regressions for the relation of Ba/Ca and I/Ca to temperature were calculated (element/Ca ratios given in μmol mol^{-1}; temperature given in °C):

$$\mathrm{Ba/Ca} = 26.89 - 0.574T, \quad R^2 = 0.98$$

$$\mathrm{I/Ca} = 1.49 + 0.244T, \quad R^2 = 0.97.$$

Discussion

Our results demonstrate a strong influence of both temperature and salinity on the incorporation of trace

Table 1 *Sepia officinalis*. Analysis of variance (ANOVA) table summarizing the effect of salinity and temperature treatments on statolith element/Ca ratios

Source	*F*	*P*
Sr/Ca		
Temperature	2.93	0.10
Salinity	1.20	0.34
Ba/Ca		
Temperature	13.52	0.002
Salinity	1.98	0.18
I/Ca		
Temperature	13.33	0.002
Salinity	20.02	0.002

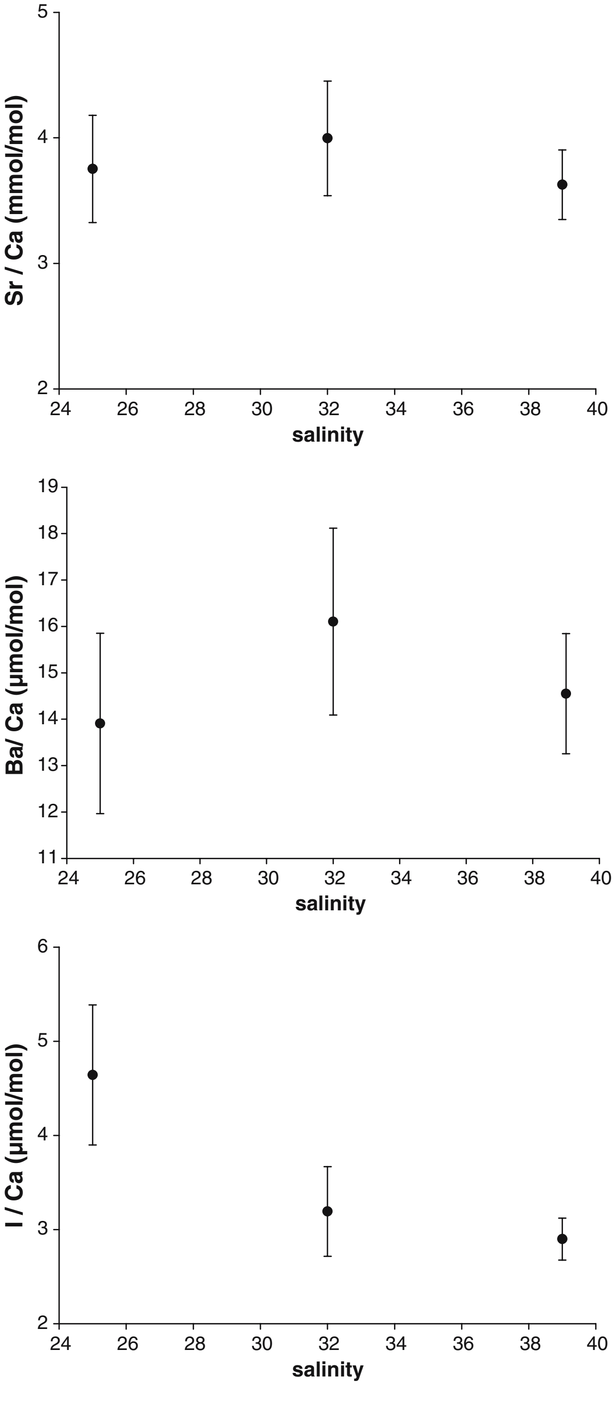


Fig. 3 *Sepia officinalis.* Element concentrations determined by SYXRF in the distal part of the statolith lateral dome versus salinity of the surrounding water in laboratory experiments. *Points* represent means of five specimens with six analytical spots each. *Error bars* represent standard deviation

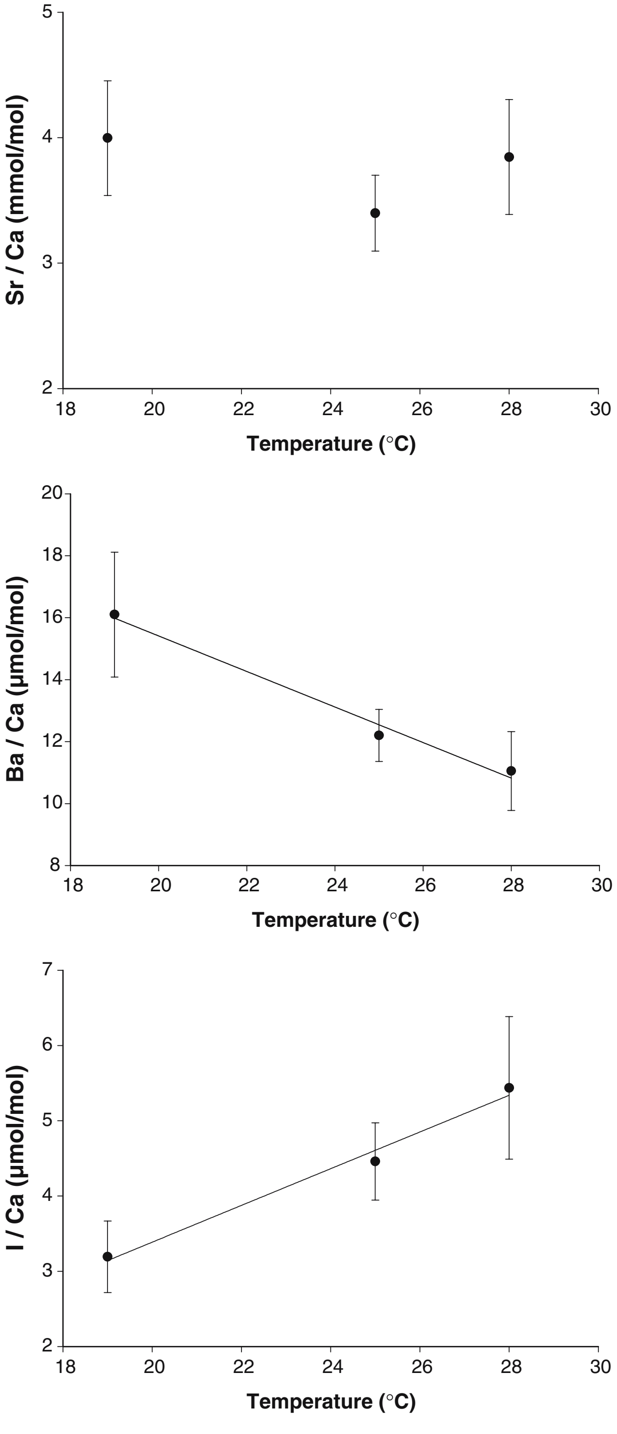


Fig. 4 *Sepia officinalis.* Element concentrations determined by SYXRF in the distal part of the statolith lateral dome versus temperature of the surrounding water in laboratory experiments. *Points* represent means of five specimens with six analytical spots each. *Error bars* represent standard deviation. Linear regressions for Ba/Ca and I/Ca are given

elements into cuttlefish statoliths. Elemental concentrations of iodine have not been determined before in statoliths. Therefore no published information on this element in statoliths is available for cross-reference. Results for strontium and barium, however, can be compared to other studies. They show some similarities but also some major differences that are important for the applications in the field.

Strontium

Several papers have addressed the influence of salinity on strontium incorporation into statoliths and otoliths. A positive relation to salinity has been proposed for cephalopods from the field (Ikeda et al. 2002a, b) as well as for several migrating fish species (reviewed by Secor and Rooker 2000). However, the relation to temperature is more ambiguous in the literature. Empirical laboratory studies have reported a negative temperature dependence of Sr for corals (Smith et al. 1979) and fish (Secor et al. 1995) as well as positive (Bath et al. 2000), non-linear (Kalish 1990; Elsdon and Gillanders 2002) and nonexistent (Gillanders and Kingsford 1996) relationships between temperature and Sr/Ca in fish otoliths. For cephalopod statoliths, a negative Sr/Ca relation to temperature has been proposed on the basis of specimens from the field (Ikeda et al. 1996a; Yatsu et al. 1998; Arkhipkin et al. 2004). However, the only spatially resolved micro-chemical study on cephalopod statoliths from rearing experiments to date reported considerable variation in Sr/Ca ratios despite of constant water temperature (Ikeda et al. 2002a, b). The underlying basis of the Sr/Ca temperature dependence is presumed to be a kinetic effect since the rate of strontium incorporation into inorganic aragonite varies inversely with temperature (Kinsman and Holland 1969), but the composition of most biomineralized tissues is obviously influenced by some form of biological fractionation (Campana 1999). Furthermore the same author points out that the ambient concentration of an element (or the element/Ca ratio) is not necessarily a good indication of its availability to the animal. In general, dissolved ions that are free of ligands are the only chemical species available for uptake by the gills (Knezovich 1994). Uptake of elements from the diet, however, may follow completely different principles. In corals, strontium is incorporated not only through a simple ion substitution for Ca, but to a considerable amount as strontianite ($SrCO_3$) (Greegor et al. 1997; but for a different view see: Finch et al. 2003). Since strontium concentrations in corals are similar to those in cephalopod statoliths, such a mechanism seems possible for these aragonite-dominated structures as well. However, no specific investigations have been made about the possible occurrence of strontianite in statoliths. Strontium is required for statolith development and thus normal swimming behaviour of hatchling cuttlefish (Hanlon et al. 1989). Therefore it obviously plays an important role in the biomineralization process of statoliths (Lipinski 1993), but the actual mechanism of statolith formation is still unknown.

Barium

The Ba/Ca ratio in coral aragonite is as a well-established proxy for water depth and accordingly upwelling due to its nutrient-like distribution (Lea et al. 1989). Furthermore, experiments on inorganic aragonite have shown that barium incorporation is negatively correlated with temperature between 10 and 50°C (Dietzel et al. 2003). Zacherl et al. (2003) were the first to demonstrate an inverse effect of temperature on barium incorporation into biogenic aragonite using protoconchs of larval neogastropods *Kelletia kelletia*. Results of the current study support this relation. Therefore Ba/Ca ratios in calcified structures may have potential for recovering temperature histories. Barium appears to be a particularly suitable proxy for the environmental temperature history of cuttlefish since our data suggest that the Ba incorporation is only influenced by temperature, and not affected by salinity, e.g. the absolute barium concentration. However, a positive relation of Ba/Ca ratio in the surrounding water to barium incorporation has been demonstrated for gastropods (Zacherl et al. 2003).

Iodine

In spite of its low concentration levels in seawater, absolute concentrations of iodine should increase with salinity. The negative relation of statolith I/Ca to salinity suggests that their incorporation is not dependent on the element/Ca ratios in the surrounding water. A negative relation to the absolute concentration in seawater, however, sounds rather uncommon. A possible explanation for this relation may be a different degree of complexation of ions in the water at different salinities. As only dissolved ions which are free of ligands are likely to pass the various membranes between seawater and the statoliths, the concentration of free ions may be the key factor. Further, iodine is known to be a metabolically well-regulated element. Anyhow, since iodine was not detectable in the water, we can only speculate about this phenomenon. As analytical possibilities will improve, this question may be addressed in future investigations.

Moreover, the I/Ca ratios in the statoliths differed not only between salinities but also between temperatures. This will probably be more important for investigations in the field, since most cephalopods are not supposed to encounter considerable salinity changes during their lives.

Iodine is located in either the organic matrix as an organi-iodo compound or possibly incorporated as iodate in the aragonite lattice. The variations with temperature may be explained by different growth rates, but the salinity variations are harder to explain without knowing if and where the iodine resides in the lattice.

Microchemistry as a fingerprinting of migration pathways

Among our experimental results, the very good linear trends between Ba, I and temperature stand out in the data set (Fig. 4). Iodine appears to be positively related to temperature, while Ba appears to be negatively related to temperature. Due to the obvious linear alignment of the mean values we estimated a linear regression for the temperature influence on Ba and I. One may argue that having only three points per element at our disposal renders the exact form of a calibration curve uncertain. The estimated least square regression lines are, however, very close to the mean values, which strongly indicates that our observations are valid. Further, our observations concerning Ba are in accordance with the linear Ba-temperature relation demonstrated convincingly for the neogastropod *Kelletia kelletia* (Zacherl et al. 2003). Anyway, we emphasize that the lines and equations given here are not meant to represent accurate correlations, but good approximations of relations that obviously affect the microchemistry of statoliths. We thus recommend keeping this uncertainty in mind when using these equations in further studies.

In the salinity experiments, no differences were found between salinity 32 and 39 for any element/Ca ratio. Since by far the most cephalopod species live within this salinity range and will never experience lower salinities (*S. officinalis* is one of the few exceptions), the temperature dependence will probably be more important for the majority of future investigations in the field. A considerable change of temperature should be experienced by most species that are performing horizontal or vertical migrations.

Because we wanted to test whether statolith composition is influenced by the ‘‘standard’’ parameters salinity and temperature, the element/Ca ratios were not manipulated and thus did not vary between the treatments. As data for these parameters are easily available with enormous spatial and temporal resolution for most areas in the world ocean, chemical zonation patterns in statoliths would be relatively straightforward to interpret. Further experimental studies should investigate the influence of element/Ca ratios in the water. On the other hand, however, the lack of trace element composition data of natural water masses can make it difficult to interpret field studies concerning migration pathways and natal origins.

The diet influences the elemental composition of cephalopod statoliths to a minor extent (Zumholz et al. 2006). However, to avoid any further source of variation, we chose an identical diet for all experiments. We cannot exclude, however, the effect of physiological changes, if uptake of trace elements is related to growth because there is a significant effect of temperature on growth rate (Forsythe and Van Heukelem 1987). There is no published information about this topic for cephalopods, but for larval spot, Bath-Martin et al. (2004) report no relations of aragonite precipitation rates and larval growth rates to Sr/Ca ratios in the otoliths. When assuming that trace element incorporation in cephalopod statoliths functions in a similar way as in fish otoliths, then temperature has been the dominant variable responsible for compositional variations in our experiments.

Our results reveal notable differences of statolith elemental incorporation compared to studies carried out on fish otoliths. This indicates different biological fractionation processes, probably due to the considerably different metabolism and physiology of cephalopods. Obviously it is not appropriate to simply adopt results from experimental otolith studies to cephalopod statoliths. Therefore, further experimental investigations on statoliths are required to evaluate the influence on other environmental factors such as growth and stress.

Suitability of SYXRF for the analysis of zoned biocrystals

The current study is the first to apply SYXRF to cephalopod statoliths. The set up as used in our study provides a unique combination of both high spatial resolution of about 12 μm, representing ~5 days in the life of the cuttlefish (Bettencourt and Guerra 2001) and very low detection limits (down to 0.5 ppm). Another main advantage of SYXRF is the simultaneous measurement of all chemical elements in the periodic table between Ca ($Z = 20$) and Pb ($Z = 82$). This means that we do not have to focus on distinct elements but can obtain an overview of the complete trace elemental composition.

Up to now, microchemical methods either provided an appropriate spatial resolution (microbeam methods like EPMA, LA-ICP-MS, SIMS or PIXE) or very low detection limits (solution based methods). Hence, most microbeam studies focussed on strontium, the most abundant element incorporated into the aragonite-matrix of statoliths. However, in cuttlefish statoliths, Sr appears not to be a useful proxy for either salinity or temperature. Solution-based methods like ICP-MS methods provide extremely low detection limits (Arkhipkin et al. 2004), but in return they cannot give any time-scale information.

Because the X-ray beam passes completely through the sample during analysis, an integrated signal over the sample thickness is obtained. Compared to ablation-type analytical methods like SIMS and LA-ICP-MS, this is a major advantage of the SYXRF for this special application. Due to the slight sample heterogeneity caused by organic substances occurring in variable amounts, SYXRF is more likely than other methods to provide reliable measurements. The SYXRF analyses provide an average value of elemental concentrations in a cylindrical volume through the sample. Thereby smaller scale elemental heterogeneities that may occur due to daily increment formation (Zumholz et al. 2007b) are averaged out. Elements are measured independently from their location in the matrix, i.e. in the organic matter or in the aragonite lattice. This principle is completely different from other microbeam methods and may be responsible for the detection here of iodine. However, the relatively higher analysed volume compared to other microanalytical methods can only be combined with an optimum spatial resolution once the beam path is aligned perpendicular to the earstone growth direction.

The analyses of these particular biocrystals require quite extensive preparation. Due to the incorporated organic matter, the ground slices are very delicate and fragile. Until now there is no practicable way to remove or replace the organic compounds without destroying the structure or influencing the chemical composition. Another peculiarity of these objects is the geometrical shape. In order to analyse a transect representing the whole lifetime, it is essential to have a thinly ground slice containing the core region. Assuming the growth rings are exactly in a right angle to the transect, SYXRF achieves a spatial resolution corresponding to the beam diameter, which means 12 μm in our study. Analysing overlapping spots can halve this spatial resolution. The analytic time involved (in our study 1,000 s per point analysis) is quite long compared to other spatially resolved methods. It is expected, however, that the analytical potential will improve with more powerful detectors and further development of the method, so that data collection times will decrease.

The excellent micro-scale resolution of several micrometers and the precise, simultaneous analysis of elements from Calcium (Ca) to Lead (Pb) makes this method a powerful tool to gather life history information of various marine organisms forming bio-crystals.

The combination of spatially resolved chemical analyses and growth increment interpretation provides a method of dating observed changes in chemical composition of statoliths. This allows reconstructing a kind of a Curriculum Vitae for the particular specimen. However, before statoliths can be used to reconstruct life histories from wild caught specimens, a quantitative calibration is needed for as many factors and as many species as possible. Therefore further rearing experiments under controlled conditions are urgently recommended.

Conclusion

This study demonstrates considerable influences of temperature and salinity on the chemical composition of cuttlefish statoliths. Among the investigated trace elements, Barium shows the best potential for field studies, since the barium concentrations in statoliths are influenced only by temperature and not by salinity. Iodine shows positive relations to temperature, but also weak negative relations to salinity. Strontium, a well-established proxy in other marine biomineralized tissues, appears not to be a useful proxy for either salinity or temperature in cuttlefish. The advanced SYXRF set-up as used in this study provides excellent prospects for trace element analyses of various calcified tissues, particularly since elements like Iodine cannot be measured with a similar spatial resolution using other methods.

Acknowledgments Thanks are due to Frank Lechtenberg for invaluable help with analytical and quantification procedures. Armelle Perrin and Juergen Beusen assisted in collecting Sepia eggs. The Kiel Aquarium team provided technical and logistical support during the experiments. This work was funded by the Deutsche Forschungsgemeinschaft (DFG PI 203/11-1, HA 2100/9-1, PI 203/11-2, PI 203-3).

References

Allison N, Finch AA, Sutton SR, Newville M (2001) Strontium heterogeneity and speciation in coral aragonite: implications for the strontium paleothermometer. Geochim Cosmochim Acta 65(16):2669–2676

Arai T, Kotake A, Lokman PM, Miller MJ, Tsukamoto K (2004) Evidence of different habitat use by New Zealand freshwater eels *Anguilla australis* and *A. dieffenbachii*, as revealed by otolith microchemistry. Mar Ecol Prog Ser 266:213–225

Arkhipkin AI, Campana SE, FitzGerald J, Thorrold SR (2004) Spatial and temporal variation in elemental signatures of statoliths from the Patagonian longfin squid (*Loligo gahi*). Can J Fish Aquat Sci 61:1212–1224

Barrea RA, Pérez CA, Ramos AY, Sánchez HJ, Grenón M (2003) Distribution and incorporation of zinc in biological calcium phosphates. XRay Spectrom 32(5):387–395

Bath GE, Thorrold SR, Jones CM, Campana SE, McLaren JW, Lam JWH (2000) Sr and Ba uptake in aragonitic otoliths of marine fish. Geochim Cosmochim Acta 64:1705–1714

Bath-Martin G, Thorrold SR, Jones CM (2004) Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). Can J Fish Aquat Sci 61:34–42

Bettencourt V, Guerra A (2000) Growth increments and biomineralization process in cephalopod statoliths. J Exp Mar Biol Ecol 248:191–205

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

Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Prog Ser 188:263–297

Campana SE, Chouinard GA, Hanson JM, Frechet A, Brattey J (2000) Otolith elemental fingerprints as biological tracers of fish stocks. Fish Res 46:343–357

Clarke MR (1978) The cephalopod statolith—an introduction to its form. J Mar Biol Assoc UK 58:701–712

Denis V, Robin JP (2001) Present status of the French Atlantic fishery for cuttlefish (*Sepia officinalis*). Fish Res 52:11–22

Dietzel M, Gussone N, Eisenhauer A (2003) Co-precipitation of Sr2+ and Ba2+ with aragonite by membrane diffusion of CO2 between 10 and 50°C. Chem Geol 203:139–151

Durholtz MD, Lipinski MR, Przybylowicz WJ, Mesjasz-Przybylowicz J (1997) Nuclear microprobe mapping of statoliths of Chokka Squid *Loligo vulgaris reynaudii* d'Orbigny, 1845. Biol Bull 193:125–140

Elsdon TS, Gillanders BM (2002) Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. Can J Fish Aquat Sci 59:1796–1808

Elsdon TS, Gillanders BM (2003) Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri.* Mar Ecol Prog Ser 260:263–272

Espen PJ van, Nullens H, Adams F (1977) A computer analysis of X-ray fluorescence spectra. Nucl Instrum Methods 142: 269–273

FAO (2005) FISHSTAT plus global data set capture production 1950–2003 (FAO yearbook Fishery Statistics). ftp://www.ftp.fao.org/fi/stat/windows/fishplus/capdet.zip

Finch AA, Allison N, Sutton SR, Newville M (2003) Strontium in coral aragonite: characterization of Sr coordination by extended absorption X-ray fine structure. Geochim Cosmochim Acta 67(6):1197–1202

Gallahar NK, Kingsford MJ (1996) Factors influencing Sr/Ca ratios in otoliths of *Girella elevata*: an experimental investigation. J Fish Biol 48:174–186

Greegor RB, Pingitore NE, Lytle FW (1997) Strontianite in coral skeletal aragonite. Science 275:1452–1454

Grieken RE van, Markowicz AA (1993) Handbook of X-ray spectrometry. Marcel Dekker, New York, Basel, Hongkong, p 704

Hanlon RT, Bidwell JP, Tai R (1989) Strontium is required for statolith development and thus normal swimming behaviour of hatchling cephalopods. J Exp Biol 141(1):187–195

Hansteen TH, Sachs PM, Lechtenberg F (2000) Synchrotron-XRF microprobe analysis of silicate reference standards using fundamental-parameter quantification. Eur J Miner 12:25–31

Ikeda Y, Arai N, Sakamoto W, Kidokoro H, Yoshida K (1996a) Relationship between statoliths and environmental variables in cephalopods. Int J PIXE 6:339–345

Ikeda Y, Arai W, Sakamoto W, Nateewathana A, Murayama T, Yatsu A, Yoshida K (1996b) PIXE analysis of trace elements in squid statoliths: comparison between Ommastrephidae and Loliginidae. Int J PIXE 6:537–542

Ikeda Y, Yatsu A, Arai N, Sakamoto W (2002a) Concentration of statolith trace elements in the jumbo flying squid during El Niño and non-El Niño years in the eastern Pacific. J Mar Biol Assoc UK 82:863–866

Ikeda Y, Okazaki J, Sakurai Y, Sakamoto W (2002b) Periodic variation in Sr/Ca ratios in statoliths of the Japanese Common Squid *Todarodes pacificus* Steenstrup, 1880 (Cephalopoda: Ommastrephidae) maintained under constant water temperature. J Exp Mar Biol Ecol 273(2):161-170

Ikeda Y, Arai N, Kidokoro H, Sakamoto W (2003) Strontium:calcium ratios in statoliths of Japanese common squid *Todarodes pacificus* (Cephalopoda: Ommastrephidae) as indicators of migratory behaviour. Mar Ecol Prog Ser 251:169–179

Jackson GD (1994) Application and future potential of statolith increment analysis in squid and sepioids. Can J Fish Aquat Sci 51:2612–2625

Kalish JM (1990) Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fish Bull US 88:657–666

Kristensen TK (1980) Periodical growth rings in cephalopod statoliths. Dana 1:39–51

Knezovich JP (1994) Chemical and biological factors affecting bioavailability of contaminants in seawater. In: Hamelink JL, Landrum PF, Bergman HL, Benson WH (eds) Bioavailability: physical, chemical and biological interactions. Lewis Publishers, London, pp 23–30

Lea DW, Shen GT, Boyle EA (1989) Coralline barium records temporal variability in equatorial Pacific upwelling. Nature 340:373–376

Lechtenberg F, Garbe S, Bauch J, Dingwell DB, Freitag F, Haller M, Hansteen TH, Ippach P, Knöchel A, Radtke M, Romano C, Sachs PM, Schmincke HU, Ullrich HJ (1996) The X-ray fluorescence measurement place at beamline L of Hasylab. J Trace Microprobe Tech 14(3):561–587

Limburg KE (1995) Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima*. Mar Ecol Prog Ser 119:25–5

Limburg KE (1996) Growth and migration of 0-year American shad (*Alosa sapidissima*) in the Hudson River estuary: otolith microstructural analysis. Can J Fish Aquat Sci 53:220–238

Limburg KE (1998) Anomalous migrations of anadromous herrings revealed with natural chemical tracers. Can J Fish Aquat Sci 55:431–437

Lipinski MR (1993) The deposition of statoliths: a working hypothesis. In: Okutani T, O'Dor RK, Kubodera T (eds) Recent advances in cephalopod fisheries biology. Tokai University Press, Tokyo

Lipinski M, Przybylowicz WJ, Durholtz MD, Mesjasz-Przybylowicz J (1997) Quantitative micro-PIXE mapping of squid statoliths. Nucl Instrum Methods Phys Res B 130:374–380

Lowenstern JB (1993) Evidence for a copper-bearing fluid in magma erupted at the Valley of Ten-Thousand-Smokes, Alaska. Contrib Mineral Petrol 114:409–421

Martin RR, Naftel SJ, Machfie SM, Jones KW, Fenh H (2006) High variability of the metal content of tree growth rings as measured by synchrotron micro x-ray fluorescence spectrometry. X-Ray Spectrometry 35(1):52–56

Nakai I,Iwata R, Tsukamoto K (1999) Ecological study of the migration of eel by synchrotron radiation induced X-ray fluorescence imaging of otliths. Spectrochim Acta [B] 54(1):167–170

Radtke RL (1983) Chemical and structural characteristics of statoliths from the short-finned squid *Illex illecebrosus*. Mar Biol 76:47–54

Secor DH, Henderson-Arzapalo A, Piccoli PM (1995) Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? J Exp Mar Biol Ecol 192:15–33

Secor DH, Rooker JR (2000) Is otolith strontium a useful scalar of life cycles in estuarine fishes? Fish Res 46(1):359–371

Shen CC, Lee T, Chen CY, Wang CH, Dai CF, Li LA (1996) The calibration of D[Sr/Ca] versus sea surface temperature relationship for *Porites* corals. Geochim Cosmochim Acta 60:3849–3858

Sholkovitz E, Shen GT (1995) The incorporation of rare earth elements in modern coral. Geochim Cosmochim Acta 59(13):2749–2756

Sohrin Y, Fujishima Y, Ueda K, Akiyama S, Mori K, Hasegawa H, Matsui M (1998) Dissolved niobium and tantalum in the North Pacific. Geophys Res Lett 25(7):999–1002

Smith SV, Buddermeier RW, Redalje RC, Houck JE (1979) Strontium-calcium thermometry in coral skeletons. Science 204:404–407

Thorn K, Cerrato RM, Rivers ML (1995) Elemental distributions in marine bivalve shells as measured by synchroton X-ray fluorescence. Biol Bull 188:57–67

Thorrold SR, Jones CM, Campana SE (1997) Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus).* Limnol Oceanogr 42(1):102–111

Tsukamoto K, Nakai I, Tesch WV (1998) Do all freshwater eels migrate? Nature 396:635–636

Vincze L, Janssens K, Adams F (1993) A general Monte Carlo simulation of energy dipersive X-Ray fluourescence spectrometers-Part I. Spectrochim Acta 48B:553–573

Yatsu A, Mochioka N, Morishita K, Toh H (1998) Strontium/Calcium ratios in statoliths of the neon flying squid *Ommastrephes bartrami* (Cephalopoda) in the North Pacific Ocean. Mar Biol 131:275–282

Zacherl DC, Paradis G, Lea DW (2003) Barium and strontium uptake into larval protoconchs and statoliths of the marine neogastropod *Kelletia kelletii*. Geochim Cosmochim Acta 67:4091–4099

Zoeger N, Wobrauschek P, Streli C, Pepponi G, Roschger P, Falkenberg G, Osterode W (2005) Distribution of Pb and Zn in slices of human bone by synchrotron μ-XRF. X-Ray Spectrometry 43(2):140–143

Zumholz K, Hansteen TH, Klügel A, Piatkowski U (2006) Food effects on statolith composition of the common cuttlefish (*Sepia officinalis*). Mar Biol 150:237–244

Zumholz K, Klügel A, Hansteen TH, Piatkowski U (2007a) Statolith microchemistry traces environmental history of the boreoatlantic armhook squid *Gonatus fabricii*. Mar Ecol Progr Ser (in press)

Zumholz K, Hansteen TH, Hillion F, Horreard F, Piatkowski U (2007b) Elemental distribution in cephalopod statoliths: NanoSIMS provides new insights into nanoscale structure. Rev Fish Biol Fisher (in press)