

The influence of environmental factors on the micro-chemical composition of cephalopod statoliths



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Foreword

This thesis has been realised with the help of several colleagues. The contributions in particular are listed below:

Chapter II

Statolith sections were analysed at the Synchrotron X-ray Fluorescence microprobe (SYXRF) set-up at HASYLAB Beamline L, Hamburg. Element quantification was conducted by Frank Lechtenberg. Dr. Peter Croot brought in his broad expertise on chemical oceanography.

Chapter III and IV

The chemical analyses were carried out by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) at the Institute of Geosciences, University Bremen. Heike Anders helped in preparing and carrying out the solution ICP-MS analyses. The calculation of elemental concentrations was conducted by Dr. Andreas Klügel.

Chapter V

Statolith samples were analysed using a NanoSIMS NS50 ion microprobe, made available by CAMECA, Paris. Measurements and evaluations were conducted by Dr. Francois Hillion.

General scientific evaluation and interpretation

The evaluation process was supported by the mineralogical and analytical expertise of Dr. Thor Hansteen and the broad expertise on cephalopod ecology of Dr. Uwe Piatkowski. Both colleagues made extensive reviews of the text. Statistical analyses were performed under the guidance of Prof. Dr. D. Schnack.

The manuscripts included in this thesis are prepared for submission to peer reviewed journals as listed below:

- Trace element incorporation into statoliths of the common cuttlefish (*Sepia officinalis*): Influence of temperature and salinity

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- Food effects on statolith composition of the common cuttlefish (*Sepia officinalis*)

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- Statolith trace element composition reflects the life-history of the boreoatlantic armhook squid *Gonatus fabricii*

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- Trace element distribution in cephalopod statoliths:
NanoSIMS provides new insights into nano-scale structure

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Chapter I

Introduction

Cephalopods are an ancient and an outstanding group of the Mollusca. They have been among the dominant large predators in the ocean at various times in geological history. Two groups of cephalopods exist today: a) the Nautiloidea with a few species of the pearly nautilus, and b) the Coleoidea, comprising the squids, cuttlefishes, octopods and vampire squids, and representing a total of about 800 species. Although there are relatively few species of recent cephalopods, they occupy a great variety of habitats in all of the world's oceans. Individual species are often very abundant and provide major targets for marine

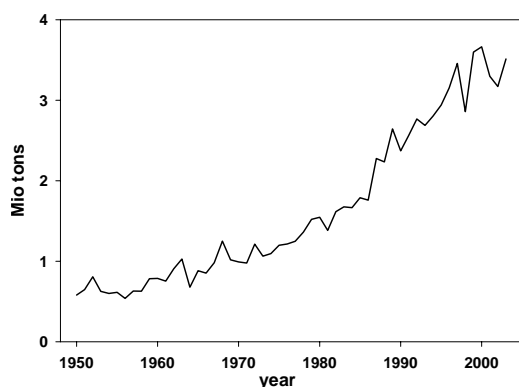


Fig. 1 World cephalopod catch (FAO, 2005)

fisheries. Commercially by far the most important species are the Japanese flying squid *Todarodes pacificus* and the Argentinean shortfin squid *Illex argentinus*. The global importance of cephalopod fisheries is increasing as traditional finfish fisheries decline, a trend that may reflect underlying shifts in marine community structure (Caddy and Rodhouse 1998; Balguerías et al. 2000). Total worldwide cephalopod landings have steadily increased since the 1950s, peaking in the year 2000 at

more than 3.6 Mio tons (Fig. 1), which represented an economic value of 6.4 billion US\$ (FAO). Traditionally the major part of cephalopod catches is sold in Asia. In recent times, however, the European market, especially in Mediterranean countries, is growing considerably.

Despite the high fishing pressure on several cephalopod resources, only patchy knowledge exists on their distribution, migrations and stock structure, preventing optimum harvesting strategies. Their overall role in the marine environment has only begun to be studied. As reviewed in Piatkowski et al. (2001), two major shortcomings are responsible for the sparse knowledge on cephalopods compared to other marine groups: (1) cephalopods are difficult to catch and are mostly taken as by-catch in surveys targeting other taxa; (2) they have complicated life cycles and distribution patterns which are only roughly understood for a few species.

However, a profound knowledge about life cycles, migration routes and spawning grounds is essential for a scientifically based management of stocks. Due to their short life cycles, resource abundance and fishing yields in many cephalopod stocks depend on the recruitment of the annual cohort. In most cephalopod populations, the entire standing stock is replaced every year (Boyle and Boletzky 1996), making long-time predictions and a suitable resource management rather complex.

This study deals with two cephalopod species, the common cuttlefish *Sepia officinalis* and the boreoatlantic armhook squid *Gonatus fabricii*.

S. officinalis is widely distributed in eastern Atlantic shelf waters (Boletzky 1983) and supports one of the largest fisheries for cephalopods in the eastern Atlantic. Official statistics for this species indicate an average (1993-2003) production of 41 000 tons per year almost equally coming from the Atlantic and the Mediterranean (FAO 2005).

G. fabricii is the most abundant squid of the arctic and subarctic waters of the North Atlantic and is considered to have some commercial potential (Kristensen 1984, Piatkowski & Wieland 1993, Zumholz & Frandsen in press). This species is not commercially exploited yet, but Spanish vessels already carried out test fishing surveys in Greenland waters (Frandsen, pers. comm.).

What is a statolith?

Statoliths ("ear-stones") are small, white structures found in the gravity receptor system in the head of cephalopods (Fig 2). They grow throughout the lifetime of the animal and provide a sense of balance to the animals in much the same way that the inner ear provides balance in humans.

Statoliths are composed of calcium carbonate in the aragonite form intergrown with organic compounds, which consist mainly of high-molecular proteins (Radtke 1983). IN most cephalopods they form concentric rings visible under a light microscope, originating from periodically changing amounts of organics incorporated into the aragonite crystal (Kristensen 1980). The more organic material is incorporated, the better the increments can be visualized (Bettencourt & Guerra 2000).

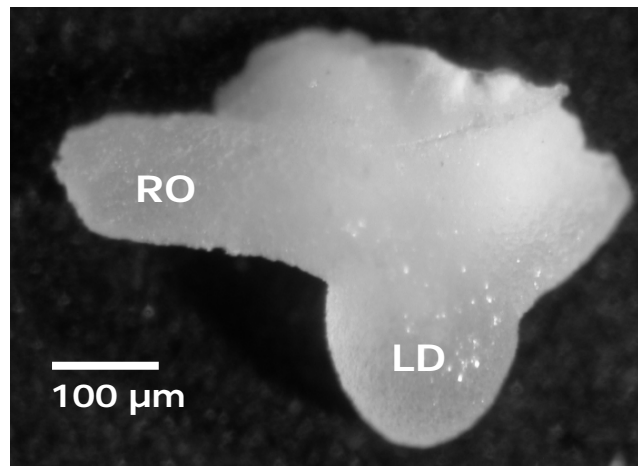


Fig. 2 Statolith of *Sepia officinalis*.
LD =lateral dome; RO = rostrum

The shape of the statolith is varying markedly between species. It changes during ontogenesis from a simple droplet shape in paralarvae to the differentiated adult statolith comprising the rostrum, dorsal and lateral domes and the wing, which are common to all squid and cuttlefish (Fig. 3). To analyse their microstructure, most statoliths need to be ground first. Various methods of statolith preparation and reading were described by Arkhipkin (1991) and Dawe & Natsukari (1991). Increment rings in statoliths of the cuttlefish *Sepia officinalis* are weakly developed in specimens from the field and even less developed in reared specimens (see Chapter II and III). In contrast, the statoliths of the squid *Gonatus fabricii* show very clear and distinct rings (see Chapter IV).

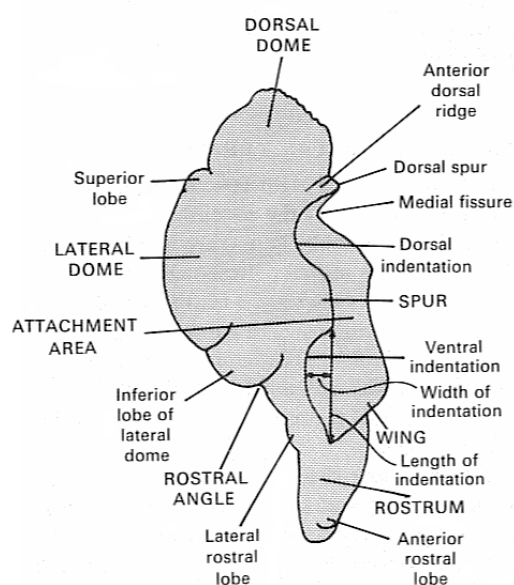


Fig. 3 Diagram of a generalized cephalopod statolith to show dimensions and terms used in descriptions. View of anterior side (from Clarke, 1978).

Diversity of information taken from statoliths

The amount of information that may be extracted from cephalopod statoliths by different methods (reviewed by Arkhipkin 2005) is quite similar to that taken from the otoliths of fishes (reviewed by Campana 1999). One of the striking differences between these two groups is their longevity. Cephalopods are typically short-lived animals (approx. one year), whereas most fishes are long-lived animals with life spans of at least several years. By analysing cephalopod statoliths, it is possible to reveal and to date many events in their life with daily precision. This is almost impossible to do in adult fishes because of the strong decrease in growth rates of otoliths in older fish and the disappearance of daily growth increments in the otoliths of fish usually older than two years (Campana and Neilson 1985).

Information from optical analyses

Age was one of the first biological parameters that was investigated from squid statoliths. After revealing growth increments within the statolith microstructure, Clarke (1966) suggested that they could be potentially used for age estimations. In analogy with the otoliths of juvenile fish (review: Campana and Neilson 1985), the hypothesis 'one growth increment – one day' was later adopted (Hurley et al. 1979, Lipinski 1980) and then validated using chemical marking (Dawe et al. 1985, Hurley et al. 1985). Where the validation has been done, the total number of growth increments within the statolith microstructure represents the age of an individual squid in days. Validation of the statolith growth increments is difficult due to the costly maintenance of squid in aquariums (Yang et al. 1986 and others) and the small return of tagged squid in the wild (Lipinski et al. 1998). However, given the similarity of growth increments in validated species with those observed in unvalidated species (especially if both species are closely related), the validity of the 'one increment – one day' hypothesis is generally accepted. Over the last fifteen years, about 52 squid species have been aged, including all known commercial squid species of the world's oceans (Arkhipkin 2004, Jackson 2004).

Three main growth zones (postnuclear, dark and peripheral) are usually observed in the statolith microstructure of adult squid. The formation of each zone generally corresponds to a major ontogenetic shift. The formation of the dark (with the widest growth increments) and peripheral (with the narrowest growth increments) zones in statoliths might be associated with decreased food intake in adult squid, and to maturation (Kristensen 1980, Morris and Aldrich 1985).

When a squid or cuttlefish hatches out of its egg, a stress mark (natal ring) appears in the statolith microstructure. By knowing the total age of a squid in days and the date of capture of that squid, it is possible to back-calculate its hatching date. Knowledge of hatching dates with a daily precision is very important, especially for short-lived animals such as squid (review: Jackson 1994).

Information from chemical analyses

Stock separation

Following the recent successful application of elemental signatures in fish otolith (Campana 1999), their potential as natural markers of cohort and population structure in squid has been studied as a compliment to more common genetic analyses using allozymes and microsatellite DNA (Carvalho and Pitcher 1989; Shaw *et al.* 2004). Geographical differences in elemental signatures in the statoliths of *Loligo gahi* appear to reflect the occurrence of two cohorts in different environments throughout a substantial portion of their lives (Arkhipkin *et al.* 2004). These cohorts were assumed to represent distinct population components with sufficient low-level intermixing of spawners to homogenise any genetic differences, which were shown earlier by genetic methods (Shaw *et al.* 2004). Trace element composition (chemical fingerprint) of the statoliths can therefore be used to identify and track specific groups of squid as they mix with other groups over short periods of time.

Ambient environmental conditions

Aragonitic hard increments of many marine taxa have been analysed for their chemical composition in order to find proxies for environmental conditions. During the last decades, several elements in coral aragonite have become well-established proxies for sea surface temperature and other parameters such as salinity upwelling events in modern and ancient times (e.g. Shen *et al.* 1996). However, the pathway of any given element or ion from the environment into cephalopod statoliths is a multi-stage process, and is characterized by a sequence of more or less independent barriers. The high degree of regulation is most evident in a phylogenetic comparison, in which the aragonitic skeleton of the most primitive animals (corals) tends to reflect the composition of the ambient water, while animals of increasing complexity (cephalopods, fish) show increasing discrimination against the most abundant elements (Campana 1999). This comparison implies that individual group-specific calibrations are needed. To predict accurately the environmental characteristics at the time of calcification, it is important to understand the influence of as many factors as possible.

Reconstruction of migration routes

In fish otoliths, micro-chemical analyses have proven to be a useful tool for the successful reconstruction of the fish's life history, ranging from temperature history (Patterson et al. 1993), to detection of anadromy (Secor 1992), use as a natural tag (Campana et al. 1995) and the determination of migration pathways (Thresher et al. 1994).

Chemical analyses of cephalopod statoliths indicate similar influences of temperature and salinity on their elemental composition as those for fish otoliths (e.g. Yatsu et al. 1998, Ikeda et al. 2002). However, no experimental data are available so far, and thus only qualitative indications from field studies exist. Therefore, rearing experiments under controlled environmental conditions are strongly needed to verify observations from the field.

Direct methods to track migrations like mark-recapture studies provide definitive patterns of movements but are generally unfeasible for cephalopods since their bodies are too fragile for any external tag. Most external recording devices on cephalopods last for less than a few days (Murata and Nakamura 1998). Furthermore the short life span of cephalopods complicates the procedure. There are many aspects that are essential for successful tagging-recapture studies in general. Problems may be caused by body-size and structure in relation to the tag or the ability of the specimen to remove the tag actively or passively. There are additional problems concerning the recapture of tagged cephalopods and a considerable fishing effort must be guaranteed. If at all, this method is suitable only for larger cephalopods like the jumbo squid *Dosidicus gigas* (Markaito et al. 2005). Another approach of telemetric tracking by acoustical microphones and transponders can only be successful for small scale migrations within a well-defined area like a bay or a fjord (Jackson et al. 2005). However, this migration scenario is only realistic for octopuses and cuttlefish, but definitely not for squids.

The main advantage of micro-chemical analysis of statoliths compared to tagging methods is that all animals are "tagged" and thus all specimens that are captured would represent a recovery. Furthermore an absolutely natural behaviour and life-cycle is guaranteed throughout the entire "tagging-period".

Analytical approach

In this thesis, several modern micro-analytical techniques are applied to statoliths in order to obtain high-precision chemical information on the environmental conditions of the animals, and further to evaluate and assess the possibilities and optimal utilization of the respective techniques. Recent applications of the micro-analytical methods include mainly geological samples,

which do not contain organic compounds. Therefore a considerable part of this project focussed on adjusting and optimising the respective methods to the analyses of biogenic aragonite intergrown with organic compounds.

The analytical methods used are Synchrotron X-ray Fluorescence Analysis (SYXRF, Chapter II), Laser ablation inductively coupled mass spectrometry (LA-ICP-MS, Chapter III and IV) and a high-resolution version of Secondary ion mass spectrometry (NanoSIMS, Chapter V). The technical differences between these techniques are addressed in the following paragraphs. The analytical potential in each case and the suitability for biological questions is discussed in Chapter VI.

SYXRF

X-ray fluorescence analysis bases on atomic inner shell processes. Once an atom has lost a core level electron by the absorption of a x-ray photon, the hole is refilled by an electron from a higher shell. The released energy can either cause a third electron to leave the atom (Auger effect) or may be emitted as a high-energy x-ray photon. The latter process is called x-ray fluorescence. The energy of a fluorescence photon is characteristic for the atom and thus every element contained in the sample leaves its characteristic fingerprint of x-ray fluorescence photons.

The x-ray tube is the most widely used photon source. It provides the experiment with monochromatic characteristic radiation from the anode material and/or a white bremsstrahlungs-continuum. The major advantages are an easy operability, low costs and mobility. Disadvantages may be the monochromatic radiation, small photon flux or small brilliance, and limited spatial resolution.

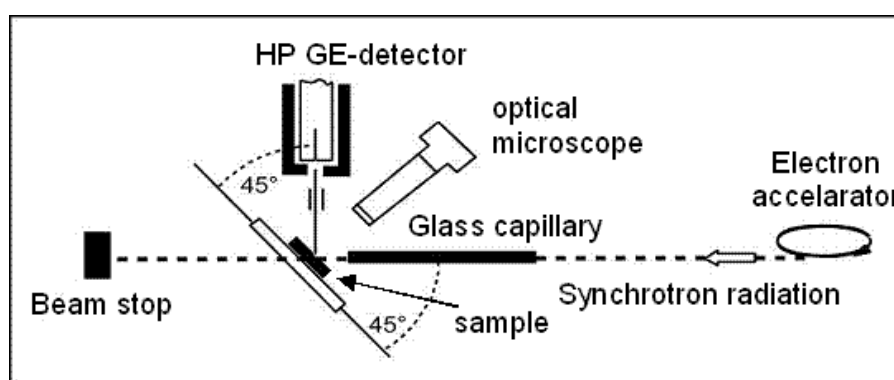


Fig. 4 Schematic SYXRF set-up at HASYLAB, Hamburg

A more powerful source with high photon flux and brilliance is the synchrotron (Fig. 4). Ultra-relativistic electrons are forced on a closed orbit of tens to hundreds of meter length. The acceleration of the electrons in the curves results in a white photon emission spectrum, comprising energies from the infrared to hard x-rays. The photons are naturally collimated and polarized, opening for a

wide range of applications. Any energy might be cut out with monochromators for specially designed experiments but also the white beam may be used as in our case at HASYLAB beamline L (Lechtenberg et al. 1996).

LA-ICP-MS

In analytical atomic spectrometry, free atoms or ions are required for the interaction with light energy. A laser beam focused by the optics of a microscope on the surface of a solid leads to the evaporation of a microscopic amount of sample. Because the laser ablation process deals only with sampling, the laser-ablated material must be transported by a carrier gas to a second excitation process (ICP). In this process, the ablated material is evaporated, atomized, excited by the second energy source and finally led into a mass spectrometer. An advantage of this two-step operation is the potential of increased efficiencies during the evaporation, atomization, and excitation steps.

LA-ICP-MS was first used in 1985 and has been an important analytical tool in the earth sciences since the early 1990s. It has been widely used as a powerful analytical technique for solid micro sampling analyses in geological, biological, environmental, nuclear, and metallurgical applications. This rapid and moderate cost technique combines the advantages of the high sensitivity multi-element capability by ICP-MS and *in-situ* micro solid sampling by LA. It is particularly attractive to scientists who want to study spatial distributions of trace elements in a micro scale area on sample surfaces.

NanoSIMS

SIMS is being used for the compositional analysis of small samples. In a SIMS instrument (or "ion microprobe") a high energy primary ion beam is directed at an area of the sample whose composition is to be determined. The interaction of the primary ions with the sample causes the ejection of some secondary particles (atoms and small molecules) from the sample. Charged particles of one polarity ("secondary ions") can then be extracted from the sputtering area with the help of an electrical field between the sample and an extraction lens. These accelerated secondary ions constitute a secondary ion beam, which is then led into a mass spectrometer. There, the secondary ions are sorted by mass (and energy) and finally counted in an ion detector (which can be an electron multiplier, a Faraday cup or a channel plate).

The CAMECA NanoSIMS 50, as used in this study, is a new ion microprobe, developed for trace element analysis of ultra-fine structures. Among the unique new features offered by the NanoSIMS 50 is the ability to extend the SIMS analysis to extremely small areas down to 50nm, while maintaining extremely high sensitivity at high mass resolution. Further, up to 5 masses (ions) can be measured in parallel.

Aim of this study

The general focus of the present study was to evaluate the ability of micro-chemical analysis of statoliths to give information about the environmental conditions during the animal's life. From this information a reconstruction of life history and individual migration routes seems possible. In particular, the influence of temperature and salinity (see **Chapter II**) and diet (see **Chapter III**) on the chemical composition of statoliths was tested in laboratory experiments under controlled conditions. By analysing statoliths of animals with a known life history from the laboratory, both a qualification and a quantification of such influences were attained.

After having evaluated the different methods and the influences of environmental factors in the lab, the analyses of statoliths was applied to cephalopods from the field (**Chapter IV**). In order to obtain new information on the chemical micro- and nanostructure of statoliths, the "high end" spatially resolved surface-analysis NanoSIMS was applied to statoliths of cuttlefish from the lab and squid from the field (**Chapter V**). The outcomes of these studies are further discussed in **Chapter VI**, thereby outlining the suitability and importance of these analytical techniques for further field studies.

The crucial questions during this project were:

- Which elements in the statolith can give evidence for the environmental factors encountered by the animal?
- Can the relation between distinct environmental parameters and element concentrations be quantified?
- Can microchemical analysis provide a kind of a Curriculum Vitae for individual animals?
- Which ecological questions can be answered with microchemical analyses of biomineralized tissues and which analytical techniques are most suitable in each case?

The results of this study will provide an essential basis for future investigations in the field. This may provide us with a better understanding of yet unknown migration patterns and spawning grounds of various cephalopod species. Such information can contribute considerably to an effective management of this valuable resource.

Chapter II

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

Abstract

This study presents the first microchemical analyses of cephalopod statoliths from laboratory experiments under different controlled environmental conditions. The results imply that statolith chemical composition is strongly related to both salinity and temperature in ambient waters. Element/Ca ratios for iodine, yttrium, zirconium, niobium and nickel are positively related to temperature and negatively to salinity. Ba/Ca ratios are negatively related to temperature and do not show a relation to salinity. Strontium showed no relation to both salinity and temperature, suggesting that the well-established proxy strontium is not as useful in cephalopods 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 (SYXRF) is introduced as a promising novel method for statolith analysis, providing a spatial resolution of 12µm combined with detection limits down to 0.5 ppm.

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 & 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 & 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 despite of constant conditions during the rearing (Ikeda et al., 2002).

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, 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., 2002; Bettencourt & Guerra, 2000), but statolith chemistry appears to be a field of growing interest. 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, diet or other factors. 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 *Todarodes 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 which 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.

Microchemical studies on statoliths so far were based on various techniques, including particle induced X-ray emission (PIXE) (Ikeda et al., 1996a, 1996b; Lipinski et al., 1997; Durholtz et al., 1997), electron probe microanalysis (EPMA) (Bettencourt & Guerra, 2000; Ikeda et al., 2002; Ikeda et al., 2003), and solution based inductively coupled plasma mass spectrometry 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., 2004), tree stems (Martin et al., 2003) 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 the 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 and temperature and salinity of the surrounding water (3) evaluate the suitability of SYXRF for this kind of analysis.

Materials and methods

Rearing experiments

Cuttlefish (*Sepia 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 240l glass tanks at a density of 1 cuttlefish per 20 litres. 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 (15 tanks in total). Constant salinity and temperature were maintained throughout the whole experiments. Temperature was controlled permanently in climate chambers. Salinity was controlled daily. Artificial seawater (InstantOcean®) 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 were measured. Due to high mortalities during experiments, replicates were pooled and five specimens from each 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 micrometers 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. Due to the trough passing x-rays, however, the influence of a contaminated surface is minimized when using SYXRF.

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 (4000) 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 $0.01 \text{ ng}\cdot\text{ml}^{-1}$.

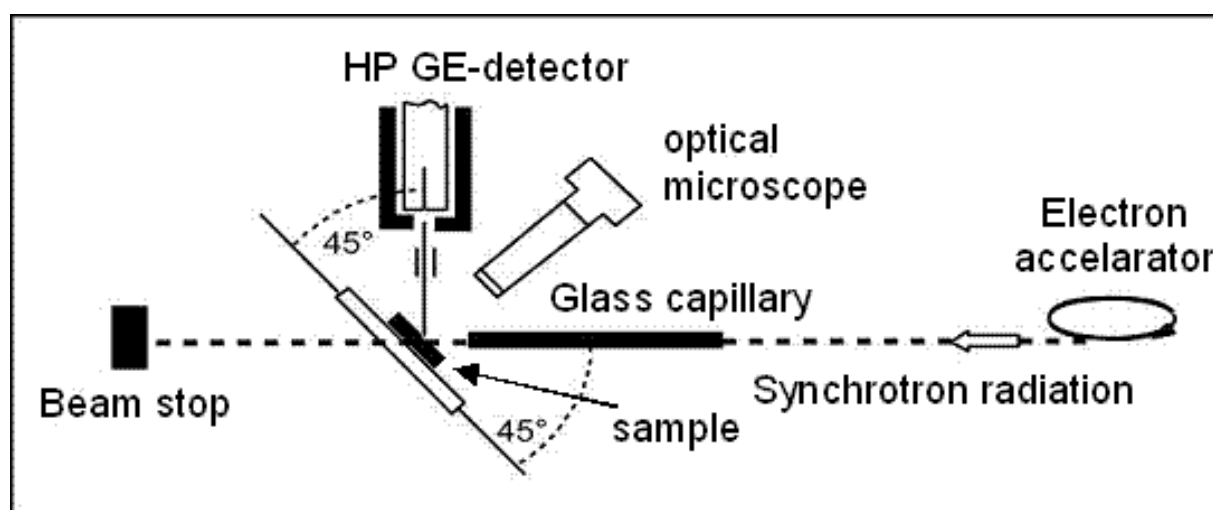


Fig. 1:
Schematic SYXRF set-up at HASYLAB, Hamburg

Synchrotron X-ray Fluorescence microprobe (SYXRF) was used to determine the distribution of trace elements in statoliths. Doubly polished, 40 micrometers thick sections were analysed at the SYXRF set-up at HASYLAB Beamline L (Fig. 1), using white synchrotron radiation and a glass capillary producing a beam diameter of about 12 micrometers. The primary beam was passed through a 200 μm thick ultra-pure Cu foil; acquisition times were typically 1000s. 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 = $(\sin 45^\circ)^{-1} * \text{sample thickness}$. 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 1000 s acquisition time are typically between 0.5 ppm and 10 ppm for elements between $Z = 27$ (Co) and $Z = 64$ (Gd) using the $K \alpha$ and $K \beta$ 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 was 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. altogether 30 points on 5 specimens were analysed per treatment.

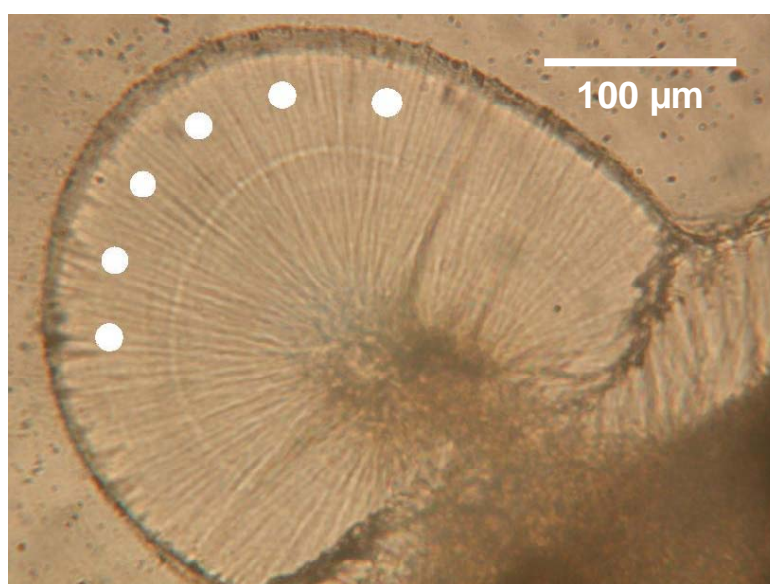


Fig. 2: *Sepia officinalis*. Ground statolith showing the location of analytical points in the outer region of the lateral dome as white dots. Location of measurements is displayed artificially, since SYXRF leaves no visible marks in or on the statolith.

Statistical analysis

Analysis of variance (ANOVA) was used to test for significant element/Ca ratio differences (1) in water between tanks and (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, strontium and yttrium 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 ($p > 0.05$). Concentrations of zirconium were close to detection limit and showed high variances, niobium and iodine were 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. 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.

Salinity effect

For iodine, yttrium, zirconium, niobium and nickel, the respective element/Ca ratios differed significantly between salinity treatments (table 1). For all five elements, post-hoc Tukey multiple comparisons revealed that the element/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

Iodine, yttrium, zirconium, niobium and nickel showed positive relations to temperature while barium is negatively related to temperature (table 1). For all six elements, post-hoc Tukey multiple comparisons revealed that determined element/Ca ratios for the 19°C treatment varied significantly from 25°C and 28°C (Fig. 4). Differences between 25°C and 28°C, however, were not significant. Strontium did not show any clear relation to temperature.

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

$$[\text{Ba}/\text{Ca}] = 26.89 - 0.574 T$$

$$[\text{I}/\text{Ca}] = 1.49 + 0.244 T$$

Table 1:

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

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
Y/Ca		
temperature	8.61	0.007
salinity	15.29	0.0007
Zr/Ca		
temperature	7.23	0.01
salinity	11.34	0.002
Nb/Ca		
temperature	7.52	0.01
salinity	7.51	0.009
Ni/Ca		
temperature	17.48	0.0005
salinity	45.61	0.0000

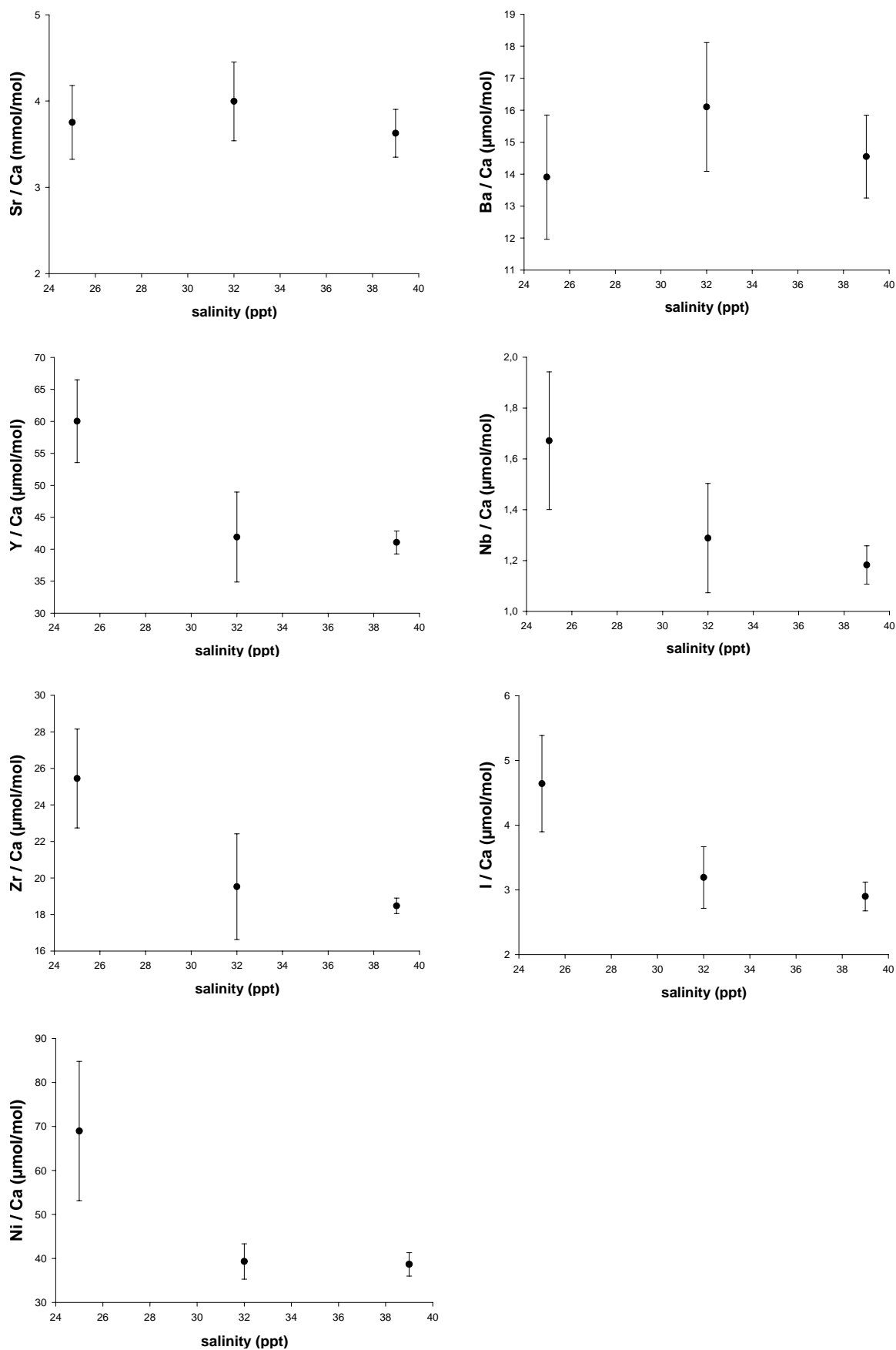
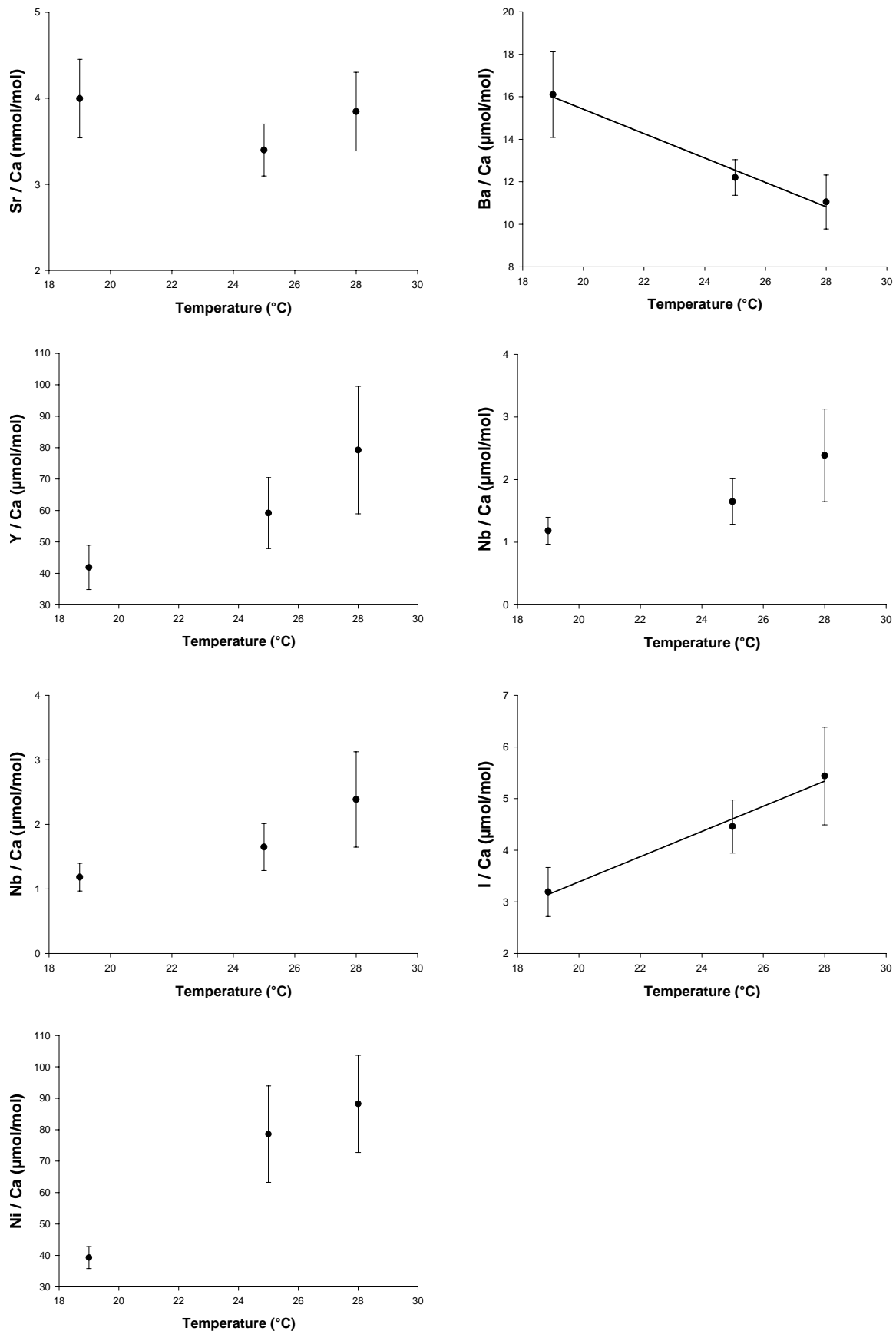


Fig. 3: *Sepia officinalis*. Element concentrations determined by SYXRF in the distal part of the statolith lateral dome versus salinity of the surrounding water in laboratory experiments. Points represent means of five specimens with six analytical spots each. Error bars represent standard deviation.

**Fig. 4:**

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.

Discussion

Our results demonstrate a strong influence of both temperature and salinity on the incorporation of elements into cuttlefish statoliths. Elemental concentrations of iodine, yttrium, zirconium and niobium have not been determined before in statoliths. Therefore no published information on these elements 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 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., 2002) 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, 1989; 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 (Yatsu et al., 1998; Ikeda et al., 1996a). 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., 2002). 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 the fact that the ambient concentration or the element/Ca ratio of an element 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 (SrCO₃) (Greeger et al., 1997; but for a different view see: Finch et al., 2003). Since strontium concentrations are similar to those in cephalopod statoliths, this 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, but the actual mechanism of statolith formation is still unknown.

Barium

The value of Ba as a proxy for water depth due to its nutrient-like distribution is well established (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, not confounded by salinity. A positive relation of Ba/Ca ratio in surrounding water to barium incorporation has been demonstrated for gastropods (Zacherl et al., 2003), but the absolute barium concentration appears to have less influence in cephalopods.

Iodine, Yttrium, Zirconium, Niobium, Nickel

For iodine, yttrium, zirconium, niobium and nickel, the element/Ca ratio of the surrounding water was constant in all treatments. The negative relations of these elements to salinity suggest that their incorporation is dependent on the absolute concentrations and not 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. Anyhow, since the analyses of these trace elements in seawater do not allow for a separation of free and bound ions, we can only speculate about this phenomenon. As analytical possibilities will improve, this question may be addressed in future investigations.

Moreover, the element/Ca ratios for these five elements 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.

Rather close relations between concentrations of yttrium, zirconium and niobium independent from treatments suggest a common mode of inclusion. The difference compared to strontium and barium may be explained by the different

incorporation process into the crystal lattice, or alternatively, a preconcentration in the organic portion of the statoliths.

Iodine is located in either the organic matrix as an organo-iodo compound or is incorporated as iodate in the lattice. The variations with temperature may be explained by different growth rates, but the salinity variation is harder to explain without knowing what the iodine resides in the lattice. However, if it was passive incorporation into the lattice from an inorganic form the salinity should not change the incorporation ratio.

Yttrium is chemically very similar to the Rare Earth Elements (REEs), which are well studied in corals and other CaCO₃ minerals (Sholkovitz and Shen, 1995). In seawater Y is mostly dissolved as carbonate complexes and so would easily be taken up into the aragonite lattice of the statolith. In principle this explains the observed behaviour of Y incorporation into the statoliths. Anorganic precipitation of aragonite would typically result in Y/Ca ratios 1-2 times higher than in seawater. The observed anomalous enrichment of Y may thus be related to biological processes. The temperature and salinity variations might be anticipated based on a decreased amount of Y(CO₃)⁺ with increasing salinity and an increase with temperature due to changes in pH (more basic solution). However, pH measurements would be needed to address this problem in a quantitative manner.

Both zirconium and niobium are present in seawater at extremely low concentrations and their chemistry is such that they would not normally be expected to occur in such elevated concentrations in the aragonite. Dissolved zirconium shows a recycled-type vertical profile in the Northwest Pacific with concentrations around 330pM, while niobium shows a more uniform profile with concentration around 3pM (Sohrin et al., 1998). They are both present at highly enriched concentrations over their metal:Ca ratios in seawater. As in Y, this enrichment is probably biologically controlled.

The role of yttrium, zirconium and niobium in the metabolism is poorly studied, so that assumptions on metabolic influences cannot be based on facts. The incorporation of these elements is obviously influenced by salinity and temperature, but further studies on the underlying processes are strongly needed for an adequate evaluation.

Microchemistry as a fingerprinting of migration pathways

In the salinity experiment, no differences were found between 32 and 39 ppt for any element/Ca ratio. Since by far the most cephalopods live within this salinity range and will never experience lower salinities (*Sepia 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.

The element/Ca ratios were not manipulated and therefore not different between the treatments in the current study. We wanted to test whether the statoliths elemental composition is influenced by the “standard” parameters salinity and temperature. Since data for these parameters are easily available with enormous spatial and temporal resolution for most areas in the world ocean, studies on chemical statolith composition would be easily interpretable. Further experimental studies should investigate the influence of element/Ca ratios in the water. But the comparably bad documentation of trace element concentrations in natural water masses makes it difficult to interpret field studies concerning migration pathways and natal origins.

Having only three points per element at our disposal, the exact form of a calibration curve is uncertain for a majority of the elements, and it thus appears inappropriate to fit distinct curves to our data alone. The relations of Y, Zr, Nb, Ni and I to salinity, however, show obvious negative trends. The same five elements show positive relations to temperature, while Ba appears to be negatively related to temperature. Among the presented relations, the very good linear trends between Ba, I and temperature stand out in the data set. Despite the mentioned uncertainties concerning the accurate curve form, we estimated a linear regression for the temperature influence on Ba and I. This relation is in accordance with the linear Ba-temperature relation demonstrated for the neogastropod *Kelletia kelletia* (Zacherl et al., 2003). Further, the estimated least square regression lines are very close to the mean values. However, it must be emphasized that the lines and equations given here are not meant to represent accurate correlations, but first approximations of relations that obviously affect the microchemistry of statoliths. Therefore we strongly recommend keeping this uncertainty in mind when using these equations in further studies.

To avoid any possible influence of diet (Chapter III) 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, than temperature has been the dominant variable in our experiment.

Our results, however, revealed 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 diet, growth and stress.

Suitability of SYXRF for the analysis of zoned biocrystals:

The current study is the first to apply SYXRF to statoliths. The set up as used in our study provides a unique combination of both high spatial resolution around 12µm, according to approximately five 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 elements in the periodic table of elements 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, strontium appears to be no useful proxy for neither temperature nor salinity. 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. 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. To analyse transect representing the whole lifetime, it is essential to have a thinly ground slice containing the core region. Furthermore, we have to find a transect for our measurements that forms a right angle to the growth rings. 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 μ m in our study. Analysing overlapping spots can halve this spatial resolution. 12 μ m in the earstone are equivalent to approximately 5 days in the life of cephalopods, depending on species and individual growth rates.

The finding of hitherto not determined elements, given a high spatial resolution for statoliths in this study expresses the suitability of SYXRF. 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 SYXRF analyses provide an average value of elemental concentrations in a cylindrical volume through the sample. Thereby smaller scale heterogeneities that may occur due to daily increment formation (Chapter V) 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 finding of iodine and the comparably high concentrations of yttrium, zirconium and niobium.

The combination of spatially resolved chemical analyses and growth increment interpretation provides a method of dating observed changes in elemental 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 strongly required.

Conclusion

This study demonstrated considerable influences of temperature and salinity on the elemental composition of cuttlefish statoliths. We detected four elements (Iodine, Yttrium, Zirconium and Niobium) that showed negative relations to salinity and positive relations to temperature. Strontium, a well-established proxy in other marine biomineralized tissues, appears to be no useful proxy for both salinity and temperature in cuttlefish. Barium, however, may bear the best potential for field studies, since the barium concentration in statoliths is influenced only by temperature and not by salinity. The advanced SYXRF set-up as used in this study provides excellent prospects for trace element analyses of calcified tissues, particularly since most of the detected elements cannot be measured with other methods given a similar spatial resolution.

Acknowledgements

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Chapter III

Food effects on statolith composition of the common cuttlefish (*Sepia officinalis*)

Abstract

The concentration of trace elements within cephalopod statoliths can provide a record of the environmental characteristics at the time of calcification. To reconstruct accurately the environmental characteristics at the time of calcification, it is important to understand the influence of as many factors as possible. To test the hypothesis that the elemental composition of cuttlefish statoliths could be influenced by diet, juvenile *Sepia officinalis* were fed either shrimp *Crangon sp.* or fish *Clupea harengus* under equal temperature and salinity regimes in laboratory experiments. Element concentrations in different regions of the statoliths (core – lateral dome – rostrum) were determined using laser ablation - inductively coupled plasma mass spectrometry (LA- ICPMS). The ratios of Sr/Ca, Ba/Ca, Mn/Ca and Y/Ca in the statolith's lateral dome of shrimp-fed cuttlefish were significantly higher than in the statolith's lateral dome of fish-fed cuttlefish. Moreover, significant differences between statolith regions were found for all analysed elements. The fact that diet adds a considerable variation especially to Sr/Ca and Ba/Ca must be taken into account in future micro-chemical statolith studies targeting cephalopod's life history.

Introduction

Statoliths, the earstones of cephalopods, are composed mostly of calcium carbonate (aragonite form) but also include protein and minor and trace elements. The concentrations of elements in statoliths can provide substantial information for the reconstruction of cephalopod's environmental histories (Ikeda et al 2003, Zumholz et al. Chapter II and IV). Furthermore, stock separations based on statolith microchemistry appear to be possible (Arkhipkin et al. 2004).

Statolith composition studies generally assume that element incorporation into the statolith is mainly influenced by chemical and physical parameters of the surrounding water (Yatsu et al 1998, Zumholz et al. Chapter II). However, strontium is also incorporated into the statolith matrix via the cephalopod's diet (Hurley et al 1985). In their study, artificially enriched diet (shrimp soaked in strontium chloride solution) was used to chemically mark distinct statolith regions. Therefore a positive effect of Sr in the diet on the statolith Sr/Ca was revealed, but a quantification of this influence has not been shown yet. Furthermore, strontium is the only element tested for a dietary effect up to now.

Research on fish otoliths has shown that many factors can affect otolith chemistry (for a detailed review, see Campana 1999). In addition to temperature and salinity, growth rate (Sadovy & Severin, 1992; Mugiya & Satoh, 1997), gonad development (Kalish, 1991), stress (Kalish, 1991; Townsend et al. 1992) and diet (Limburg, 1995; Gallahar & Kingsford, 1996, Buckel et al. 2004) are all thought to influence incorporation of elements into otoliths. The magnitude of the effect of these factors on cephalopod statoliths, and how precisely they can be analysed, will be important in determining how reliable statoliths can be used in recording environmental history.

The general effect of diet on fish otolith chemistry is still being debated; of the six studies that have addressed this question (reviewed by Buckel et al. 2004), three have found a dietary effect on otolith Sr/Ca (Limburg 1995, Gallahar and Kingsford 1996) or Sr/Ca and Ba/Ca (Buckel et al 2004). Three other studies on different species, however, found no effect of artificially enriched diet on otolith composition (Hoff and Fuiman 1995, Farrell and Campana 1996, Milton and Chenery 2001).

For spatially resolved microchemical analyses of statoliths, the evaluation of the elemental composition in different statolith regions like core, rostrum and lateral dome is essential. Considerable spatial variations in strontium contents, but not in magnesium were found in statoliths of squid and cuttlefish (Durholtz et al. 1997, Bettencourt and Guerra 2000). Distributions of other elements, however, have not been investigated systematically up to now.

Cuttlefish are among the most important cephalopod resources in European waters (Denis and Robin 2001). Official statistics for this species indicate an average (1993-2003) production of 41 000 tons per year almost equally coming from the Atlantic and the Mediterranean (FAO, 2005). As well as representing an important fisheries resource, cuttlefish have a potentially important ecological role both as predators on shrimp and fish (Castro and Guerra 1990) and as prey (e.g. Clarke and Pascoe 1985). Since cuttlefish is rather unproblematic in rearing experiments, compared to other cephalopods, this species is regularly used as a model organism for cephalopods studies. Thus, cuttlefish provide an opportunity to examine the effect of natural prey types on statolith chemistry using fish and shrimp. The dietary effect of natural prey types, a crucial question for future micro-analytical statolith studies targeting cephalopod's life history, has never been investigated before. The aim of this study therefore was to (1) test the null hypothesis that statolith chemistry of cuttlefish is independent of diet, and (2) to quantify and compare the elemental composition in different regions of *S. officinalis* statoliths.

Materials and Methods

Rearing experiments

Cuttlefish eggs were collected in coastal waters of Normandy, France in July 2005 and transported to the aquarium at IFM-GEOMAR, Kiel. Hatching took place in the aquarium in a common tank in running water from the main aquarium water circuit (salinity: 28ppt, temperature 19°C). Hatchlings were initially fed with live mysid shrimps for the first 20 days. After that time they were randomly distributed among six experimental tanks and were fed either shrimp (*Crangon sp.*) or fish (*Clupea harengus*) prey over a 60-day period. For each food treatment three replicate tanks with a stocking of three specimens each were used. The light: dark cycle was 12:12h for the duration of the experiment. Both prey types were caught in the North Sea and stored frozen. The temperature during the experiments was maintained at 21.3°C (standard deviation: 0.6°C), salinity was 28.4ppt (standard deviation: 0.4ppt). Both temperature and salinity were controlled daily. At the end of the experiment, the animals were killed and statoliths were removed immediately.

Statolith processing

One statolith from each cuttlefish was used, i.e. altogether 18 statoliths were analysed. Statoliths were attached concave side up onto a microscopic glass slide with thermoplastic resin (Buehler[®]) and were ground on one side with wet waterproof lapping film (3M[®]; 30µm, 15µm, 3µm) until the ground surface extended to the edge (i.e. the most extended point) of the dorsal dome and the focus could be seen just beneath the ground surface. Ground statoliths were

observed under a Leitz Laborlux K microscope (250× magnification) to locate the analytical regions.

Statolith analyses

The polished statoliths used for element analysis were triple-rinsed in MilliQ water. Eight analytical points were placed on each statolith (Fig. 1). One point was placed into the nucleus, i.e. the region laid down before hatching. Four points were placed into the outer lateral dome and three points were placed into the rostrum, both regions representing the time of experiments.

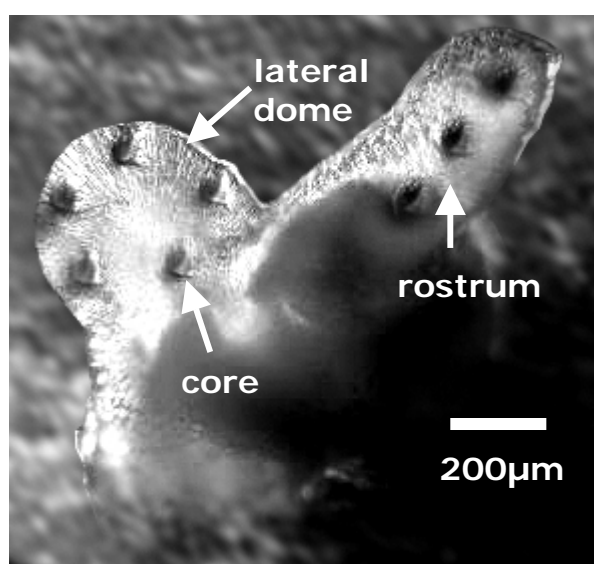


Fig. 1:
Ground statolith of *Sepia officinalis* showing the laser ablation craters at analytical points.

The analyses were carried out by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) 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. Helium was used as sample gas in the ablation cell ($0.4 \text{ l}\cdot\text{min}^{-1}$) and Argon was subsequently added ($0.8 \text{ l}\cdot\text{min}^{-1}$) to the gas flow. Ablation parameters included a laser energy of 2-2.5 mJ (Q-switched mode) and a pulse rate of 5 Hz resulting in crater sizes of 60- 80µm. Eleven isotopes were recorded during 90 seconds with five samples near the center of each peak and a total dwell time of 50 ms per isotope. Owing to the lack of matrix-matched standards, we used the glass reference materials NIST610 (for ^{23}Na , ^{43}Ca , ^{88}Sr) and NIST612 (for ^{23}Na , ^{25}Mg , ^{43}Ca , ^{55}Mn , ^{64}Zn , ^{89}Y , ^{90}Zr , ^{93}Nb , ^{138}Ba , ^{238}U) 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.

We used the GeoPro™ software 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%. We preferred single-point over two-point calibration because the two differently coloured NIST standards show distinct ablation behaviour and element fractionation at 266 nm laser wavelength (Guillong et al., 2003). The external analytical precision was determined through replicate analyses of the homogeneous standard reference materials NIST612 and GOR128G (basaltic glass; Jochum et al. (2000)). Our data indicate a precision of better than 3 % for concentrations above 0.5-1 $\mu\text{g}\cdot\text{g}^{-1}$ and up to 13 % for concentrations above 0.01 $\mu\text{g}\cdot\text{g}^{-1}$.

Prey and water analyses

Bulk analyses of prey and water 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. Samples of whole shrimp and fish pieces as used for the feeding were freeze-dried and hackled. The dry samples were weighted (between 146 and 368 mg) and mixed with 5 ml of concentrated ultrapure nitric acid in teflon beakers for more than a day at room temperature. For subsequent pressure digestion the samples were heated in about 10 minutes to 210 °C in a MLS Ethos™ microwave and the temperature held for further 10 minutes. The solutions were diluted and spiked with indium as internal standard with the final analyte solutions containing 0.29-0.74 $\text{mg}\cdot\text{ml}^{-1}$ of total dissolved solids.

For sample introduction we used a PEEK cyclonic spray chamber with a micro-flow nebulizer operating in self-aspirating mode. In order to avoid mass interferences, Mg, Mn, Ni and Zn were measured at medium resolution (4000) 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 $\text{ng}\cdot\text{ml}^{-1}$.

Statistical analyses

Molar element/Ca ratios were calculated and values per statolith region were averaged by specimen. We compared statolith element/Ca ratios using a nested ANOVA to test for the hypotheses of no overall effect of diet on element incorporation into statolith aragonite. For comparisons between statolith locations a one-way analysis of variance (ANOVA) and post-hoc Tukey multiple

comparisons were used to test for significant differences in element/Ca ratios among the core, the lateral dome and the rostrum. The assumptions of ANOVA were met: the data were normally distributed and variances were homogeneous among factor levels.

Results

Water chemistry

Element/Ca ratios in the water did not differ significantly between treatments or between replicates for all analysed elements (p-values between 0.08 and 0.87). The elemental composition of the rearing water is listed in table 1.

Table 1:

Mean \pm s.d. element/Ca ratios in the rearing water determined by ICP-MS

elements	Tank water fish diet	Tank water shrimp diet
Sr/Ca (mmol·mol ⁻¹)	17.0 \pm 0.9	16.3 \pm 0.4
Y/Ca (nmol·mol ⁻¹)	134.6 \pm 3.0	147.9 \pm 32.4
Zr/Ca (nmol·mol ⁻¹)	968.8 \pm 125.6	916.5 \pm 73.1
Nb/Ca (nmol·mol ⁻¹)	15.5 \pm 1.6	18.3 \pm 2.9
Ba/Ca (μ mol·mol ⁻¹)	96.5 \pm 8.4	87.4 \pm 6.9
U/Ca (nmol·mol ⁻¹)	782.3 \pm 23.2	644.6 \pm 79.3
Mg/Ca (mol·mol ⁻¹)	11.1 \pm 0.1	11.2 \pm 0.4
Mn/Ca (μ mol·mol ⁻¹)	1.55 \pm 0.27	1.1 \pm 0.35
Zn/Ca (μ mol·mol ⁻¹)	2.61 \pm 0.39	7.28 \pm 4.96

Prey composition

The concentrations of all analysed elements except from U differed between the two prey types (table 2). Shrimp prey contained significantly more Ca, Sr, Ba, Mg, Mn, Y, Zr, Nb and less Zn than fish prey (t-test, $p < 0.05$). Not only element concentrations, but also the respective element/Ca ratios differed significantly between both groups.

Table 2:

Mean \pm s.d. element concentrations for fish (*Clupea harengus*) and shrimp (*Crangon sp.*) prey

element	fish	shrimp
Ca	1184 \pm 162 μ g·g ⁻¹	1570 \pm 271 μ g·g ⁻¹
Mg	83.5 \pm 10.8 μ g·g ⁻¹	148.5 \pm 10.8 μ g·g ⁻¹
Mn	0.45 \pm 0.14 μ g·g ⁻¹	1.23 \pm 0.22 μ g·g ⁻¹
Zn	6.4 \pm 1.0 μ g·g ⁻¹	2.8 \pm 0.3 μ g·g ⁻¹
Sr	3.1 \pm 0.4 μ g·g ⁻¹	42.1 \pm 7.6 μ g·g ⁻¹
Y	0.14 \pm 0.05 ng·g ⁻¹	3.3 \pm 2.7 ng·g ⁻¹
Zr	0.86 \pm 0.65 ng·g ⁻¹	9.6 \pm 4.4 ng·g ⁻¹
Nb	< 0.02 ng·g ⁻¹	0.86 \pm 0.4 ng·g ⁻¹
Ba	59 \pm 14 ng·g ⁻¹	300 \pm 60 ng·g ⁻¹
U	0.86 \pm 0.23 ng·g ⁻¹	0.76 \pm 0.4 ng·g ⁻¹

Statolith analyses

Dietary effect:

Sr/Ca, Ba/Ca, Mn/Ca, and Y/Ca are significantly higher in the statolith's lateral dome of shrimp-fed cuttlefish compared to fish-fed cuttlefish (Fig. 2). Though statistically significant, the absolute differences were comparably low, ranging between 5 and 15%. However, no significant difference between treatments could be found in the rostrum and in the core for any element (table 3). When averaging the elemental composition over the whole statolith, i.e. all analytical points from all regions, again no significant difference between treatments was observed.

Table 3:

Analyses of Variances (ANOVA) table summarizing the dietary effect (p-values for shrimp-fed vs. fish-fed specimens) on the element/Ca ratios in different statolith regions of *Sepia officinalis*

Element/Ca	core	lateral dome	rostrum
Sr/Ca	0.36	0.0006	0.22
Ba/Ca	0.31	0.005	0.82
Mg/Ca	0.85	0.42	0.24
Na/Ca	0.18	0.43	0.065
Mn/Ca	0.26	0.004	0.25
Y/Ca	0.68	0.00004	0.064
U/Ca	0.69	0.059	0.24
Zr/Ca	0.83	0.41	0.43
Nb/Ca	0.21	0.84	0.40
Zn/Ca	0.22	0.63	0.70

Spatial variations:

Significant differences in element/Ca ratios between the three distinguished statolith regions (core – lateral dome – rostrum) were observed for all determined elements (table 4). Element/Ca ratios in the core were significantly higher than in both the lateral dome and the rostrum for Sr, Mg, Y, U and Mn (Fig. 2). Na/Ca ratios in the core and in the rostrum are significantly higher than in the lateral dome. Ba/Ca ratios are significantly lower in the core compared to the lateral dome and the rostrum. For Zn/Ca, Zr/Ca and Nb/Ca, only a significant difference between the core and the lateral dome was found. Their element/Ca ratios in the lateral dome and in the rostrum are not significantly different.

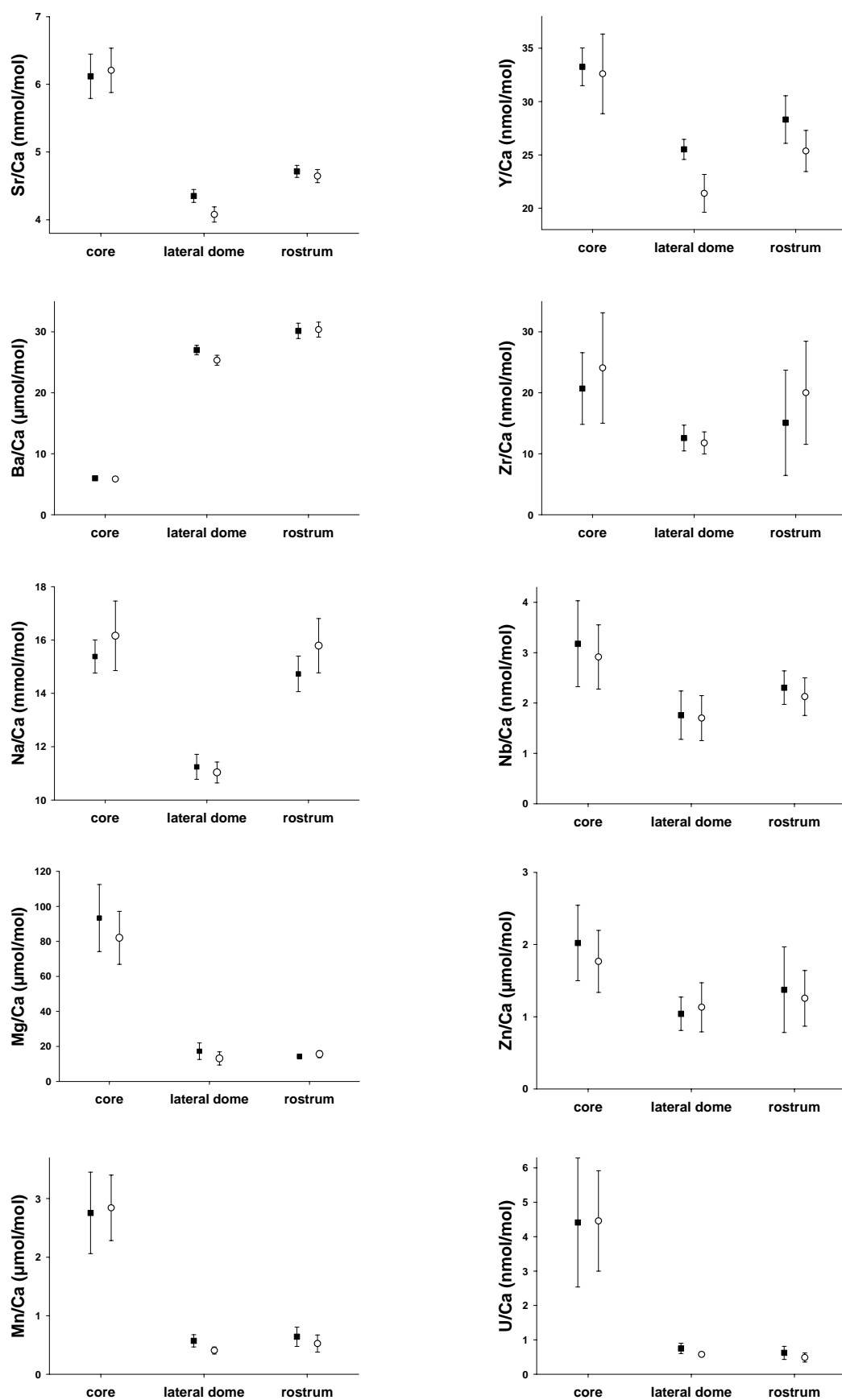


Fig. 2: Element/Ca ratios measured by LA-ICPMS in different regions of *Sepia officinalis* statoliths from shrimp-fed (filled squares) and fish-fed (open circles) specimens. Each point represents the mean of measurements in 9 statoliths; error bars indicate 95% confidence intervals.

Table 4:

Analysis of variance (ANOVA) table summarizing the effect of *Sepia officinalis* statolith location on element/Ca ratios. All 18 statoliths from both food treatments were pooled for this analysis.

element	df	Anova between core- LD -rostrum		Post-hoc Tukey-multiple comparison		
		F	p	core vs. LD p	core vs. RO p	LD vs. RO p
strontium	2	248.41	<0.0001	0.0001	0.0001	0.0001
barium	2	2099.1	<0.0001	0.001	0.001	0.001
magnesium	2	108.39	<0.0001	0.0001	0.0001	0.99
sodium	2	96.34	<0.0001	0.0001	0.35	0.0001
manganese	2	51.09	<0.0001	0.0001	0.0001	0.95
zinc	2	4.26	0.019	0.031	0.114	0.83
yttrium	2	40.56	<0.0001	0.0001	0.0001	0.007
zirconium	2	3.79	0.029	0.017	0.135	0.64
uranium	2	27.30	<0.0001	0.0001	0.0001	0.99
niobium	2	7.76	0.001	0.002	0.01	0.80

Discussion

Dietary effects

Our data show that the hypothesis that statolith chemistry is independent of diet can be rejected for cuttlefish. Especially Sr and Ba, elements that are almost routinely used as proxy for salinity or temperature of the surrounding water for fish, corals and bivalves, should be viewed with some caution in future cephalopod investigations. Although it is not possible to quantify the dietary influence on statolith composition, our data suggest that this effect seems to be rather small compared to e.g. the temperature effect on Ba concentration

(around - 5% per degree °C) (Chapter II). The dietary effect on statolith Ba/Ca as indicated by our data ranges around 10% for completely different diets with Ba values being one order of magnitude higher in shrimps than in fish. Therefore a complete diet shift from crustaceans to fish would result in an inaccuracy of around 2°C when using Ba as a temperature proxy.

In contrast to Ba/Ca, the Sr/Ca ratio in *S. officinalis* statoliths is apparently not affected by both temperature and salinity (Chapter II). Thus the dietary effect on Sr/Ca (which is up to 10% in our study) could be an important proxy even under changing water conditions. Clearly, a diet composed exclusively of fish or crustaceans appears unlikely in the field. However, for many cephalopod species including *Sepia officinalis*, a distinct shift in feeding habits due to ontogenetic development is reported (e.g. Castro and Guerra 1990). The present results suggest that a shift from an invertebrate to a piscivorous diet would be detectable with modern microanalytical methods in cuttlefish statoliths if their past environmental history (water temperature and chemistry) was to a certain extent stable. Combined with optical ageing, based on statolith growth increments, feeding habits may be assigned to the individual age. The onset of piscivory is considered an important event during cephalopod ontogeny. The determination of the age at which the shift from invertebrate to fish diet had occurred would allow, for example, testing the importance of the timing of the diet shift to future survival (Buckel et al. 2004).

Yttrium, though present in very low concentrations, was significantly influenced by diet. Y/Ca in *Sepia officinalis* statoliths varies not only with diet, but with both temperature and salinity as well (Chapter II). Taking into account at least these three sources of variation and the very low observed concentrations, we suggest that Y is rather unsuited for further ecological investigations of statolith chemistry targeting these factors. However, Y is routinely detectable in cephalopod statoliths and further experimental investigations may provide its prospect for other questions.

The source of the elements in cuttlefish statoliths for which no dietary effect was found may be solely from water. Alternatively, some of these elements are

derived from the food but are effectively regulated on their way to the statoliths through several membranes. The differences in statolith element/Ca ratios induced by the diet (around 5 to 15 %) are significant, but they are much smaller for all elements than the differences of these ratios in the diet itself and the spatial variations within a single statolith.

Spatial variations

The considerable spatial variation of elemental composition has substantial relevance for further studies. Solution-based methods obtain a pooled signal over the whole statolith and do not differentiate between regions. This means that spatial variations add to all other sources of variation. The mean elemental composition from all analysed points in our study does not necessarily represent the composition of a whole dissolved statolith as analysed in solution based methods; but the averaging of analytical points demonstrates that statistically significant differences in the elemental composition of the lateral dome can be masked by the spatial variation.

The Sr/Ca values determined in this study are higher in the rostrum than in the lateral dome. Further, the wing is reported to contain more Sr than the lateral dome (Bettencourt and Guerra 2000). Both our results and the results from Bettencourt and Guerra (2000) are contradictory to the concept by Lipinski (1993), who suggested that good increment visibility in cephalopod statoliths is associated with high Sr contents. This hypothesis was confirmed for statolith of the chokka squid *Loligo vulgaris reynaudii* (Durholtz et al. 1997), but at least in *Sepia officinalis* statoliths, increments are best visible in the lateral dome and almost indistinguishable in the wing and the rostrum. Cohen et al. (2001) and Ferrier-Pages et al. (2002) proposed that Sr incorporation into coral skeletons is inversely correlated with the rate of calcification. Bettencourt and Guerra (2000) proposed that good increment visibility is associated with high organic matter contents. Combining these points with our results, we suggest that enhanced calcification rates, related to higher organic matter incorporation and higher Sr contents, are associated with better increment visibility.

The observation that Mg/Ca did not vary significantly outside the core region is in accordance with the results from Bettencourt and Guerra (2000), who compared the Mg/Ca ratios in the wing and the lateral dome. While Mg is considered to be the key factor in statolith calcification (Morris 1991a), hypotheses on statolith deposition are still controversial (Arkhipkin 2005) and convincing evidence has not been supplied yet.

Ba/Ca ratios outside the statolith core of reared *Sepia officinalis* are much higher than in comparable statolith regions of the squid *Gonatus fabricii* collected from the field (20-25 and 6-7 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively; Chapter IV), whereas values for Mn (~ 1 and 4-6 $\mu\text{mol}\cdot\text{mol}^{-1}$) and Mg (~ 20 and 200-300 $\mu\text{mol}\cdot\text{mol}^{-1}$) were

substantially lower. These variations may be due to interspecies differences, but it is more likely that they were caused by the different elemental composition of rearing water. Ba concentrations and Ba/Ca in the experimental tank water were considerably higher and Mn, Mn/Ca, Mg and Mg/Ca were considerably lower than in natural seawater. Results suggest that the incorporation of Ba, Mn and Mg in statoliths is related to the respective concentrations or element/Ca ratios in the surrounding water. To test this hypothesis, however, further systematic experimental investigations are required.

Core composition

The conformity in the elemental composition of core regions between treatments confirms that material deposited into the statolith is metabolically inert after deposition and not influenced by later processes. The same principle has been demonstrated in statoliths of the squid *Gonatus fabricii* from the field (Chapter IV). The strongly different elemental composition in the statolith core region of *Sepia officinalis*, laid down during embryo development inside the egg, compared to the regions deposited after hatching may be related to the transfer of almost completely developed eggs from the Atlantic to experimental tanks in Kiel. However, the capsule of cuttlefish eggs is known to act as a shield to protect embryos against influx of elements such as zinc and cadmium (Bustamente et al. 2002). The extremely low Ba concentrations in the core suggest a similar protection against barium. U concentrations, however, are significantly higher in the core than in the outer regions, suggesting that this shield does not protect against U. If the same protection would be active against U, our results would indicate substantially higher U concentrations in the cuttlefish's spawning sites. However, these mechanisms have to be experimentally tested, before distinct assumptions are possible.

Sr contents in the core were significantly higher in the core than in outer regions as well. While there is evidence that the yolk composition likely influences Sr concentrations in fish otoliths more than the surrounding water (Kalish 1990), there is also evidence that environmental conditions, specifically temperature, can affect Sr contents in statoliths of encapsulated marine neogastropod larvae (Zacherl et al. 2003). For further evaluation it would be desirable to analyse the elemental composition of the liquid inside the eggs, its dependence on the surrounding water composition and temperature and its influence on the statolith composition.

Conclusion

Our results imply that further statolith composition studies have to take into account the dietary factor as a significant, albeit not major, source of elemental variation for barium, manganese and yttrium. Sr, however, appears to be useful proxy for the diet even under changing water conditions. Furthermore the demonstrated spatial variation within statoliths is particularly relevant for further studies with spatially resolved analyses, concerning the positioning of measurements. Despite the considerable dietary effect on elemental composition, the lateral dome appears to be the most suitable region for chemical analysis, since in the lateral dome the growth rings are best readable for age determination in most cephalopod species including *Sepia officinalis* (Bettencourt and Guerra 2001). This enables to accurately place analytical spots in particular areas, which can easily be assigned to a corresponding time-window in the cephalopod's life.

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Chapter IV

Statolith trace element composition reflects the life-history of the boreoatlantic armhook squid *Gonatus fabricii*

Abstract

Statoliths of the gonatid squid *Gonatus fabricii* from Disko Bay, West Greenland were analysed by laser ablation- inductively coupled plasma mass-spectrometry (ICP-MS) to determine the concentrations and spatial distributions of nine 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 the individual squid's life. Significant variations in concentrations of all measured elements except from Na were found between different regions of the statolith. Variations in Ba/Ca ratios confirm that juveniles inhabit surface waters, while larger specimens move to deeper waters. U/Ca and Sr/Ca ratios increase towards the outer statolith region, suggesting migration of adult squid into colder waters. Mg/Ca ratios decrease progressively from the core to the edge, most likely related to statolith growth rates. In this study, laser- ablation ICP-MS was applied for the first time to cephalopod statoliths. Results confirm the strong potential of spatially resolved statolith analyses to gather information on life-history, migrations and habitat use of cephalopods.

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, in press). This species occurs frequently in the stomachs of marine mammals and fishes (Dawe et al., 1998; Bjørke, 2001; Simon et al., 2003) and is likely to play an important role in the ecosystem off West Greenland. *G. fabricii* is caught frequently as a 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 characterized by well-defined ontogenetic vertical migrations. Hatching is proposed to take place in great depths (Arkhipkin & Bjørke, 1999). Its early life stages are found in large numbers in west Greenland epipelagic waters (Kristensen, 1983; Piatkowski & Wieland 1993), while larger squids occur mainly in mesopelagic and bathypelagic water layers (Kristensen, 1984).

Trace element analysis of biomineralized tissues has become a well-established 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 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). 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, Ikeda et al. 1995, 1997, 2002a, 2002b, Durholtz et al. 1997, Lipinski et al. 1997, Yatsu et al. 1998, Arkhipkin et al. 2004). Fundamental results on the biomineralization 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 and 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. 1996, 1999, 2003). However, no study to date has shown experimental evidence that Sr/Ca ratios or any other elemental signals in squid statoliths are temperature dependent.

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 the Patagonian longfin squid *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.

By optical increment analysis, Kristensen (1980) and Arkhipkin & Bjørke (2000) defined four 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 60mm pen length). Z4 is formed during the sub adult and adult period.

No published information on the chemical composition of *G. fabricii* statolith was available up to now. In this study, transects on ground *G. fabricii* statoliths from the core to the edge were analyzed, 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 in *G. fabricii* statoliths and (2) to evaluate whether spatially resolved elemental analysis can provide useful information on habitat use and life history of individuals.

Materials and Methods

Sample collection

Specimens were sampled on board of RV "Paamiut" as by-catch during a bottom trawl survey targeting deep-water prawn (*Pandalus borealis*) in July-August 2003 in Disko Bay, West Greenland (Fig.1). Cephalopods were frozen on board immediately after capture and later defrosted in the laboratory. Species identification, measurements and further analysis were performed at IFM-GEOMAR, Kiel. Sex was determined and maturity stages (I, immature; II-III, maturing; IV-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 1mm; 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 3) and 10 females (all specimens were assigned to maturity stage 2). The pen length of analysed squid ranged between 177 and 310 mm, corresponding to a weight range of 84 to 470 g. All specimens were caught within three hauls on the same day in the same area. The statoliths were dissected from the cartilaginous skull, washed in distilled water, stored in eppendorf-caps and kept dry.



Fig. 1: Area where specimens of *Gonatus fabricii* were sampled from bottom trawl hauls in Disko Bay, West Greenland in 2003. Box indicates study site. The map was plotted with OceanDataView® (Schlitzer 2004).

Statolith processing and reading

A total of 26 statoliths were prepared and analysed. One statolith from each pair was attached concave side up onto a microscopic glass slide with thermoplastic resin (Buehler[®]) and was ground on one side with wet waterproof lapping film (3M[®]; 30 μ m, 15 μ m, 3 μ m) until the ground surface extended to the edge (i.e. the most extended point) of the dorsal dome and the focus could be seen just beneath the ground surface. Ground statoliths were finally polished with Buehler[®] micropolish II (0.3 μ m and 0.05 μ m) and observed under a Leitz Laborlux K microscope (250 \times 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. Five analytical points were located along a transect from the core to the edge of each statolith (Fig. 2). The first point was placed into the nucleus, i.e. the region laid down before hatching, according to Kristensen (1980) the zone Z1. Points 2 and 3 were placed into the post-nuclear zones Z2 and Z3, while points 4 and 5 both were located in Z4, representing the subadult and adult phase in the squid's life (Kristensen 1980, Arkhipkin & Bjørke 2004). In some cases, especially at the nucleus, the measured element ratios changed after some time when the laser drilled into a different zone. Here only the first, homogeneous part of the time series was integrated for data quantification to obtain a true two-dimensional profile.

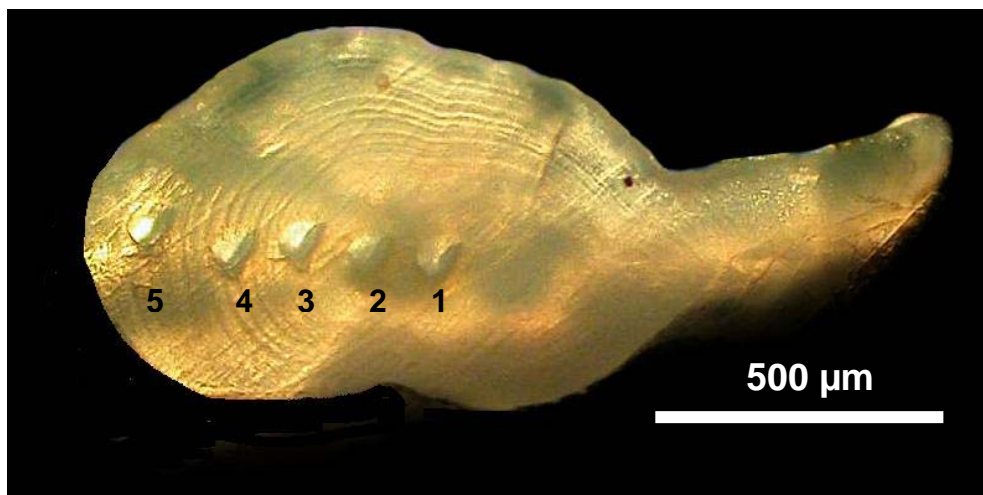


Fig. 2: Ground statolith of *Gonatus fabricii* showing the location of analytical points as laser ablation craters. Points are numbered from the core to the edge. point 1 = growth zone Z1; point 2 = growth zone Z2; point 3 = growth zone Z3; points 4 and 5 = growth zone Z4.

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. Helium was used as sample gas in the ablation cell ($0.4 \text{ l}\cdot\text{min}^{-1}$) and Argon was subsequently added ($0.8 \text{ l}\cdot\text{min}^{-1}$) to the gas flow. Ablation parameters included a laser energy of 2-2.5 mJ (Q-switched mode) and a pulse rate of 5 Hz resulting in crater sizes of 60- 80 μm . Eleven isotopes were recorded during 90 seconds with five 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}Na , ^{43}Ca , ^{88}Sr) and NIST612 (for ^{23}Na , ^{25}Mg , ^{43}Ca , ^{55}Mn , ^{64}Zn , ^{89}Y , ^{90}Zr , ^{93}Nb , ^{138}Ba , ^{238}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. GeoProTM 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 two-point calibration because the two differently coloured NIST standards show distinct ablation behaviour and element fractionation at 266 nm laser wavelength (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 $0.5\text{-}1 \mu\text{g}\cdot\text{g}^{-1}$ and up to 13 % for the lowest concentrations measured in this study ($0.01\text{-}0.05 \mu\text{g}\cdot\text{g}^{-1}$). 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).

Table 1a:

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.

Element	Average ($\mu\text{g}\cdot\text{g}^{-1}$)	Ref. Value ($\mu\text{g}\cdot\text{g}^{-1}$)	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

Table 1b:

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 two 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.

Element	LA-ICP-MS ($\mu\text{g}\cdot\text{g}^{-1}$)	Solution 1 ($\mu\text{g}\cdot\text{g}^{-1}$)	Solution 2 ($\mu\text{g}\cdot\text{g}^{-1}$)
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

A powdered *Arctica islandica* bivalve shell was also analyzed by both LA-ICP-MS, using the same analytical method and parameters as for statolith analyses, and solution ICP-MS calibrated with concentration-matched pure element standards (Table 1b). Even though the measured concentrations cover a wide range of 10^{-3} to $10^3 \mu\text{g}\cdot\text{g}^{-1}$, the laser ablation data show excellent to moderate accuracy. Again, the limiting factors appear to be the calibration with standards having concentrations much higher (Y, Zr, Nb, U) or much lower (Sr) than those of the samples, as well as the different matrices of standards and samples causing different elemental fractionation.

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). The assumptions of ANOVA were met: data were normally distributed and variances were homogeneous among factor levels.

Results

Nine minor and trace elements (Sr, Ba, Mg, Mn, U, Zr, Y, Zn and Na) were analysed in the statoliths by laser ablation ICP-MS. The ranges of element/Ca ratios are given in Table 2. The lateral resolution achieved in this study, approximately 70 μm , was adequate to sample single ontogenetic stages on the polished statolith surface.

Table 2.

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

element	concentration range	element/ Ca ratio range	element/Ca ratios of <i>L. gahi</i> statoliths (Arkhipkin et al. 2004)
sodium	3200-5050 $\mu\text{g}\cdot\text{g}^{-1}$	15-22 $\text{mmol}\cdot\text{mol}^{-1}$	
strontium	5500-7100 $\mu\text{g}\cdot\text{g}^{-1}$	6.3-8.1 $\text{mmol}\cdot\text{mol}^{-1}$	~ 8- 8.5 $\text{mmol}\cdot\text{mol}^{-1}$
magnesium	25-140 $\mu\text{g}\cdot\text{g}^{-1}$	110-590 $\mu\text{mol}\cdot\text{mol}^{-1}$	~ 70-350 $\mu\text{mol}\cdot\text{mol}^{-1}$
barium	7.8-11.3 $\mu\text{g}\cdot\text{g}^{-1}$	5.7 - 8.2 $\mu\text{mol}\cdot\text{mol}^{-1}$	~ 3-8 $\mu\text{mol}\cdot\text{mol}^{-1}$
zinc	0.6-2.9 $\mu\text{g}\cdot\text{g}^{-1}$	0.9-4.5 $\mu\text{mol}\cdot\text{mol}^{-1}$	
manganese	1.8-3.7 $\mu\text{g}\cdot\text{g}^{-1}$	3.2-6.8 $\mu\text{mol}\cdot\text{mol}^{-1}$	~ 1-3 $\mu\text{mol}\cdot\text{mol}^{-1}$
yttrium	0.02-0.14 $\mu\text{g}\cdot\text{g}^{-1}$	56-81 $\text{nmol}\cdot\text{mol}^{-1}$	
zirconium	0.01-0.026 $\mu\text{g}\cdot\text{g}^{-1}$	12-29 $\text{nmol}\cdot\text{mol}^{-1}$	
uranium	0.005-0.022 $\mu\text{g}\cdot\text{g}^{-1}$	2.2 -9.2 $\text{nmol}\cdot\text{mol}^{-1}$	
lead	0.013-0.076 $\mu\text{g}\cdot\text{g}^{-1}$	6.3-37 $\text{nmol}\cdot\text{mol}^{-1}$	~ 2-30 $\text{nmol}\cdot\text{mol}^{-1}$

Table 3.

Analysis of variance (ANOVA) table summarizing the effect of *Gonatus fabricii* statolith measurement location on element/Ca ratios.

element	df	F	p
strontium	4	16.65	0.0001
barium	4	4.90	0.0009
magnesium	4	2.88	0.026
sodium	4	0.20	0.936
manganese	4	15.16	0.0001
zinc	4	3.54	0.009
yttrium	4	3.79	0.0059
zirconium	4	5.99	0.0002
uranium	4	32.23	0.0001

The two-way ANOVA test found no significant effects of specimens on the statolith elemental composition. Furthermore, no significant differences between statolith composition of males and females were found. The location of measurements, however, showed significant effects for all analysed elements except for sodium (table 3).

Post-hoc Tukey multiple comparisons revealed significant differences in the elemental composition between distinct regions in the statoliths (Fig. 3):

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

Ba/Ca ratios show considerable variation within the post-hatch zones Z2-Z4. Concentrations in the outer regions (points 4 and 5) are significantly higher than point 2 and 3 ($p < 0.007$). However, variations between the core and the rest are statistically not significant.

Mg/Ca ratios are generally high in the nucleus, decreasing toward the edge with lowest values in the outermost region. Significant differences were observed only between points 1 and 5 ($p = 0.01$).

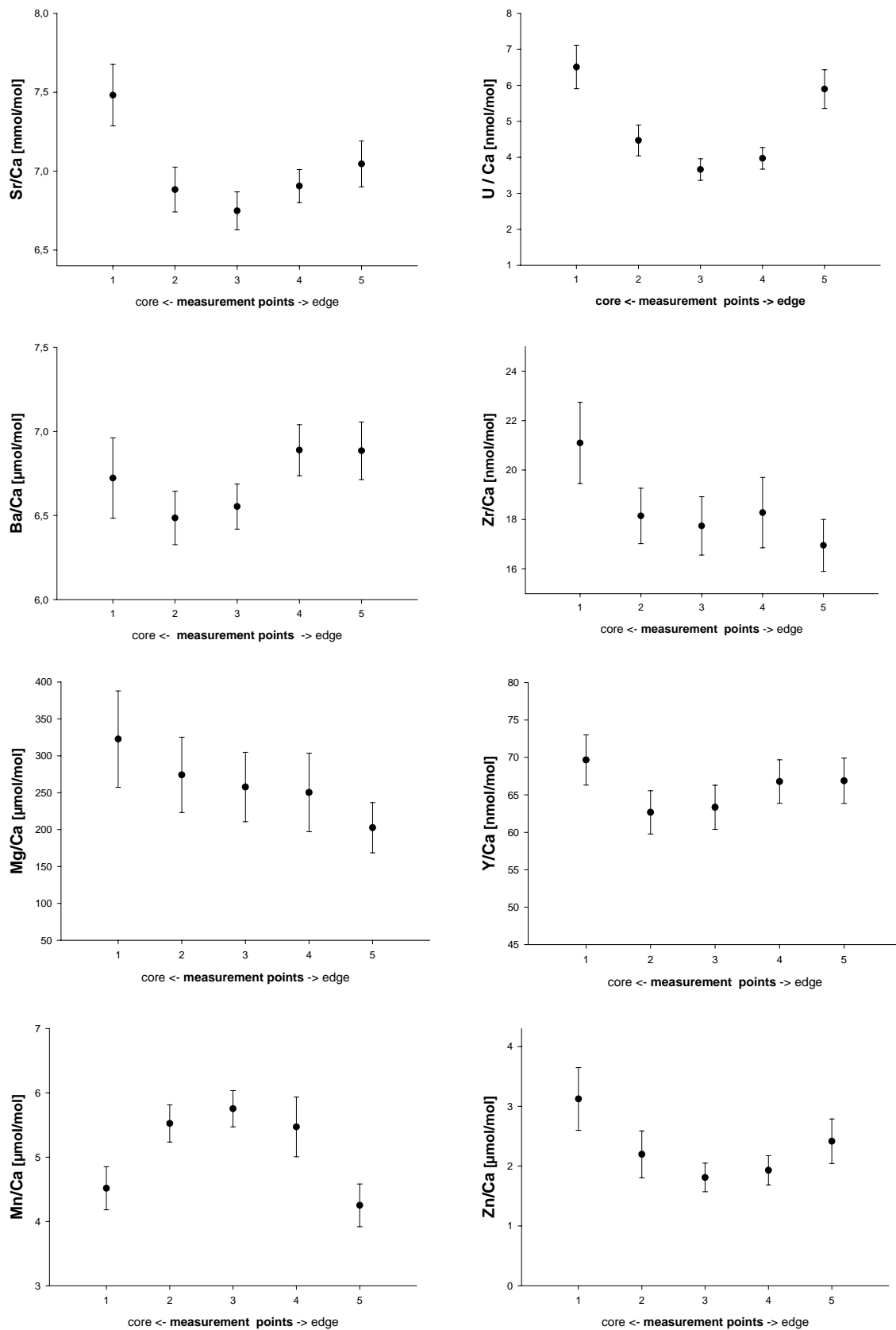
Mn/Ca ratios show a dome-shaped pattern along the transect with low values in the core and the outer region and highest values in the middle. This pattern is negatively related to the Sr distribution. Values for point 1 and 5 are significantly different from points 2, 3 and 4 ($p < 0.003$).

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

Zr/Ca ratios show highest values in the core, decreasing to the edge. Core values were significantly higher than all other points ($p < 0.002$).

Y/Ca ratios in the core are significantly higher than in growth zones 2 and 3 ($p < 0.008$), but not different from growth zone 4.

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

**Fig. 3:**

Element/Ca ratios measured by LA-ICPMS in different regions of *Gonatus fabricii* statoliths from the core to the edge. Point numbers refer to numbers given in Fig. 2. Each point represents the mean of measurements in 26 statoliths; error bars indicate 95% confidence intervals.

Discussion

Pre-hatch zone

Analyses reveal that the elemental composition differs considerably between the core region (representing time before hatching) and the outer parts. Concentrations of seven elements were significantly different between the pre-hatch zone and the post-hatch zones. This change is likely 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. In cuttlefish eggs the capsule was shown to act as a shield to protect embryos against trace metals like zinc and cadmium (Bustamente et al. 2002). Since yolk composition may well be influenced by genetic factors, the elemental signature of the statolith core could be representative for separate populations. However, 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.

No significant variations between the cores of different specimens were observed in this study, possibly indicating that specimens belong to the same stock. However, with larger sample sizes from a broader geographical area it may be possible to carry out a stock separation based on variations in the elemental core composition as demonstrated for fish (Campana et al. 1994).

Strontium

Gonatus fabricii statoliths, similar to other cephalopods, corals and sclerospores, contain more Sr (Sr/Ca ratio $\sim 6.3\text{--}8.1 \text{ mmol}\cdot\text{mol}^{-1}$) than fish otoliths ($\sim 2\text{--}2.5 \text{ mmol}\cdot\text{mol}^{-1}$, Campana 1999) or bivalve shells ($\sim 0.8\text{--}1.6 \text{ mmol}\cdot\text{mol}^{-1}$, 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* ($8.5\text{--}10 \text{ mmol}\cdot\text{mol}^{-1}$; Ikeda et al. 2003) and with a species of benthic octopus, *Octopus dofleini* ($10.6\text{--}14.3 \text{ mmol}\cdot\text{mol}^{-1}$; Ikeda et al. 1999) but quite high compared with the common cuttlefish *Sepia officinalis* ($2.5\text{--}5 \text{ mmol}\cdot\text{mol}^{-1}$; Chapter II). However, statolith Sr/Ca data in the current study are very similar to those of loliginid squid species (Ikeda et al., 1997, Biemann and Piatkowski 2001, Arkhipkin et al. 2004).

Applying the generally suggested negative correlation between Sr/Ca and temperature and the positive relation between Sr/Ca and salinity, the higher

Sr/Ca in the outer region compared to growth zone 3 indicates a migration into colder, more saline waters. This scenario is supported by Ba/Ca and U/Ca patterns and confirms the proposed life-cycle of *Gonatus fabricii*. Although results presented in chapter II imply no Sr relation to either salinity or temperature in *Sepia officinalis* statoliths, those values represent completely different conditions. They are probably not simply adaptable for the current study since this is a different species in substantially colder waters under natural conditions. However, since no experimental data for this species in this region under these conditions is available, the Sr pattern may be influenced by other factors as well.

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 strontium 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. In corals, Ba is therefore used as an indicator for upwelling events (Lea et al. 1989, Fallon et al. 1999). Experimental data indicate a positive correlation of Ba/Ca in gastropod larvae protoconchs (Zacherl et al. 2003b) and fish otoliths (Bath et al. 2000, Bath-Martin et al 2004, Elsdon and Gillanders 2003) with Ba/Ca in the seawater. The Ba/Ca distribution found in the current study confirms the previously stated ontogenetic migrations from surface waters during juvenile phase to meso-bathy-pelagic waters in the sub adult and adult phase 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 *Loligo gahi*.

Magnesium

Magnesium has been assumed to be important for biomineralization in squid statoliths (Morris 1991). Statolith core regions are characterized 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 and Guerra (2000) showed that Mg^{2+} 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 and Guerra 2000),

concentrations of magnesium would be expected to decrease correspondingly, which is in accordance with the data presented in this study.

Mitsuguchi et al. (1996) reported that Mg/Ca ratios were positively correlated with temperature in coral skeletons, 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 important and highly regulated. Moreover, given the long residence time of Mg in ocean waters, it is improbable that squid experienced varying concentrations of dissolved Mg^{2+} during their lives.

The Mg/Ca values in the core are 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 ($\sim 1\text{--}3 \mu\text{mol}\cdot\text{mol}^{-1}$; Arkhipkin et al., 2004) determined by ICP-MS. However, the values of both data sets are almost one order of magnitude lower than in *T. pacificus* ($22\text{--}35 \mu\text{mol}\cdot\text{mol}^{-1}$) and *O. dofleini* ($22\text{--}40 \mu\text{mol}\cdot\text{mol}^{-1}$) determined by proton-induced X-ray emission (Ikeda et al. 1995, 1999). It seems unlikely that these differences reflect environmental variability in dissolved Mn concentrations, given the distribution of Mn in the world's oceans (Donat and Bruland 1995). Rather, these differences may either due to taxon-specific differences in elemental uptake or perhaps to an instrumental artefact. In statoliths of *Loligo gahi* (Arkhipkin et al 2004) a decrease of Mn/Ca with increasing squid size was observed. This is in accordance to the current study, considering that Arkhipkin et al. analysed the whole statolith of specimens > 7.5 cm mantle length. These statoliths already contain the zone Z1-3 as constant factor, 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 uranium in the world's oceans have been reported to be rather uniformly $3.4\pm 0.3 \text{ ng}\cdot\text{g}^{-1}$ (Hossain et al. 2002). Reef-building corals incorporate uranium in the form of uranyl ions (UO_2^{2+}) as impurity by replacing calcium (Ca^{2+}) (Ohde et al. 2003) in a concentration range of $2\text{--}3.5 \mu\text{g}\cdot\text{g}^{-1}$ (Shen & Dunbar 1995). Uranium concentrations in statoliths from this study are two orders of magnitude lower, but they show considerable spatial variation.

Shen & Dunbar (1995) propose a possible U/Ca temperature dependence of + 3-4% per 1°C cooling for coral aragonite. Moreover, the same authors identified a probably positive salinity influence on coral U/Ca, which suggests that uranium uptake depends on the total uranium 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 phase) 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 ($r=0.56$, $p<0.01$) exists between U/Ca and Sr/Ca, which agrees with previous studies on corals (Min et al. 1995, Fallon et al. 1999). The combined use 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. Results further show 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 do not vary significantly along the post-hatch ontogenetic stages. However, core values for all of these elements are significantly higher than in the outer regions. Zinc has been determined before in statoliths (Ikeda et al. 1996), but convincing relations to environmental factors have not been proposed yet. Yttrium and zirconium have never before been determined in cephalopod statoliths, but spatial variations found in this study suggest some potential for future use as environmental proxy. These elements should be further investigated in biogenic aragonite to evaluate their incorporation process.

Lead could not reliably be measured in *Gonatus fabricii* statoliths. Preliminary data give Pb/Ca of 6.3-37 nmol·mol⁻¹ for two specimens, which is comparable to Pb/Ca ratios found in *Loligo gahi* statoliths with solution based ICPMS (~ 2-30 nmol·mol⁻¹; Arkhipkin et al. 2004).

Hydrography

The hydrographical conditions off West Greenland and particularly in Disko Bay are highly variable (Ribergaard & Buch, 2004), making assumptions of the squid's habitat use complex. In summer, rather warm surface water (up to 7°C) in Disko Bay is caused by solar heating of the 20–30 m thin low-saline surface layer. However, in offshore areas, which would be easily reached by the squids, there are completely different conditions influenced by a warm current northwards and a cold current southward. Therefore speculations about possible migrations appear not appropriate at this stage for this particular region.

Suitability of LA-ICPMS

Laser ablation 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 $<0.01 \mu\text{g}\cdot\text{g}^{-1}$ to some thousand $\mu\text{g}\cdot\text{g}^{-1}$. A spatial resolution of about $70\mu\text{m}$ was chosen to be able to differentiate between the squid's main ontogenetic stages. A higher spatial resolution down to about $10\mu\text{m}$ is possible by simply using a smaller laser beam diameter. However, this 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's nucleus. Therefore the set-up used here represented a reasonable compromise between spatial resolution and detection limits. In order to obtain true two-dimensional profiles, the laser beam should ideally ablate along a raster in the nucleus and along lines parallel to the growth lines. However, this is not possible with the laser system used by us.

In comparison to solution based ICPMS analyses of *Loligo gahi* statoliths (Arkhipkin et al. 2004), the current study obtained the same magnitudes of concentrations by laser ablation ICP-MS (table 2). The main advantage given by laser ablation, the spatial resolution, is thus not counterbalanced by the slightly poorer accuracy and detection limits as compared to solution ICP-MS analyses. LA-ICPMS is therefore suitable to assign element composition *in situ* to distinct statolith regions, corresponding to ontogenetic stages of the individuals.

Conclusions

This study proves that LA-ICP-MS is a very useful tool to gather life history information on cephalopods by spatially resolved trace element analyses of statoliths. The *in-situ* Ba/Ca distribution in *Gonatus fabricii* statoliths convincingly confirms the previously stated ontogenetic migrations from surface waters during juvenile phase to meso-bathy-pelagic waters in the sub adult and adult phase of (Kristensen, 1984). This highlights the enormous potential for further studies, particularly on the many cephalopod species, for which almost no information on life-history and migration patterns is available.

Acknowledgements

We are grateful to Kai Wieland and the crew of RV "Paamiut" for collecting the cephalopods, and Rikke Frandsen for assistance with dissecting specimens. Heike Anders helped with solution ICP-MS analyses, and Valérie Epplé provided the *Arctica islandica* sample. Jörn Schmidt is acknowledged for assistance with figure 1.

Chapter V

Trace element distribution in cephalopod statoliths: NanoSIMS provides new insights into nano-scale structure

Abstract

In this study we applied the novel method NanoSIMS for the first time to cephalopod statoliths to analyse their chemical microstructure. We performed measurements of trace elements with a spatial resolution of 400 nm. This technique makes it possible to analyse in situ elemental distributions between organic or inorganic layers, and also nano-scale chemical variations within the heterogeneous structures. As a case study we analysed statoliths of the boreoatlantic armhook squid *Gonatus fabricii*. We found distinct concentration patterns indicating a periodicity in strontium and sodium distributions. Sr and Na show a negative relation, both elements showing alternating patterns, probably corresponding to daily increments. These increments vary in width between 1 and 5 μm . This study demonstrates the excellent suitability of NanoSIMS for nano scale microchemical analyses of aragonite. Possible future applications include not only cephalopod statoliths, but virtually all biomineralized tissues in aquatic organisms like fish otoliths, gastropod statoliths, bivalve shells, foraminifers and corals.

Introduction

Cephalopod statoliths (“ear-stones”) grow throughout the lifetime of the animal and provide a sense of balance to the animals in much the same way that the inner ear provides balance in humans. Statoliths are essentially composed of calcium carbonate crystallised as aragonite with a small percentage of organic material that has been ascertained to be protein (Radtke, 1983). Similar to fish otoliths, statoliths form concentric rings visible under a light microscope, originating from periodically changing amounts of organics incorporated into the aragonite crystal (Kristensen, 1980; Bettencourt & Guerra, 2000). These growth rings are frequently used for age determination since a daily deposition of increments has been validated for several cephalopod species (Bettencourt & Guerra, 2001, Jackson, 1994). The remarkable diversity of information about various aspects of squid life history, which is recorded in these small calcareous structures, has been reviewed comprehensively (Jackson 2004, Arkhipkin 2005).

Numerous studies have shown that cephalopod statoliths contain a number of minor and trace elements (e.g. Hurley et al., 1985; Durholtz, 1997; Lipinski et al., 1997; Yatsu et al., 1998; Ikeda et al., 2003; Arkhipkin et al., 2004). Knowledge about statoliths microstructure, however, is mainly based on optical analyses so far (Kristensen, 1980; Arkhipkin and Bjoerke, 2000). Fundamental results on the biomineralization process have been reported 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). Lipinski (1993) proposed that strontium is directly or indirectly responsible for the definitions of growth layers and increments in the statoliths. Furthermore, or alternatively, magnesium may be a key factor in the calcification process within the statocyst (Morris, 1988, 1991a,b; Bettencourt and Guerra 2000).

Apart from further understanding of the crystallization process, information on the distribution of trace elements in statoliths is essential for several applied studies. The characteristic trace element pattern in bio-crystals of many marine organisms is dependent on the composition and temperature of the surrounding sea water (e.g. Smith et al., 1979; Zacherl et al., 2003, Bath-Martin et al., 2004). Statoliths can deposit information on the changing environmental factors during their life-cycle. Hence, they can be used to detect distribution and migration pathways through different water masses (Yatsu et al. 1998, Ikeda et al., 2003) or to distinguish between stocks and cohorts (Arkhipkin et al., 2004).

In this study, the modern technique NanoSIMS was applied to analyse the chemical microstructure of cephalopod statoliths for the first time. This method represents an outstanding further development of the well-established SIMS (secondary ion mass spectrometry) technique. SIMS is one of the most widespread surface analysis techniques for advanced material research. It has

been used traditionally for a variety of geological questions and recently on fish otoliths (Weber et al., 2002, 2005). However, NanoSIMS as used in this study presents an entirely new analytical dimension regarding the fine-scale spatial resolution, as demonstrated for coral aragonite microstructures (Meiboom et al., 2004). The aim of this study was to obtain new information on the sub-micrometer scale distribution of strontium and sodium related to growth structures in cephalopod statoliths.

Materials and Methods

Using a CAMECA NanoSIMS NS50 ion microprobe (Hillion et al., 1993), we produced images of Ca, Sr, and Na distributions in the statoliths of the common cuttlefish *Sepia officinalis* and the boreoatlantic armhook squid *Gonatus fabricii*. Statoliths were mounted in epoxy and ground and polished in the sagittal plane. Analysis were performed by using a 16 keV O⁻ primary ion beam of about 10 pA, focused to a diameter of about 0.4 micron. This beam was rastered across a pre-sputtered area on the gold-coated sample. Secondary ions of ²³Na⁺, ⁴⁰Ca⁺ and ⁸⁸Sr⁺ were collected simultaneously in the mass spectrometer and detected by means of electron multipliers. The mass resolving power of the mass spectrometer was about 3000 during all analyses; no mass-interferences on the measured peaks were observed.

The *G. fabricii* specimen (a maturing male, pen length 215mm, weight 226g) was collected with a bottom trawl in Disco Bay, West Greenland during a shrimp survey performed by RV "Paamiut" in June 2003. The *S. officinalis* specimen (a juvenile, length 61mm and weight 30g) was taken from laboratory rearing facilities at IFM-GEOMAR.

Results

A striking μm -scale zoning is visible in both Sr and Na concentrations in the *Gonatus fabricii* statoliths (Figure 1). Fluctuations vary in width between 1 and 5 μm . Further, the distributions of Sr and Na are negatively correlated; i. e. they show clearly alternating patterns (Fig. 2). This distinct zoning pattern is ubiquitous in the statolith.

In *Sepia officinalis* statoliths, patterns are not as clear as in *G. fabricii* statoliths. Hints of a banding pattern are visible (Fig. 3), but the general image is more confuse and patchy.

Neither iodine nor organic compounds (normally detected due to their elevated CN⁻ contents) were detectable on the surface of any statolith sample. This is most probably due to the applied preparation technique, involving wet mechanical grinding and polishing.

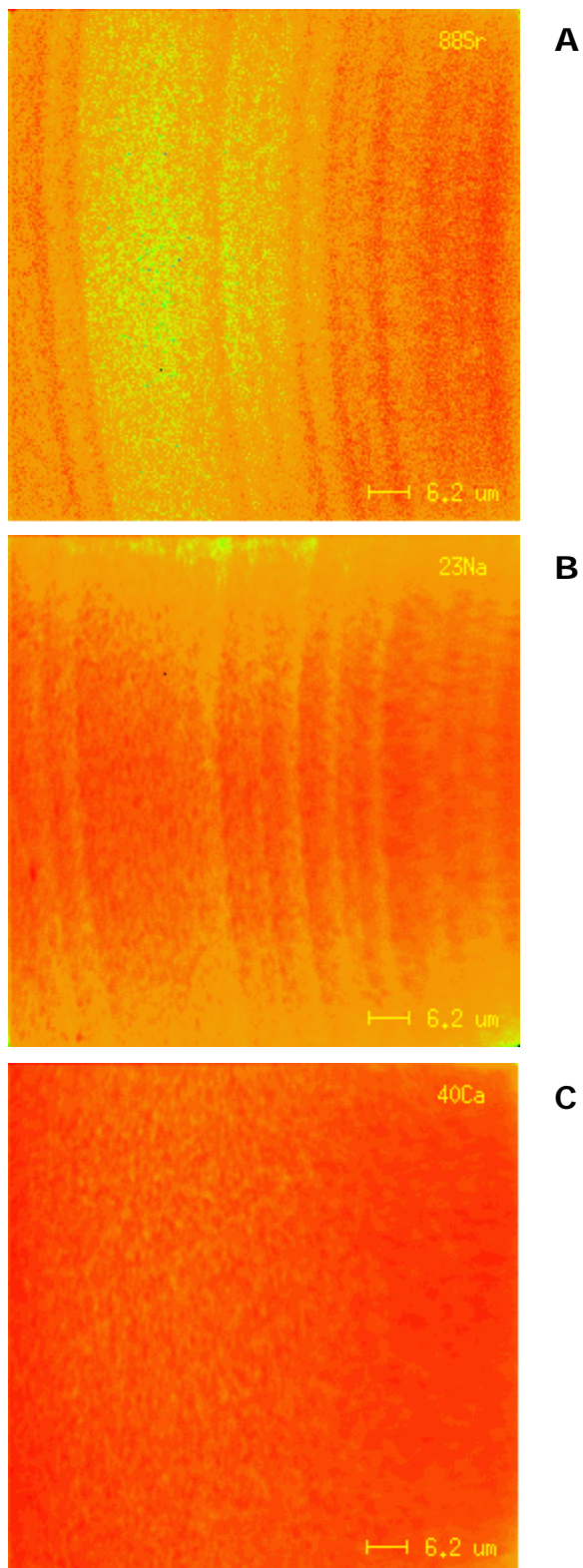


Fig. 1

The distribution of **(A)** Sr, **(B)** Na, and **(C)** Ca in the statolith of *Gonatus fabricii*. Green and yellow correspond to relatively low concentrations; orange and red correspond to increasingly high concentrations. Scale bars are 6.2 μm .

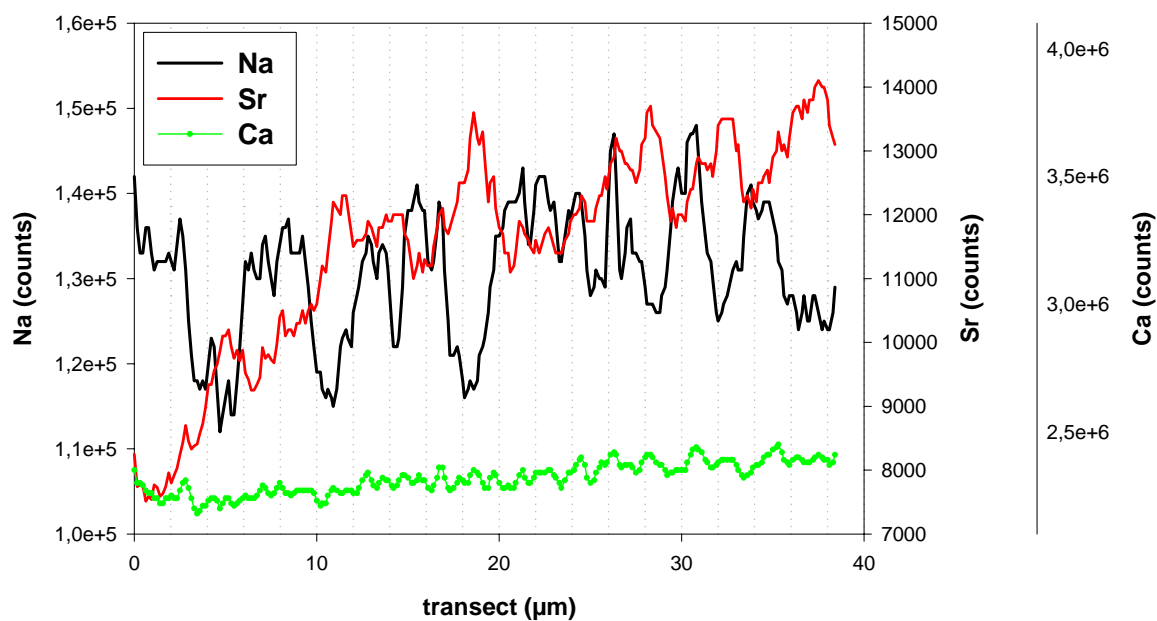


Fig. 2

The distribution of Sr, Na, and Ca in the statolith of *Gonatus fabricii*. Ca, Sr and Na line scan analyses correspond to the same area that is given in Fig 1.

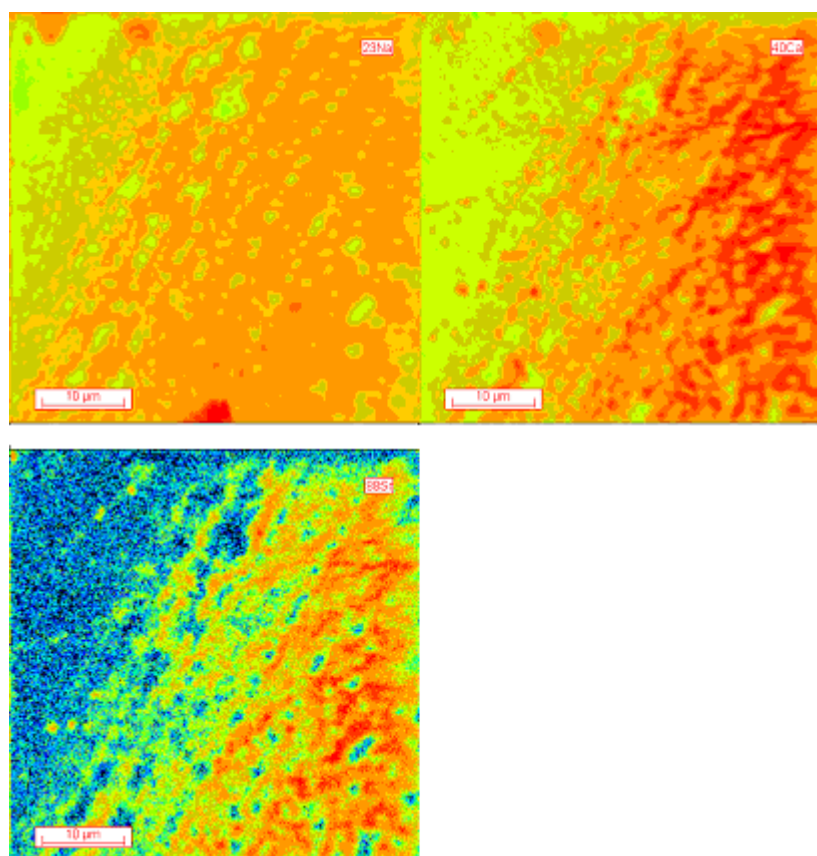


Fig. 3

The distribution of (A) Na, (B) Ca and (C) Sr in a statolith of *Sepia officinalis*. Dark blue colours correspond to relatively low concentrations; green, yellow and red colours correspond to increasingly high concentrations. Scale bars are 10 µm.

Discussion

Gonatus fabricii

Fluctuations found in the current study vary in width between 1 and 4 μm , most probably corresponding to daily increments. The size of compositional bands corresponds well with the microscopically visible increments, which are presumed albeit not confirmed to be daily increments (Kristensen, 1980; Arkhipkin and Bjoerke, 2000). However, the general hypothesis that one increment is laid down each day is largely accepted for many cephalopod species (Arkhipkin, 2005).

The time-span involved in formation of the statolith structures (Fig. 1) is in the order of one day. During this time period, external environmental parameters are likely to change significantly for these highly mobile squids. *Gonatus fabricii* is known to perform considerable diurnal vertical migrations (Kristensen, 1984); therefore the animal may experience daily temperature changes of up to 5°C off West Greenland where the squid was sampled (Ribergaard and Buch, 2004). Such temperature variations, however, would probably not be reflected by changes in the Na concentration in the statoliths (Chapter IV). Concentrations of this highly regulated element in the animal's tissue are believed to be largely independent from the temperature and composition of the surrounding water. The striking inverse relations between oscillating patterns for Na and Sr documented in Figures 1 and 2 imply a common origin. They are probably not due to changes in the thermodynamic equilibrium between aragonite and seawater. More likely they result from a strong biological control over the transport of both Sr and Na to the statocyst fluid and finally into the growing statolith matrix.

A temperature dependence of strontium incorporation into statoliths has not been validated yet (Chapter II), although a few field studies propose a negative relation of statolith Sr/Ca to temperature (e.g. Ikeda et al., 2003). In the Japanese common squid *Todarodes pacificus*, however, the statolith Sr/Ca is not related to daily vertical migrations (Ikeda 2002). Oscillating variations in the Sr/Ca ratios have also been observed in statoliths of *T. pacificus* (Ikeda et al., 2002). The observed undulation followed a 2.7-day cycle in captive squid, maintained under constant temperature and salinity, and a 2.4-day cycle in collected wild squid. Therefore the hypothesis that statolith Sr/Ca reflects the vast diurnal vertical migrations of *T. pacificus* through considerable temperature gradients has been rejected. However, these authors used a completely different analytical method (Electron microprobe) that provides a spatial resolution of 1-3 μm only, and counting statistics for trace elements are not comparable to NanoSIMS. Analyzing a continuous line scan along the growth axis with a beam diameter of 1 μm (typically reflecting a spatial resolution of 2-4 μm), they found repetitive fluctuations. Small-scale concentration fluctuations in the order of 10

percent relative, however, as presented in this study, may have been simply not detectable.

It is not likely that the observed variations in both Sr and Na concentrations are simply due to localized organic matter in the statoliths; i.e., a strong matrix effect influencing the yield of secondary ions. If this was the case, variations in the Sr and Na signal would be correlated to the Ca signal (Meiboom et al., 2004). This has not been observed in our study (Fig.1). Moreover, no organic compounds were detectable on the surface. Not even traces of organic compounds (measured as CN^-) and iodine were detectable on the analysed surface. Since C and N are major elements in the organic matter, they would definitely have been detectable by NanoSIMS NS50, if organic material occurred at the surface. The only possible explanation is that the organic compounds were almost completely removed during preparation. Amounts of up to 20% organic compounds are reported for statoliths of *S. officinalis* and other cephalopod species (Radtko, 1983; Bettencourt & Guerra, 2000). As statoliths contain considerable amounts of iodine (Chapter II), it seems likely that iodine is incorporated into the organic compounds and has been removed during the statolith preparation as well.

As suggested for Mg in coral aragonite (Meiboom et al., 2004), the banded micro-distribution might reflect either systematic differences in the amount of Sr and Na in solid solution within the aragonite crystal structure and/or the preferential concentration of both elements at the surfaces of individual aragonite fibers at the initiation or termination of each fibrous growth layer. This mechanism has been observed for magnesium in mollusk shells (Rosenberg et al., 2001) and foraminifers (Eggins et al., 2004). Similar small-scale oscillating distribution patterns for Mg, but not for Sr, have been found in corals by NanoSIMS (Meibom et al., 2004). In contrast to Mg, the distribution of Sr is smoother and does not display the same degree of zoning in corals. Further, the distributions of Mg and Sr are not correlated on shorter length scales in corals (Meiboom et al., 2004). Na was not determined in their study.

Qualitatively similar observations of banded or layered distributions have been observed for Mg and S in bivalves (Rosenberg et al., 2001). In the bivalve shell, Mg and S are preferentially concentrated at the termination of elongated calcite crystals. It was inferred that Mg and S-rich organic components are used actively by the mussel to control crystal size and morphology (Rosenberg et al., 2001).

Isotopic labeling experiments with corals have demonstrated that Sr^{2+} and Ca^{2+} are delivered to the mineralizing surface through the same voltage-sensitive, transcellular pathway located in the calcicoblastic ectoderm (Ferrier-Pages et al., 2002; Tambutte et al., 1996). Furthermore, in coral aragonite, which is generally much better investigated than aragonite in cephalopod statoliths, incorporation of Sr into the skeleton is inversely correlated with the rate of calcification (Ferrier-Pages et al., 2002). As a consequence of the enhanced calcification rate,

coral aragonite precipitated during the daytime is characterized by lower Sr/Ca ratios than aragonite precipitated during the night.

Gonatus fabricii is a highly active predator during the night and rests during the day (Kristensen, 1984). Therefore, distinct daily increments may be expected. If Sr incorporation into the statolith follows similar principles as in corals, then we can assume that the Sr-rich bands are laid down during the day while aragonite built during the night is characterized by lower Sr contents. This hypothesis is strongly supported by our findings in the way that Sr-rich bands are generally thinner than Sr-poor bands.

Sepia officinalis

Sr and Na distribution in *Sepia officinalis* statoliths showed substantially poorer patterns compared to *Gonatus fabricii* statoliths. Since both samples have been prepared and analyzed in exactly the same way, handling artifacts are most unlikely. However, when comparing the optical appearance of increments, there is a considerable difference as well. Increments in *S. officinalis* statoliths are deposited daily, but they are generally poorly defined compared to many squid species (Bettencourt and Guerra, 2001). This may be due to the very irregular crystalline structure coupled with a comparably low percentage of organic matter (Bettencourt and Guerra, 2000). The visibility is even poorer in statoliths of cultured specimens, since the day-night rhythm is not as pronounced as in the natural environment. Increment visibility is suggested to be positively related to the amount of both organic compounds (Bettencourt and Guerra, 2000) and strontium (Lipinski, 1993) incorporated. However, Lipinski's suggestion, though supported by Durholtz et al. (1997) has been contradicted by Bettencourt and Guerra (2000) and the results from Chapter III.

Hypotheses on statolith deposition are still controversial. According to the hypothesis from Bettencourt and Guerra (2000), the aragonite deposited during the day (rest period) is richer in organic matter than the aragonite deposited during the night (active period) in *S. officinalis* statoliths. While Morris (1993) considers magnesium to be the key factor in statolith calcification, Lipinski (1993) proposed that statolith biomineralization is controlled by strontium. Results from Bettencourt and Guerra (2000) rather support Morris' hypothesis than Lipinski's, but convincing evidence has not been supplied yet.

Suitability of NanoSIMS

The lateral resolution of ca. 400 nm combined with impressively low detection limits demonstrates the tremendous possibilities of NanoSIMS NS50. This instrument is not only suitable for the micro-chemical analysis of statoliths and

corals, but virtually all calcified tissues in aquatic organisms like fish otoliths, gastropod statoliths, bivalve shells, and foraminifers. Importantly, the observations presented here indicate that both Na and Sr distributions show distinct heterogeneities on a substantially smaller spatial scale than any analytical instrument was able to determine up to now. These heterogeneities should be considered in further spatially resolved analyses of cephalopod statoliths and probably fish otoliths as well.

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Chapter VI

General Discussion

The present thesis outlines several aspects, which contribute considerably to further understanding of elemental incorporation into cephalopod statoliths. Furthermore, new analytical approaches for fine-scale spatially resolved determination of trace elements in aragonitic bio-crystals have been developed.

Influences of different environmental factors

Despite continuously improving technical possibilities, the interpretation of micro-chemical statolith data from the field can only be interpreted when compared to experimental data. The main focus of the current study was therefore to give qualitative relations and as far as possible quantitative equations for individual environmental factors. It was possible to qualify the respective influences of salinity, temperature and diet on several trace elements.

Barium appears to be the most powerful indicator for temperature, since the statolith Ba/Ca ratio is only influenced by temperature and not by salinity. A dietary effect on Ba/Ca was detectable as well, but it was rather small compared to the temperature effect. Strontium, however, a well established, proxy for temperature and salinity in other biomineralized tissues, appears not to be a suitable indicator for salinity and temperature in cephalopod statoliths. The cephalopod's food influences the strontium incorporation into the statolith to a greater extent than the surrounding water. This makes strontium a potential proxy for the diet even under changing water conditions.

The fact that the incorporation of most other elements like iodine, yttrium, zirconium, niobium and nickel, appears to be influenced by more than one factor complicates the interpretation of data from the field. However, most cephalopod species are not supposed to encounter significant changes in salinity during their lives. Moreover, the dietary effect on the incorporation of these elements is comparably small. Thus, temperature may be the dominant factor in most cases.

This study outlines the importance of field-data to be compared with experimental data. The experimental data presented here are derived from only one cephalopod species and should be supported by experiments with other species. However, since rearing experiments with most cephalopod species, especially squids, are almost not achievable, the common cuttlefish may be used

as a model species for cephalopods. The provided equations may therefore be used for other species as well until data from the particular species are available.

Improved methods for trace element analyses of aragonite bio- crystals

The development of specially designed analytical set-ups in this study provides new prospects for statolith trace element analyses. Apart from analysing the well-established elements strontium and barium, the new applications now enable a fine-scale spatially resolved and quantitative determination of trace element concentrations ranging in a few ppm, in some cases even tens of ppb.

The necessity of developing advanced techniques arose from the fact that traditional methods for the spatially resolved determination of trace elements in calcified tissues revealed several drawbacks. None of the methods currently available is perfect for all applications, different specimens and all scientific questions. The tendency to attempt everything on one instrument is obviously doomed to failure.

Therefore, it appears necessary to critically compare advantages and disadvantages of the different methods and advanced applications. The analytical methods used in this study are the Synchrotron X-ray Fluorescence Analysis (SYXRFA), Laser-ablation inductively coupled mass spectrometry (La-ICP-MS) and an advanced device for Secondary ion mass spectrometry (NanoSIMS). None of these methods has ever been applied to cephalopod statoliths before. The differences between these techniques and their respective suitability are discussed below.

SYXRF (Synchrotron x-ray fluorescence)

At first glance, the “old-fashioned” x-Ray fluorescence analysis seems to be a very sensitive and effectful method to detect element traces in the multi-element mode. But in praxis the application range is restricted, if x-Ray tubes are used as excitation sources. To overcome this situation, it is necessary to improve the conditions of excitation and to reduce the background, produced by different scattering effects. SYXRF, which allows multi-element analysis in the trace- and ultra trace region, is using this strategy. The x-Ray fraction of synchrotron radiation is used as excitation source, giving the opportunity to improve the conditions of excitation as well as to reduce the background by using the high polarisation of the beam.

SYXRF as used in Chapter II combines a number of properties which are very useful for the current questions: The set-up used for this study gives an

exceptionally high spatial resolution (around 12 μ m) of all elements from Calcium (Ca) to Lead (Pb) with concentrations down to 1 ppm.

Although it is unlikely that this synchrotron-based method, which requires high intensity sources of X-rays, will ever be widespread enough to accommodate the potential applications in fisheries and other ecological studies, the ability to screen and detect multiple elements at low concentrations simultaneously, and at a fine scale necessary in statoliths and otoliths, facilitates this method to further directed pilot studies. It is expected, however, that the analytical potential will improve with more powerful detectors and refinements to the method, so that data collection times will decrease.

LA-ICP-MS (Laser ablation inductively coupled mass spectrometry)

In Chapter III and IV of this thesis the LA-ICP-MS was used, because a spatial resolution of 70 μ m is absolutely adequate for the separation of different phases in the life of individual cuttlefish and squids. When the area of interest for measurements is large enough, the LA-ICP-MS is obviously the method of choice. Given the spatial resolution used in this study, LA-ICP-MS provides excellent detection limits of many elements, down to 10ppb for uranium. The development of new laser sampling devices moved rather fast during the last years. The new laser generation is already able to sample considerably smaller areas. When using such a laser for a raster along growth increments, the temporal resolution within a statolith can be reduced substantially. Out of the three techniques used in this study, the LA-ICP-MS probably has the best potential to become an established method for statolith analysis. Advantages compared to SYXRF are most of all lower analytical costs, lower preparation effort and a generally better availability to scientists around the world.

NanoSIMS (Secondary ion mass spectrometry)

The spatial resolution offered by NanoSIMS provided striking insights into a completely new dimension of chemical structures in cephalopod statoliths (Chapter V). This extremely expensive and extensive method is, at least presently, certainly not suitable for the analysis of larger quantities of statoliths. However, it gave us the unique opportunity to find nano-scale structures and patterns that are not visible with any other method currently available. At present, only a few of these advanced analytical instruments exist worldwide. Nevertheless, as technical development moves on, someday this method may be routinely applied to a broader range of questions.

Future applications of chemical statolith analyses

Modern technology has opened up many fields of research that allow us to follow the movements of mobile marine animals in ways not possible a short time ago. Most cephalopods live for a relatively short period of time, leading to a high rate of turnover and resulting in populations that are highly dynamic and extremely variable in terms of both recruitment levels and individual characteristics. We need tools to identify regions or environments that individuals have been utilising to grow, feed and spawn in order to explore where and how such variability arises. From a population level such information may assist us in understanding the spatial and temporal links between populations, including gaining insights into stock structure and the determination of source and sink areas for commercially fished populations. The investigation on squids from Greenland waters (Chapter V) demonstrates the excellent potential of micro-chemical analyses for reconstructing environmental histories of wild-caught cephalopods. Given the many successful applications on corals and fish otoliths and the tremendous technical development nowadays, potential future applications of the presented techniques are manifold.

Migration history

Migration analysis is obviously one of the most powerful applications of statolith microchemistry, though it is strongly dependent on the possibilities of analytical methods. Reconstruction of migration histories using the elemental or isotopic composition along a statolith growth axis combines a series of elemental assays along a statolith transect to the growth chronology recorded, thus allowing the reconstruction of migration pathways structured by age or date.

Several forms of migration analysis have been reported for fish. One of the most successful has been the comparison of elemental trajectories among fish in search of a common history, or alternatively, the determination of the age or date at divergence (e.g. Limburg 1995, Thorrold et al. 1997). This is a particularly robust application, since it requires no knowledge of the animal's past environment.

A potentially more powerful approach is one in which the cephalopod statolith elemental trajectory is linked to shifts in temperature and water chemistry along possible migratory routes. Such an approach was the basis for the detection of anadromy in fishes, in which the shift between freshwater and saltwater environments is clearly evident in the fish otolith Sr:Ca ratios (e.g. Tsukamoto et al. 1998). Although several cephalopod species perform vast migrations, only very few species are regularly exposed to considerable salinity changes. However, migration through a more homogeneous environment as given in cephalopods should also be detectable. With further experimentation and the

advanced analytical techniques presented in this thesis, it should be achievable to factor out more and more aspects that influence elemental composition, thus simplifying the interpretation of possible migration pathways.

Stock Identification

To the extent that groups of cephalopods inhabit different environments, the statolith elemental composition should serve as a natural marker or tag of those groups. Environmental responses, recorded permanently in the statolith, imply that the statolith concentrations of selected elements and isotopes (the “elemental fingerprint”) can be used as a biological tag to discriminate among groups of cephalopods which have spent at least part of their lives in different environments (Arkhipkin et al. 2004). However, it is probably inappropriate to refer to the use of elemental fingerprints as stock discriminators, since genetic differences are not implied and spatial heterogeneity in the stock environment can result in different fingerprints for different stock components (Campana et al. 1999). Since the elemental fingerprint reflects the exposure of the individual animal to both the environment and its own physiology, it would be expected to differ among any groups, which have experienced different histories, whether or not the groups come from the same population. As a result, it seems fair to categorize statolith elemental fingerprints as powerful discriminators of groups when differences exist, but of negligible value when differences cannot be detected. Where differences are detected, additional information would be required to determine if the groups actually corresponded to stocks or populations.

Age determination

Age determination studies based on isotopic concentrations are motivated by the ongoing requirement for accurate age-structured information in support of many fisheries studies. Radiochemical dating of bio-mineralised tissue is based on the radioactive decay of naturally occurring radioisotopes, which are incorporated during growth. Assays of fish otolith cores appear to provide objective, accurate age estimates (e.g. Milton et al. 1995). Nevertheless, the isotopic concentrations are exceedingly low, requiring very advanced assay methods. Current discriminatory power is probably slightly better than 5 yr for ^{210}Pb : ^{226}Ra and 1-2 yr for ^{228}Th : ^{228}Ra , over age ranges of 0-40 and 0-8 yr, respectively as reported by Campana (1999). Nevertheless, this approach is best suited to long-lived species where the age interpretations are widely divergent, such as in redfish *Sebastes sp.* (Stransky et al., 2005) or orange roughy *Hoplostethus sp.* (Fenton et al. 1991).

However, since most cephalopods are short-lived species with a life span of only 1-2 years, the currently used methods of age determination are obviously inappropriate for cephalopods. One of the few examples for a useful application may be the giant squid *Architeuthis sp.* (Landmann et al. 2004), since proposed life spans for this genus differ considerably between authors and a life span of up to 14 years seems probable.

Outlook

The present thesis addressed manifold aspects of elemental incorporation into cephalopod statoliths. Environmental influences of several elements have been shown and considerable technical improvements have been achieved. The results of this study provide an essential basis for future investigations in the field, probably leading into further understanding of yet unknown migration patterns and spawning grounds of various cephalopod species. Applying these future approaches could establish a consolidated biological knowledge on cephalopod species and stocks, and therefore may contribute to an effective and sustainable management of this valuable resource.

Summary

The statoliths of cephalopods are calcified bio mineral structures found in the gravity receptor system in the head of cuttlefish and squid. They grow throughout the lifetime of the animal and deposit microscopically visible daily increments similar to the annual rings in tree stems.

Statolith growth and composition are linked to environmental factors. Thus, the statolith reveals the biological history of each individual cephalopod. If a relationship can be established between the composition of a growth layer of the statolith and the ambient water properties, then the statolith chemistry becomes a predictor of the surrounding water chemistry and/or temperature. Provided that statolith material is not altered or resorbed after deposition, the statolith becomes a permanent archive of environmental conditions and may provide information on habitat use, timing of exposure to a pollutant, and timing of migrations.

Several micro analytical state-of-the-art techniques have been applied in this thesis to investigate the spatially resolved chemical composition of cephalopod statoliths. Recent applications of these methods include mainly geological samples, which do not contain organic compounds. Therefore a considerable part of this project focussed on adjusting and optimising the respective methods to the analyses of biogenic aragonite intergrown with organic compounds.

In this thesis, the influence of different environmental factors on the chemical composition of cephalopod statoliths was investigated. On the basis of laboratory experiments under controlled conditions, it is now possible to qualify the influences of salinity, temperature and diet on the concentrations of several elements in the statoliths. Analytical results indicate that the incorporation of a number of elements is influenced by environmental factors. Barium and iodine appear to be the most suitable indicators for temperature. The incorporation of strontium into cephalopod statoliths, however seems to be influenced to a greater extent by diet than by the surrounding water. This is contradictory to results from corals and fish otoliths, where strontium is a well-established indicator for both temperature and salinity.

The suitability of statolith micro-chemistry for field-studies has been proven as well. Statolith trace element compositional zoning reflects very well the life history and ontogenetic habitat-shifts of the boreoatlantic armhook squid *Gonatus fabricii*.

Further, this thesis gives valuable insights into the microstructure of statoliths and the elemental nano-scale distribution in daily increments. For the first time, the application of NanoSIMS NS50 provided distribution patterns of calcium, strontium and sodium in cephalopod statoliths with a spatial resolution of 400nm.

The results of this study provide an essential basis for future investigations in the field, probably leading into further understanding of yet unknown migration patterns and spawning grounds of various cephalopod species. Applying these future approaches could establish a consolidated biological knowledge on cephalopod species and stocks, and therefore may contribute to an effective and sustainable management of this both ecologically and economically valuable resource.

Zusammenfassung

Statolithen sind calcifizierte "Gehörsteinchen" im Gleichgewichtsorgan der Cephalopoden. Sie wachsen ein Leben lang und bilden mikroskopisch sichtbare Tagesringe aus, sehr ähnlich zu den deutlich bekannteren Jahresringen in Bäumen.

Das Wachstum und die chemische Zusammensetzung von Statolithen sind abhängig von Umweltfaktoren. Daher können Statolithen in gewissem Maße die Lebensgeschichte des individuellen Cephalopoden widerspiegeln. Wenn eine direkte Beziehung zwischen der Zusammensetzung von Statolithen und den chemischen und physikalischen Eigenschaften des umgebenden Meerwassers gefunden wird, so kann der Statolith als Indikator für Umweltbedingungen dienen. Davon ausgehend, dass einmal gebildetes Material weder verändert noch resorbiert wird, kann man den Statolithen somit als permanentes Archiv für Umweltbedingungen ansehen. Dieses Archiv kann zeitlich aufgelöste Informationen über Habitat-Nutzung, Wanderbewegungen und Kontakt zu Schadstoffen speichern.

Im Rahmen dieser Arbeit wurden mehrere moderne mikroanalytische Methoden auf Tintenfisch- Statolithen angewandt, um ortsauflösend ihre chemische Zusammensetzung zu untersuchen. Die bisher vor allem in den Geowissenschaften angewandten Methoden wurden an die besonderen Eigenschaften von Statolithen angepasst und für die jeweiligen Anwendungen optimiert.

In dieser Arbeit wurde der Einfluss von unterschiedlichen Umweltfaktoren auf die chemische Zusammensetzung der Statolithen des gemeinen Tintenfisches *Sepia officinalis* untersucht. Basierend auf Laborexperimenten unter kontrollierten Bedingungen, ist es nun möglich, den jeweiligen Einfluss von Salinität, Temperatur und Nahrung auf die Einlagerung mehrerer Spurenelemente zu qualifizieren. Die Ergebnisse verdeutlichen, dass die Einlagerung mehrerer Elemente von Umweltbedingungen beeinflusst wird. So ist die Einlagerung von Barium und Jod vor allem von der Temperatur abhängig. Die Anreicherung von Strontium hingegen, einem Element, das in Korallen und Fisch- Otolithen fast routinemäßig als Indikator für Temperatur und Salinität genutzt wird, scheint in Tintenfisch- Statolithen eher von der Nahrung als vom umgebenden Meerwasser abhängig zu sein.

Weiterhin wurde die hervorragende Eignung dieser Methoden für Untersuchungen an Tieren aus dem Freiland demonstriert. Die chemische Zusammensetzung in verschiedenen Bereichen von Statolithen des nordischen Köderkalmars (*Gonatus*

fabricii) spiegelte in beindruckender Weise die Lebensgeschichte und ontogenetisch variierende Habitatsnutzung dieser Kalmare wider.

In dieser Dissertation wurden außerdem wertvolle neue Erkenntnisse über die Mikrostruktur von Statolithen und die Elementverteilung im Größenbereich von Tagesringen gewonnen. Das erstmals für diese Zwecke eingesetzte hochmoderne Analyse-Gerät NanoSIMS NS50 lieferte zweidimensionale Elementverteilungen von Calcium, Strontium und Natrium mit einer nie zuvor erreichten Ortsauflösung von 400nm.

Die Resultate dieser Studie bilden eine substantielle Basis für weitere Untersuchungen an Tieren aus der freien Wildbahn. Dies wird voraussichtlich zu vertieften Kenntnissen von bisher unbekanntem Wanderungswegen und Laichgebieten führen und kann somit zu einem effektiven und nachhaltigen Management dieser sowohl ökologisch als auch ökonomisch wertvollen Ressource beitragen.

References

- Allison, N., Finch, A.A., Sutton, S.R., and 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, P.M., Miller M.J., and 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. Progr. Ser.* **266**: 213-225.
- Arkhipkin, A.I. (1991) Methods for cephalopod age and growth studies with emphasis on statolith ageing techniques. In 'Squid Age Determination using Statoliths. Proceedings of the International Workshop, Mazara del Vallo, Italy, 9–14 October 1989'. (Eds P. Jereb, S. Ragonese and S. von Boletzky.) pp. 83–96. N.T.R.- I.T.P.P. Special publication No.1, Istituto di Tecnologia della Pesca e del Pescato, Mazara del Vallo.
- Arkhipkin, A.I. (2004). Diversity in growth and longevity in short-lived animals: squid of the suborder Oegopsina. *Mar. Freshw. Res.* **55**: 341–355.
- Arkhipkin, A.I. (2005) Statoliths as 'black boxes' (life recorders) in squid. *Mar. Freshw. Res.* **56**: 573-583.
- Arkhipkin, A.I., and Bjørke, H. (1999) Ontogenetic changes in morphometric and reproductive indices of the squid *Gonatus fabricii* (Oegopsida, Gonatidae) in the Norwegian Sea. *Polar Biol.* **22**: 357-365.
- Arkhipkin, A.I., and Bjørke, H. (2000) Statolith shape and microstructure as indicators of ontogenetic shifts in the squid *Gonatus fabricii* (Oegopsina, Gonatidae) from the Norwegian Sea. *Polar Biol.* **23**: 1–10.
- Arkhipkin, A.I., Campana, S.E., FitzGerald, J., and Thorrold, S.R. (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.
- Balguerías, E., Quintero, M.E., and Hernandez-Gonzalez, C.L. (2000) The origin of the Saharan Bank cephalopod fishery. *ICES J Mar Sci* **57**: 15-23.
- Barrea, R.A., Pérez, C.A., Ramos, A.Y., Sánchez, H.J., and Grenón, M. (2003) Distribution and incorporation of zinc in biological calcium phosphates. *X-Ray Spectrometry* **32** (5): 387–395.
- Bath, G.E., Thorrold, S.R., Jones, C.M., Campana, S.E., McLaren, J.W., and Lam, J.W.H. (2000) Sr and Ba uptake in aragonitic otoliths of marine fish. *Geochim. Cosmochim. Acta* **64**: 1705-1714.

- Bath-Martin, G., Thorrold, S.R., and Jones, C.M. (2004) Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). *Can. J. Fish. Aquat. Sci.* **61**: 34-42.
- Beck, J.W., Edwards, R.L., Ito, E., Taylor, F.W., Recy, J., Rougerie, F., Joannot, P., and Henin, C. (1992) Sea-surface temperature from coral skeletal strontium/calcium ratios. *Science (Wash., DC)*, **257**: 644–647.
- Becker, B.J., Fodrie, F.J., McMillan, P.A., and Levin, L.A. (2005) Spatial and temporal variation in trace elemental fingerprints of mytilid mussel shells: A precursor to invertebrate larval tracking. *Limnol. Oceanogr.*, **50**(1): 48–61.
- Bettencourt, V., and Guerra, A. (2000) Growth increments and biomineralization process in cephalopod statoliths. *J. Exp. Mar. Biol. Ecol.* **248**: 191-205.
- Bettencourt, V., and 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.
- Biemann, M.D., and Piatkowski, U. (2001) Amounts and composition of trace elements in the statoliths of loliginid squids: reflection of environmental conditions? ICES CM 2001 / K:05.
- Bjørke, H. (2001) Predators of the squid *Gonatus fabricii* (Lichtenstein) in the Norwegian Sea. *Fish. Res.* **52**: 113-120.
- Boletzky, S.v. (1983) *Sepia officinalis*. In: Boyle, P. R. Cephalopod life Cycles. Vol.1. 31-52. London, Academic Press.
- Boyle, P.R., and Boletzky, S.v. (1996) Cephalopod populations: definitions and dynamics. *Phil Trans R Soc Lond B* **351**: 985-1002.
- Buckel, J.A., Sharack, B.L., and Zdanowicz, V.S. (2004) Effect of diet on otolith composition in *Pomatomus saltatrix*, an estuarine piscivore. *J. Fish Biol.* **64**: 1469-1484.
- Bustamante, P., Teyssié, J.L., Fowler, S.W., Cotret, O., Danis, B., Miramand, P., and Warnau, M. (2002) Biokinetics of zinc and cadmium accumulation and depuration at different stages in the life cycle of the cuttlefish *Sepia officinalis*. *Mar. Ecol. Progr. Ser.* **231**:167-177.
- Caddy, J.F., and Rodhouse, P.G. (1998) Cephalopod and groundfish landings: evidence for ecological change in global fisheries. *Rev Fish Biol Fish* **8**: 431-444.
- Campana, S.E. (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar. Ecol. Progr. Ser.* **188**: 263-297.
- Campana, S.E. (2005) Otolith science entering the 21st century. *Mar. Freshw. Res.* **56**: 485–495.

- Campana, S. E., and Neilson, J. D. (1985) Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.* **42**: 1014–1032.
- Campana, S.E., Gagne, J.A., and McLaren, J.W. (1995) Elemental fingerprinting of fish otoliths using ID-ICPMS. *Mar. Ecol. Progr. Ser.* **122**:115-120.
- Campana, S.E., Fowler, A.J., and Jones, C.M. (1994) Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. *Can. J. Fish. Aquat. Sci.* **51** (9): 1942-1950.
- Campana, S.E., Chouinard, G.A., Hanson, J.M., Frechet, A., and Bratney, J. (2000) Otolith elemental fingerprints as biological tracers of fish stocks. *Fish. Res.* **46**: 343-357.
- Carvalho, G. R., and Pitcher, T. J. (1989) Biochemical genetic studies on the Patagonian squid *Loligo gahi* d'Orbigny. II. Population structure in Falkland waters using isozymes, morphometrics and life history data. *J. Exp. Mar. Biol. Ecol.* **126**: 243–258.
- Castro, B.G., and Guerra, A. (1990) The diet of *Sepia officinalis* (Linnaeus, 1758) and *Sepia elegans* (D'Orbigny, 1835) (Cephalopoda, Sepioidea) from the Ria de Vigo (NW Spain). *Sci. Mar.* **54** (4): 375-388.
- Clarke, M.R. (1966) A review of the systematics and ecology of oceanic squids. *Adv. Mar. Biol.* **4**: 91–300.
- Clarke, M.R. (1978) The cephalopod statolith — an introduction to its form. *J. Mar. Biol. Assoc. UK* **58**: 701–712.
- Clarke, M.R., and Pascoe, P.L. (1985) The Stomach contents of a Risso's dolphin (*Grampus griseus*) stranded at Thurlestone, South Devon. *J. Mar. Biol. Ass. UK*, **65**: 663-665.
- Cohen, A.L., Layne, G.D., Hart, S.R., and Lobel, P.S. (2001) Kinetic control of skeletal Sr/Ca in a symbiotic coral: implications for the paleotemperature proxy. *Paleoceanography* **16** (1): 20–26.
- Correge, T., Quinn, T., Delcroix, T., Le Cornec, F., Recy, J., and Cabioch, G. (2001) Little ice age sea surface temperature variability in the southwest tropical Pacific. *Geophys. Res. Lett.* **28** (18): 3477-3480.
- Dawe, E.G., O'Dor, R.K., O'Dense, P.H., and Hurley, G.V. (1985) Validation and application of an ageing technique for short-finned squid (*Illex illecebrosus*). *Journal of the Northwest Atlantic Fishery Science* **6**, 107–116.
- Dawe, E.G., and Natsukari, Y. (1991) Light microscopy. In 'Squid Age Determination using Statoliths. Proceedings of the International Workshop, Mazara del Vallo, Italy, 9–14 October 1989'. (Eds P. Jereb, S. Ragonese and S. von Boletzky.) pp. 83–96. N.T.R.- I.T.P.P. Special publication No.1, Istituto di Tecnologia della Pesca e del Pescato, Mazara del Vallo.

- Dawe, E.G., Bowering, W.R., and Joy J.B. (1998) Predominance of squid (*Gonatus spp.*) in the diet of Greenland halibut (*Rheinhardtius hippoglossoides*) on the deep slope of the northeast Newfoundland continental shelf. *Fish. Res.* **36**: 267-273.
- Denis, V., and Robin, J.P. (2001) Present status of the French Atlantic fishery for cuttlefish (*Sepia officinalis*). *Fish. Res.* **52** :11-22.
- Dietzel, M., Gussone, N., and 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.
- Donat, J.R., and Bruland, K.W. (1995) Trace elements in the oceans. *In* Trace elements in natural waters. *Edited by* B. Salbu and E. Steinnes. CRC Press, Boca Raton, Fla. 247–281.
- Durholtz, M.D., Lipinski, M.R., Przybylowicz, W.J., and Mesjasz-Przybylowicz, J. (1997) Nuclear microprobe mapping of statoliths of Chokka Squid *Loligo vulgaris reynaudii* d'Orbigny, 1845. *Biol. Bull.* **193**: 125-140.
- Eggins, S.M., Sadekov, A., and Deckker, P.D. (2004) Modulation and daily banding of Mg/Ca in *Orbulina universa* tests by symbiont photosynthesis and respiration: A complication for seawater thermometry, *Earth Planet. Sci. Lett.* **225**: 411 –419.
- Elsdon, T.S., and Gillanders, B.M. (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, T.S., and Gillanders, B.M. (2003) Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. *Mar. Ecol. Progr. Ser.* **260**: 263–272.
- Espen, P.J. van, Nullens, H., and Adams, F. (1977) A computer analysis of x-ray fluorescence spectra. *Nucl. Instr. Meth.* **142**: 269-273.
- FAO (2005) FISHSTAT Plus Global data set Capture production 1950-2003 (FAO yearbook Fishery Statistics). <ftp://ftp.fao.org/fi/stat/windows/fishplus/capdet.zip>.
- Fallon, S.J., McCulloch, M.T., van Woosik, R., and Sinclair, D.J. (1999) Corals at their latitudinal limits: laser ablation trace element systematics in *Porites* from Shirigai Bay, Japan. *Eart Planet. Sci. Lett.* **172**: 221-238.
- Farrell, J., and Campana, S.E. (1996) Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. *Comp. Biochem. Phys. A* **115**: 103–109.

- Fenton, G.E., Ritz, D.A., and Short, S.A. (1991) Age determination of orange roughy, *Hoplostethus atlanticus* (Pisces: Trachichthyidae) 292 using Pb-210:Ra-226 disequilibria. *Mar. Biol.* **109**: 197-202.
- Ferrier-Pages, C., Boisson, F., Allemand, D., and Tambutte, E. (2002) Kinetics of strontium uptake in the scleractinian coral *Stylophora pistillata*, *Mar. Ecol. Prog. Ser.* **245**: 93– 100.
- Finch, A.A., Allison, N., Sutton, S.R., and 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.
- Forsythe, J.W., Van Heukelem, W.F. (1987) Growth. In: Boyle, P.R. (ed) *Cephalopod Life Cycles*, vol. 2. Academic, London: 135-156.
- Gallahar, N.K., and Kingsford, M.J. (1996) Factors influencing Sr/Ca ratios in otoliths of *Girella elevata*: an experimental investigation. *J. Fish Biol.* **48**: 174– 186.
- Gregor, R.B., Pingitore, N.E., and Lytle, F.W. (1997) Strontianite in Coral skeletal aragonite. *Science* **275**: 1452-1454.
- Grieken, R.E. van, and Markowicz, A.A. (1993) *Handbook of X-ray Spectrometry*. Marcel Dekker, New York, Basel, Hongkong, 704 p.
- Guillong, M., Horn, I., and Günther, D. (2003) A comparison of 266 nm, 213 nm and 193 nm produced from a single solid state Nd:YAG laser for laser ablation ICP-MS. *J. Anal. Atom. Spectr.* **18**: 1224-1230.
- Hanlon, R.T., Bidwell, J.P., and 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, T.H., Sachs, P.M., and Lechtenberg, F. (2000) Synchrotron-XRF microprobe analysis of silicate reference standards using fundamental-parameter quantification. *Eur. J. Mineral.* **12**: 25-31.
- Hillion, F., Daigne, B., Girard, F., and G. Slodzian (1993) A new high performance instrument: The Cameca Nanosims 50, in *Proceedings of the 9th SIMS Conference*, edited by A. Benninghoven et al., pp. 254– 257, John Wiley and Sons, New York.
- Hoff, G.R., and Fuiman, L.A. (1995) Environmentally induced variation in elemental composition of red drum (*Sciaenops ocellatus*) otoliths. *Bull. Mar. Sci.* **56**: 578–591.
- Hossain, M.M.M., and Ohde, S. (2002) Uranium in pore water from coastal sediments determined by chemical activation analysis. *J. Radioanal. Nuclear Chem.* **251**: 333-336.

- Hurley, G.V., Odense, P.H., O'Dor, R.K., and Dawe, E.G. (1985) Strontium labelling for verifying daily growth increments in the statolith of the short-finned squid (*Illex illecebrosus*). *Can. J. Fish. Aquat. Sci.* **42** (2): 380-383.
- Ikeda, Y., Arai, N., Sakamoto, W., Murayama, T., Maeda, K., and Yoshida, K. (1995) Preliminary report on the PIXE analysis of the squid statoliths. *Int. J. PIXE* **5**: 159–162.
- Ikeda, Y., Arai, N., Sakamoto, W., Kidokoro, H., and 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., and Yoshida, K. (1996b) PIXE analysis of trace elements in squid statoliths: Comparison between Ommastrephidae and Loliginidae. *Int J PIXE* **6**: 537-542.
- Ikeda, Y., Arai, N., Sakamoto, W., Kidokoro, H., Yatsu, A., Nateewathana, A., and Yoshida, K. (1997) Comparison on trace elements in squid statoliths of different species' origin as available key for taxonomic and phylogenetic study. *Int. J. PIXE* **7**: 141–146.
- Ikeda, Y., Arai, N., Sakamoto, W., Mitsuhashi, M., and Yoshida, K. (1999) Preliminary report on PIXE analysis for trace elements of *Octopus dofleini* statoliths. *Fish. Sci.* **65**: 161–162.
- Ikeda, Y., Yatsu, A., Arai, N., and 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 Ass UK* **82**: 863-866.
- Ikeda, Y., Okazaki, J., Sakurai, Y., and 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., and Sakamoto, W. (2003) Strontium:calcium ratios in statoliths of Japanese common squid *Todarodes pacificus* (Cephalopoda: Ommastrephidae) as indicators of migratory behaviour. *Mar. Ecol. Progr. Ser.* **251**: 169-179.
- Jackson, G.D. (1994) Application and future potential of statolith increment analysis in squid and sepioids. *Can. J. Fish. Aquat. Sci.* **51**: 2612–2625.
- Jackson, G.D. (2004) Advances in defining the life histories of myopsid squid. *Mar. Freshw. Res.* **55**: 357–365.
- Jackson, G.D., O'Dor, R.K., and Andrade, Y. (2005) First tests of hybrid acoustic/archival tags on squid and cuttlefish. *Mar. Freshw. Res.* **56**: 425-430.
- Jochum, K.P., Dingwell, D.B., Rocholl, A., Stoll, B., Hofmann, A.W., Becker, S., Besmehn, A., Bessette, D., Dietze, H.J., Dulski, P., Erzinger, J., Hellebrand, E., Hoppe, P., Horn, I., Janssens, K., Jenner, G.A., Klein, M., McDonough, W.F.,

- Maetz, M., Mezger, K., Munke, C., Nikogosian, I.K., Pickhardt, C., Raczek, I., Rhede, D., Seufert, H.M., Simakin, S.G., Sobolev, A.V., Spettel, B., Straub, S., Vincze, L., Wallianos, A., Weckwerth, G., Weyer, S., Wolf, D., and Zimmer, M. (2000) The preparation and preliminary characterisation of eight geological MPI-DING reference glasses for in-site microanalysis. *Geostand. Newslett.* **24**: 87-133.
- Kalish, J.M. (1990) Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. *Fish. Bull. US* **88**: 657-666.
- Kalish, J.M. (1991) Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus*. *Mar. Ecol. Progr. Ser.* **74**: 137-159.
- Kristensen, T.K. (1980) Periodical growth rings in cephalopod statoliths. *Dana* **1**: 39-51.
- Kristensen, T.K. (1983) *Gonatus fabricii*. In: *Cephalopod life cycles (1), Species accounts*. Edited by P.R. Boyle. Academic Press, London. pp.159-173.
- Kristensen, T.K. (1984) Biology of the squid *Gonatus fabricii* (Lichtenstein, 1818) from West Greenland waters. *Meddr Grønland, Biosci* **13**: 1-20.
- Knezovich, J.P. (1994) Chemical and biological factors affecting bioavailability of contaminants in seawater. In: *Bioavailability: physical, chemical and biological interactions*. Hamelink, J.L., Landrum, P.F., Bergman, H.L., and Benson, W.H. (eds). Lewis Publishers, London: 23-30.
- Landman, N. H., Cochran, J. K., Cerrato, R., Mak, J., Roper, C. F. E., and Lu, C. C. (2004). Habitat and age of the giant squid (*Architeuthis sanctipauli*) inferred from isotopic analyses. *Marine Biology* **144**, 685-691.
- Lea, D.W., Shen, G.T., and Boyle, E.A. (1989) Coralline barium records temporal variability in equatorial Pacific upwelling. *Nature* **340**: 373-376.
- Lechtenberg, F., Garbe, S., Bauch, J., Dingwell, D.B., Freitag, F., Haller, M., Hansteen, T.H., Ippach, P., Knöchel, A., Radtke, M., Romano, C., Sachs, P.M., Schmincke, H.U., and Ullrich, H.J. (1996) The X-ray fluorescence measurement place at beamline L of Hasylab. *Journal of Trace and Microprobe Techniques* **14** (3): 561-587.
- Limburg, K.E. (1995) Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima*. *Mar. Ecol. Progr. Ser.* **119**: 25-5.
- Limburg, K.E. (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, K.E. (1998) Anomalous migrations of anadromous herrings revealed with natural chemical tracers. *Can. J. Fish. Aquat. Sci.* **55**: 431-437.

- Lipinski, M.R. (1979) Universal maturity scale for commercially important squids (Cephalopoda: Teuthoidea). ICNAF Res. Doc. **79/II/38**: 53-64.
- Lipinski, M.R. (1980) A preliminary study on age of squids from their statoliths. NAFO SCR Doc. No. 80/II/22, 17 pp. Northwest Atlantic Fisheries Organization, Dartmouth, Nova Scotia.
- Lipinski, M.R. (1993) The deposition of statoliths: a working hypothesis. In: Recent Advances in Cephalopod Fisheries Biology, T. Okutani, R. K. O'Dor, and T. Kubodera, eds. Tokai University Press, Tokyo.
- Lipinski, M., Przybylowicz, W.J., Durholtz, M.D., and Mesjasz-Przybylowicz, J. (1997) Quantitative micro- PIXE mapping of squid statoliths. Nucl. Instr. Meth. Phys. Res. B **130**: 374-380.
- Lipinski, M.R., Durholtz, M.D., and Underhill, L.G. (1998) Field validation of age readings from the statoliths of chokka squid (*Loligo vulgaris reynaudii* D'Orbigny, 1845) and an assessment of associated errors. ICES Journal of Marine Science **55**: 240–257.
- Lowenstern, J.B. (1993) Evidence for a copper-bearing fluid in magma erupted at the Valley of Ten-Thousand-Smokes, Alaska. Contr. Min. Petrol. **114**: 409-421.
- Markaida, U., Rosenthal, J.J.C., Gilly, W.F. (2005) Tagging studies on the jumbo squid (*Dosidicus gigas*) in the Gulf of California, Mexico. Fish. Bull. **103**: 219-226.
- Martin, R.R., Naftel, S.J., Machfie, S.M., Jones, K.W., and Feng, H. (in press) High variability of the metal content of tree growth rings as measured by synchrotron micro x-ray fluorescence spectrometry. X-Ray Spectrometry. DOI: 10.1002/xrs.817
- Meibom A., Cuif, J.P., Hillion, F., Constantz, B.R. Juillet-Leclerc, A., Dauphin, Y., Watanabe, T., and Dunbar, R.B. (2004) Distribution of magnesium in coral skeleton, Geophys. Res. Lett., **31**, L23306, doi:10.1029/2004GL021313.
- Milton, D.A., Short, S.A., O'Neill, M.F., Blaber, S.J.M. (1995) Ageing of three species of tropical snapper (Lutjanidae) from the Gulf of Carpentaria, Australia, using radiometry and otolith ring counts. Fish. Bull. **93**: 103-115.
- Milton, D.A., and Chenery, S.R. (2001) Sources and uptake of trace metals in otoliths of juvenile barramundi (*Lates calcarifer*). J. Exp. Mar. Biol. Ecol. **264**: 47–65.
- Min, G.R., Edwards, R.L., Taylor, F.W., Recy, J., Gallup, C.D., and Beck, J.W. (1995) Annual cycles of U/Ca in coral skeletons and U/Ca thermometry. Geochim. Cosmochim. Acta **59** (10): 2025-2042.
- Mitsuguchi, T., Matsumoto, E., Abe, O., Uchida, T., and Isdale, P.J. (1996) Mg/Ca thermometry in coral skeletons. Science **274**: 961-963.

- Morris, C.C. (1988) Statolith growth lines and statolith function in the Cephalopoda. Ph.D. Dissertation, University of Cambridge, U. K. 140 pp.
- Morris, C.C. (1991a) Statocyst fluid composition and its effects on calcium carbonate precipitation in the squid *Alloteuthis subulata* (Lamarck, 1798): towards a model for biomineralization. *Bull. Mar. Sci.* **49** (1-2): 379–388.
- Morris, C.C., (1991b) Methods for in situ experiments on statoliths increment formation, with results for embryos of *Alloteuthis subulata*. In 'Squid Age Determination using Statoliths. Proceedings of the International Workshop, Mazara del Vallo, Italy, 9–14 October 1989'. (Eds P. Jereb, S. Ragonese and S. von Boletzky.) pp. 83–96. N.T.R.- I.T.P.P. Special publication No.1, Istituto di Tecnologia della Pesca e del Pescato, Mazara del Vallo.
- Morris, C.C., and Aldrich, F.A. (1985) Statolith length and increment number for age determination in squid *Illex illecebrosus* (Lesueur, 1821) (Cephalopoda: Ommastrephidae). *NAFO Scientific Council Studies* **9**: 101–106.
- Mugiya, Y., and Satoh, C. (1997) Strontium accumulation in slow-growing otoliths in the goldfish *Carassius auratus*. *Fish. Sci.* **63**: 361–364.
- Murata, M., and Nakamura, Y. (1998) Seasonal migration and diel vertical migration of the neon flying squid, *Ommastrephes bartramii* in the North Pacific. In 'Large Pelagic Squid'. (Ed. T. Okutani.) pp. 13–30. (Japan Marine Fishery Resources Research Center: Tokyo.)
- Nakai, I., Iwata, R., and Tsukamoto, K. (1999) Ecological study of the migration of eel by synchrotron radiation induced X-ray fluorescence imaging of otoliths. *Spectrochimica Acta Part B* **54** (1): 167-170.
- Nesis, K.N. (1965) Distribution and feeding of young squids *Gonatus fabricii* in the Labrador Sea and the Norwegian Sea. *Oceanology* **5**: 102-108.
- Ohde, S., Hossain, M.M.M., Ozaki, H., and Masuzawa, T. (2003) Uranium in coral skeletons determined by epithermal neutron activation analysis. *J. Radioanal. Nucl. Chem.* **258** (2): 275-280.
- Patterson, W.P., Smith, G.R., and Lohmann, K.C. (1993) Continental paleothermometry and seasonality using the isotopic composition of aragonitic otoliths of freshwater fishes. *Geophys. Mono.* **78**: 191-202.
- Pearce, N.J.G., Perkins, W.T., Westgate, J.A., Gorton, M.P., Jackson, S.E., Neal, C.L., and Chenery, S.P. (1997) A compilation of new and published major and trace element data for NIST SRM 610 and NIST SRM 612 glass reference materials. *Geostand. Newsl.* **21**: 115-144.
- Piatkowski, U., and Wieland, K. (1993) The boreoatlantic squid *Gonatus fabricii*: distribution and size off West Greenland in summer 1989 and in summer and autumn 1990. *Aquat. Living Resour.* **6**: 109-114.

- Piatkowski, U., Pierce, G.J., Morais da Cunha, M. (2001) Impact of cephalopods in the food chain and their interaction with the environment and fisheries: an overview. *Fish. Res.* **52**: 5-10.
- Radtke, R.L. (1983) Chemical and structural characteristics of statoliths from the short-finned squid *Illex illecebrosus*. *Mar. Biol.* **76**: 47–54.
- Ribergaard, M.H., and Buch, E. (2005) Oceanographic Investigations off West Greenland 2004. NAFO Scientific Council Documents 05/019.
- Rodhouse, P.G.K., Robinson, K., Gajdatsy, S.B., Daly, H.I., and Ashmore, M.J.S. (1994) Growth, age structure and environmental history in cephalopod *Martialia hyadesi* (Teuthoidea: Ommastrephidae) at the Antarctic Polar Frontal Zone and on the Patagonian Shelf Edge. *Antarctic Science* **6**: 259–268.
- Rosenberg, G.D., Hughes, W.W., Parker, D.L., and Kay, B.D. (2001) The geometry of bivalve shell chemistry and mantle metabolism. *Am. Malacol. Bull.* **16**: 251– 261.
- Sadovy, Y., and Severin, K.P. (1992) Trace elements in biogenic aragonite: correlation of body growth rate and strontium levels in the otoliths of the white grunt, *Haemulon plumieri* (Pisces: Haemulidae). *Bull. Mar. Sci.* **50**: 237–257.
- Schlitzer, R. (2004) Ocean Data View (ODV); <http://www.awi-bremerhaven.de/GEO/ODV>.
- Secor, D.H. (1992) Application of otolith microchemistry analysis to investigate anadromy in Chesapeake Bay striped bass *Morone saxatilis*. *Fish. Bull. U.S.* **90**: 798-806.
- Secor, D.H., Henderson-Arzapalo, A., and Piccoli, P.M. (1995) Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? *J. Exp. Mar. Biol. Ecol.* **192**: 15–33.
- Secor, D.H., and Rooker, J.R. (2000) Is otolith strontium a useful scalar of life cycles in estuarine fishes? *Fish. Res.* **46** (1): 359-371.
- Shaw, P.W., Arkhipkin, A.I., Adcock, G.J., Burnett, W.J., Carvalho, G.R., Scherbich, J.N., and Villegas, P.A. (2004) DNA markers indicate that distinct spawning cohorts and aggregations of Patagonian squid, *Loligo gahi*, do not represent genetically discrete subpopulations. *Mari. Biol.* **144**: 961–970.
- Shen, G.T., and Dunbar, R.B. (1995) Environmental controls on uranium in reef corals. *Geochim. Cosmochim. Acta* **59**: 2009-2024.
- Shen, C.C., Lee, T., Chen, C.Y., Wang, C.H., Dai, C.F., and Li, L.A. (1996) The calibration of D[Sr/Ca] versus sea surface temperature relationship for *Porites* corals. *Geochim. Cosmochim. Acta* **60**: 3849-3858.

- Sholkovitz, E., and Shen, G.T. (1995) The incorporation of rare earth elements in modern coral. *Geochim. Cosmochim. Acta* **59** (13): 2749-2756.
- Simon, M.J., Kristensen, T.K., Tendal, O.S., Kinze, C.C., and Tougaard, S. (2003) *Gonatus fabricii* (Mollusca, Teuthida) as an important food source for sperm whales (*Physeter macrocephalus*) in the Northeast Atlantic. *Sarsia* **88**: 244-246.
- Smith, S.V., Buddermeier, R.W., Redalje, R.C., and Houck, J.E. (1979) Strontium-calcium thermometry in coral skeletons. *Science* **204**: 404-407.
- Sohrin, Y., Fujishima, Y., Ueda, K., Akiyama, S., Mori, K., Hasegawa, H., and Matsui, M. (1998) Dissolved niobium and tantalum in the North Pacific. *Geophys. Res. Letters* **25** (7): 999-1002.
- Smith, S.V., Buddermeier, R.W., Redalje, R.C., and Houck, J.E. (1979) Strontium-calcium thermometry in coral skeletons. *Science* **204**: 404-407.
- Stransky, C., Kanisch, G., Krüger, A. and Purkl, S. (2005) Radiometric age validation of golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*) in the Northeast Atlantic. *Fish. Res.* **74**: 186-197.
- Tambutte, E., Allemand, D., Mueller, E., and Jaubert, J. (1996) A compartmental approach to the mechanism of calcification in hermatypic corals, *J. Exper. Biol.* **199**: 1029– 1041.
- Thorn, K., Cerrato, R.M., and Rivers, M.L. (1995) Elemental distributions in marine bivalve shells as measured by synchrotron X-ray fluorescence. *Biol. Bull.* **188**: 57-67.
- Thorrold, S.R., Jones, C.M., and Campana, S.E. (1997) Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). *Limnol. Oceanogr.* **42** (1): 102-111.
- Thresher RE, Proctor CH, Gunn JS, Harrowfield IR (1994) An evaluation of electron probe microanalysis of otoliths for stock delineation and identification of nursery areas in a southern temperate groundfish, *Nemadactylus macropterus* (Cheilodactylidae). *Fish. Bull.* **92**: 817-840.
- Townsend, D.W., Radtke, R.L., Corwin, S., and Libby, D.A. (1992) Strontium: calcium ratios in juvenile Atlantic herring *Clupea harengus* L. otoliths as a function of water temperature. *J. Exp. Mar. Biol. Ecol.* **160**: 131–140.
- Tsukamoto, K., Nakai, I., and Tesch, W.V. (1998) Do all freshwater eels migrate? *Nature* **396**: 635–636.
- Vander Putten, E., Dehairs, F., André, L., and Baeyens, W. (1999) Quantitative in situ microanalysis of minor and trace elements in biogenic calcite using infrared laser ablation inductively coupled plasma mass spectrometry: a critical evaluation. *Anal. Chim. Acta* **378**: 261-272.

- Vander Putten, E., Dehairs, F., Keppens, E., and Baeyens, W. (2000) High resolution distribution of trace elements in the calcite shell layer of modern *Mytilus edulis*: Environmental and biological controls. *Geochim. Cosmochim. Acta* **64**: 997-1011.
- Vincze, L., Janssens, K., and Adams, F. (1993) A general Monte Carlo simulation of energy dispersive X-Ray fluorescence spectrometers - Part I. *Spectrochim. Acta* **48B**: 553-573.
- Weber, P.K., Hutcheon, I.D., McKeegan, K.D., Ingram, B.L. (2002) Otolith sulfur isotope method to reconstruct salmon (*Oncorhynchus tshawytscha*) life history. *Can. J. Fish. Aquat. Sci.* **59**: 587–591.
- Weber, P.K., Bacon, C.R., Hutcheon, I.D., Ingram, B.L., Wooden, J.L. (2005) Ion microprobe measurement of strontium isotopes in calcium carbonate with application to salmon otoliths. *Geochim. Cosmochim. Acta* **69** (5): 1225–1239.
- Yang, W.T., Hixon, R.F., Turk, P.E., Krejci, M.E., Hulet, W.H., and Hanlon, R.T. (1986) Growth, behavior, and sexual maturation of the market squid, *Loligo opalescens*, cultured through life cycle. *Fishery Bulletin* **84**: 771–798.
- Yatsu, A., Mochioka, N., Morishita, K., and 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, D.C., Paradis, G., and Lea, D.W. (2003a) Barium and strontium uptake into larval protoconchs and statoliths of the marine neogastropod *Kelletia kelletii*. *Geochim. Cosmochim. Acta* **67**: 4091-4099.
- Zacherl, D.C., Manriquez, P.H., Paradis, G.D., Day, R.W., Castilla, J.C., Warner, R.R., Lea, D.W., and Gaines, S.D. (2003b) Trace elemental fingerprinting of gastropod statoliths to study larval dispersal trajectories. *Mar. Ecol. Progr. Ser.* **284**: 297-303.
- Zoeger, N., Wobrauschek, P., Strelci, C., Pepponi, G., Roschger, P., Falkenberg, G. and 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., and Frandsen, R.P. (in press). New information on the life history of cephalopods off west Greenland. *Polar Biol.* DOI : 10.1007/s00300-005-0036-y.

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Erklärung

Hiermit erkläre ich, dass die vorliegende Dissertation selbständig von mir angefertigt wurde. Die Dissertation ist nach Form und Inhalt meine eigene Arbeit und es wurden keine anderen als die angegebenen Hilfsmittel verwendet. Diese Arbeit wurde weder ganz noch zum Teil einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach Fischereibiologie erfolgen. Des Weiteren erkläre ich, dass ich Zuhörer bei der Disputation zulasse.

Kiel, den 16. Dezember 2005

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Karsten Zumholz

Lebenslauf

Name	Zumholz
Vorname	Karsten
Geburtsort	Berlin
Geburtsdatum	28.03.1974
Staatsangehörigkeit	deutsch
Familienstand	verheiratet, zwei Kinder

1981-1987	Grundschule in Berlin
1987-1993	Humboldt-Gymnasium in Berlin, Abschluss Abitur
1994-1995	Zivildienst im Dominikus-Krankenhaus in Berlin
1995-1997	Studium der Biologie an der Humboldt-Universität zu Berlin
1997-2001	Studium der Biologie an der Christian- Albrechts- Universität zu Kiel Abschluss Diplom mit dem Hauptfach Fischereibiologie
Dez 2001	Diplomarbeit am Institut für Meereskunde, Kiel mit dem Thema: „Fischereibiologische Untersuchungen zur Cephalopoden- Fauna der Nordsee“ unter der Betreuung von Prof. Dr. H. Rosenthal
2002	Technischer Angestellter am Institut für Seefischerei der Bundesforschungsanstalt für Fischerei, Hamburg
Jan- Feb 2003	wissenschaftlicher Angestellter im EU-Projekt „CEPHSTOCK: Cephalopod Stocks in European Waters: Review, Analysis, Assessment and Sustainable Management“ am Institut für Meereskunde, Kiel
März 2003	Beginn der Doktorarbeit am Institut für Meereskunde, Kiel in dem DFG-finanzierten Projekt: „Der Einfluss von Umweltfaktoren auf die mikrochemische Zusammensetzung der Statolithen von <i>Sepia officinalis</i> “

Auslandsaufenthalte

Jul- Aug 2000	Gastaufenthalt am Zoological Department der University of Aberdeen, Schottland
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Mitgliedschaft in wissenschaftlichen Arbeitsgruppen

seit 2002	Working Group on Cephalopod Fisheries and Life History (WGCEPH) des International Council for the Exploration of the Sea (ICES)
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