

**Geochemical properties of shells of *Arctica islandica* (Bivalvia) –  
implications for environmental and climatic change**

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*To DAAD and CSC!*

*for the purpose of harmony relationship among people of all countries in the world  
by the way of cooperation and mutual understanding*



## Abstract

Trace elemental concentrations of bivalve shells content a wealthy of environmental and climatic information of the past, and therefore the studies of trace elemental distributions in bivalve shells gained increasing interest lately. However, after more than half century of research, most of the trace elemental variations are still not well understood and trace elemental proxies are far from being routinely applicable. This dissertation focuses on a better understanding of the trace elemental chemistry of *Arctica islandica* shells from Iceland, and paving the way for the application of the trace elemental proxies to reconstruct the environmental and climatic changes.

Traits of trace elemental concentrations on *A. islandica* shells were explored and evaluated. Then based the geochemical traits of the shells, four non-environmental/climatic controlling is indentified. (1) Trace elemental concentrations of bivalve shells are effected by early diagenesis by the leach or exchange of elemental ions, especially in shell tip part, even with the protection of periostrucum; (2) The analytical methods also affect the results of trace elemental concentrations, especially for the element, such as Mg, which is highly enriched in organic matrices; (3) Shell organic matrices are found play a dominating role on the concentration of trace elements on *A. islandica* shells. Most trace elements only occurred in insoluble organic matrices (IOM), although others are only found in the carbonate fraction. IOM of *A. islandica* shells is significantly enriched in Mg, while Li and Na are more deplete in IOM, but enriched in shell carbonate. Ba is more or less even contented in IOM and shell carbonate. The concentrations of certain elements vary between primary layer and secondary layer; (4) The vital /physiological controlling on trace elemental distributions of bivalve shells is also confirmed. Six elemental (B, Na, Mg, Mn, Sr, and Ba) concentrations show significant correlation (exponential functions) with ontogenetic age and shell grow rates (logarithmic equations). It is worthy to remark that B, Mg, Sr and Ba concentrations are negatively correlated with shell growth rate, positive with ontogenetic age, while the concentrations of Na and Mn show the opposite trends.

At last, all the controlling described above can be taken into account and corrected to extract the environmental and climatic signal by a kind of standardization. The derived six exponential functions of the high correlations between six trace elemental concentrations and ontogenetic year are applied to make the standardization of these element-Ca ratios. The gotten standardized indices are compared with the variations of environmental and climatic

parameters in this region, and many correlations are found. Standardized indices of Sr/Ca ratios are strongly related to the sun spot number, autumn NAO, autumn Europe surface air temperature (SAT) and Arctic sea surface temperature anomaly (TA), and those of Mg/Ca ratios are strongly associated with Arctic TA, Europe SAT and Solar variation (irradiance). The variations of autumn Europe SAT demonstrated more similarity with standardized indices of B/Ca than other parameters. Except for the SAT index of Arctic, the standardized indices of Na/Ca showed no distinct relation to temperature. European precipitation and the Arctic sea level pressure index compared well the Na/Ca ratios of the shells, and so did the autumn NAO. Standardized indices of Mn/Ca were correlated with the number of hurricanes in the North Atlantic, Northern Europe SAT and sun spot number.

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# 1 General introduction

During the past decade, the number of bivalve-sclerochronological studies has strongly increased and special volumes have been published (Schöne & Surge, 2005; Gröcke & Gillikin, 2008; Oschmann, 2009). Originated from the studies on corals (Buddemeier et al., 1974; Hudson et al., 1976), the term ‘sclerochronology’ was recently refined as “*the study of physical and chemical variations in the accretionary hard tissues of organisms and the temporal context in which they formed*” in order to “*deduce organismal life history traits as well as to reconstruct records of environmental and climatic change through space and time*” by Douglas Jones, William Arnold, Irvy Quitmyer, Bernd Schöne and Donna Surge at the 1st International Sclerochronology Conference in 2007 in St. Petersburg, FL, USA.

However, reliable proxies of past environmental conditions other than  $\delta^{18}\text{O}$  values are still not available. High-potential candidates which are frequently used in coral sclerochronology are metal-to-calcium (Me/Ca) ratios (e.g., Beck et al., 1992; Guilderson et al. 1994). Trace element variations of bivalve shells provided inconsistent results and are very likely affected by non-environmental effects such as kinetic effects, biological effects and other effects.

This dissertation focuses on a better understanding of factors that control the trace metal chemistry of *Arctica islandica* shells from Iceland. Results of this thesis may help to better understand the relation between the environment and Me/Ca ratios.

## 1.1 State of the art

Knowledge of past environmental and climate variability is one of the key aspects to model possible changes in the future. The reasons why bivalve mollusks gained so much popularity can be summarized as follows (cf. Schöne et al., 2004, 2005a, c, d):

- (1) Shells provide precisely temporally aligned records of their environment.
- (2) Environmental data are recorded in multiple ways, i.e. in the form of variable shell growth rates, isotope ratios and trace elements.
- (3) Individual shells of long-lived bivalve species such as *A. islandica* can potentially provide century long climate records.

- (4) The broad biogeographic distribution makes bivalves ideal environmental recorders. They inhabit rivers, lakes and the sea, live in shallow and deep seas as well as near the poles and the equator.

Bivalve shell geochemistry can potentially provide quantifiable data on environmental variables for times prior to instrumental recordings (e.g., Odum, 1951, 1957a, 1957b; Lee and Wilson, 1969; Stanton Jr and Dodd, 1970; Lorens and Bender, 1980; Carriker et al., 1991; Richardson, 2001; Henderson, 2002; Gillikin, 2005; Gröcke and Gillikin, 2008). However, even after more than half a century of studies of shell geochemistry, most of the trace elemental variations are still not well understood and trace elemental proxies are far from being routinely applicable. To name but a few issues:

- (1) The mechanism which dominates the process of biomineralization of bivalve shells is still largely unknown, but has most likely are large impact on the trace elemental content of shells.
- (2) Interrelationships between different trace elements are not well comprehended.
- (3) The effects of non-environmental factors controlling the elemental distribution in shells have not been quantified, and it is impossible to subtract all the other signals from the pure environmental signal.

The most important results of geochemical studies on bivalve shells as well as caveats and challenges are listed below.

- (1) Stable isotopes. Most bivalves secrete their shells in oxygen isotopic equilibrium with the ambient water (Epstein et al., 1953; Grossman and Ku, 1986; Weidman et al., 1994; Al-Aasm et al., 1998; Andrus and Rich, 2008; Owen et al., 2008). Metabolic activity exerts no effect on the oxygen isotope fractionation during the biomineralization of the bivalve shells (Klein et al., 1996; Surge and Waler, 2006). Therefore, the  $\delta^{18}\text{O}$  values of bivalve shells can be used to reconstruct paleotemperatures. Many researches have successfully applied this proxy to infer past temperatures (e.g. Stecher III et al., 1996; Klein et al., 1997; Schöne et al., 2004, 2005d; Wanamaker Jr et al., 2007). However, oxygen isotope-derived temperature reconstructions require knowledge the  $\delta^{18}\text{O}$  value of the water in which the bivalve lived. This value underlies large variations and is rarely known. Variations of shell



oxygen isotopes may therefore indicate changes of ambient water temperature and/or salinity.

Stable carbon isotopes are more difficult to interpret than oxygen isotopes. They may be determined by food, dissolved inorganic carbon (DIC), metabolic activity and ontogenetic age. The  $\delta^{13}\text{C}$  values of bivalve shells are usually more positive than their diet and the dissolved inorganic carbon (DIC) because of the release of the lighter isotope,  $^{12}\text{C}$ , by metabolic activity (Putten et al., 2000; McConnaughey and Gilikin, 2008; Owen et al., 2008). The fraction of respired carbon decreases with age. More intensive metabolic activity in lateral margins of the mantle than in ventral margins leads to higher  $\delta^{13}\text{C}$  values in the latter (Klein et al., 1996; Putten et al., 2000; McConnaughey and Gilikin, 2008). However, because the food and DIC are correlated with salinity and other seasonal variations, the  $\delta^{13}\text{C}$  values of bivalves can potentially provide environmental and climatic information (Klein et al. 1996; McConnaughey and Gilikin, 2008; Owen et al., 2008; Tynan et al., 2008).

(2) Minor and trace elements (MTE).

(a) It is generally accepted that the incorporation of **Strontium** into bivalve shells is affected by physiological factors (Lowenstam, 1954a; Thompson and Chow, 1955; Swan, 1956; Pilkey and Goodell, 1963; Dodd, 1967; Klein et al., 1996; Purton et al., 1999; Elliot et al., 2002; Tripathi et al., 2004; Freitas et al., 2005; Gillikin et al., 2005a; Watanabe et al., 2005; Tanabe et al., 2007; Carroll and Romanek, 2008). Furthermore, Sr is strongly enriched in the major growth lines, i.e. in winter and reproduction lines (Swann et al., 1991; Coote and Trompetter, 1993; Palacios et al., 1994; Elliot et al., 2002). Sr concentrations of bivalve shells have also been demonstrated to be controlled by shell growth rate (Swan, 1956; Harriss, 1965; Purton et al., 1999; Tripathi et al., 2004; Carre et al., 2006; Freitas et al., 2006; Strasser et al., 2008) and ontogenetic age (Klein et al. 1996; Purton et al., 1999; Pearce and Mann, 2006). Despite all these affects, the Sr variation of bivalve shells contains environmental and climatic signals, e.g., information on the ambient water chemistry (Odum, 1951; Pilkey and Harriss, 1966; Dodd, 1967; Lee and Wilson, 1969; Carriker et al., 1980; Fujikura et al. 2003; Langlet et al., 2005; Pearce and Mann, 2006; Carroll and

Romanek, 2008) and salinity (Odum, 1957a; 1957b; Dodd, 1967; Dodd and Crisp, 1982; Klein et al. 1996; Limburg, 2004; Tanabe et al., 2007). In addition, Sr/Ca ratios have been found to reflect changes in water temperature. Sr/Ca ratios of calcitic shells are mainly positively correlated with temperature (Dodd, 1965; Stecher III et al., 1996; Freitas et al., 2006), while those of aragonitic shells show the opposite (Dodd, 1965; Lerman, 1965; Hallam and Price, 1968; Stecher III et al., 1996; Richardson et al., 2004; Tripathi et al., 2004; Nagler et al., 2006).

(b) **Magnesium** of bivalve shells seems to be affected by multiple factors including temperature (Chave, 1954; Dodd, 1965; Klein et al., 1996; Putten et al., 1999; Lazareth et al., 2003; Richardson et al., 2004; Pearce and Mann, 2006), ambient water chemistry (Dodd, 1965; 1967; Romeril, 1971; Carriker et al., 1980; Dodd and Crisp, 1982; Carriker et al., 1991; Immenhauser et al., 2005), crystal morphology (Chave, 1954; Harriss, 1965; Dodd, 1967; Lorens and Bender, 1980; Carriker et al., 1991; Tanabe et al., 2007), species (Chave, 1954; Pilkey and Goodell 1963; Dodd, 1967; Freitas et al., 2006; 2007), shell growth kinetics (Moberly Jr, 1968; Carre et al., 2006; Strasser et al., 2008), physiological processes (Dodd, 1967; Lorens and Bender, 1977; Rosenberg et al., 2001; Freitas et al., 2005; Watanabe et al., 2005), ontogenetic age (Dodd, 1965; Carriker et al. 1991; Takesue et al., 2007; Klünder et al., 2008; Wanamaker Jr et al., 2008) and diagenetic effects (Brand and Morrison, 1987; Elorza and Garcia-Garmilla, 1996).

(c) **Barium**. The Ba/Ca values of bivalve shells are associated with phytoplankton blooms (Stecher III et al., 1996; Putten et al., 1999; 2000; Lazareth et al., 2003; Gillikin, 2006; Tanabe et al., 2007) and might be an indicator of the Ba concentration of the ambient water, salinity or other environmental properties (Hendry et al., 2001; Epple, 2004; Jimenez-Berrocoso et al., 2004; Pearce and Mann, 2006; Gillikin et al., 2006; 2008a, b; Tanabe et al., 2007; Tynan et al., 2008). Furthermore, the Ba concentration of bivalve shells can vary among species (Pilkey and goodell, 1963; Tanabe et al., 2007), depend on growth rate (Carre et al., 2006; Strasser et al., 2008), ontogenetic age (Strasser et al., 2008)

and other unknown environmental forcings (Gillikin et al., 2008b). However, Ba does not seem to be altered by diagenesis (Brand and Morrison, 1987).

- (d) **Sodium.** Na distributions across bivalve shells show correlation with temperature (Rucker and Valentine, 1961). Na ions might be located in lattice defects (Busenberg and Plummer, 1985) or are adsorbed onto crystal surfaces (Lorens and Bender, 1980). Experiments demonstrated that Na/Ca ratios of bivalve shells covary with Na/Ca ratios of the ambient water (Lorens and Bender, 1980). The Na concentration in shells from contaminated water is lower than that in clean water (Almeida et al., 1998b). Na/Ca ratios increase with ontogenic age (Carriker et al., 1982) and are also thought to be effected by the periodically changing paleoenvironmental conditions (Jimenez-Berrocso et al., 2004).
- (e) **Manganese.** Mn is evenly distributed in soft tissues of bivalves (Carriker et al., 1980) and much higher concentrated than in shells. Its concentration is correlated with the shell deposition rate (Frazier 1975). Mn concentration in bivalve shells is determined by four factors: by taxon (Pilkey and Goodell, 1963; Tanabe et al., 2007; Carroll and Romanek, 2008), growth rate (Harriss, 1965; Carre et al., 2006; Freitas et al., 2008b; Strasser et al., 2008), environmental variations (Pilkey and Harriss, 1966; Carriker et al., 1980; Jimenez-Berrocso et al., 2004; Langlet et al., 2005; Madkour, 2005; Richardson et al., 2007; Carroll and Romanek, 2008; Barbink et al., 2008; Protasowicki et al., 2008) and diagenetic process (Elorza and Garcia-Gamilla, 1996). No correlation with temperature was found (Rucker and Valentine 1961). The Mn concentration in bivalve shells does not directly reflect the metal concentrations in the water in which they live (Ravera et al., 2003; Freitas et al., 2008b). Ontogenetic age affects the incorporation of Mn only during juvenile life stages (Strasser et al., 2008) or not at all (Protasowicki et al., 2008). Like Ba, high concentrations of Mn in bivalve also coincide with biomass production (Putten et al., 2000; Lazareth et al., 2003; Freitas et al., 2006; Tanabe et al., 2007).

- (f) **Lead.** Amiard et al. (1987) proposed that the pattern of the accumulation of Pb in mollusks follows a power function ( $Y = aX^b$ , where X is the concentration of Pb in the organism, Y is the concentration of metals in the environment). Prakash et al. (1994) found that the digestive gland of bivalves contains the highest amounts of Pb, followed by the gill, mantle and shell, while Yap et al. (2003) postulated that Pb levels in the shells were always higher than those of the soft tissues. Of these different components, shell nacre exhibits a strong relationship with the ambient Pb level and can be applied to monitor the ambient Pb contamination (Bourgoin 1990; Pitts and Wallace, 1994; Prakash et al., 1994 Puente et al., 1996 Yap et al., 2003). In bivalve shells, the concentration of Pb in the organic matrix is similar to that of the carbonate crystal (Lingard et al., 1992). Pb concentration in bivalve shells is determined by physiological regulation (Almeida et al., 1998b; Gundacker, 1999 Putten et al., 1999; Huanxin et al., 2000; Putten et al., 2000), bioavailability and other environmental variations (Almeida et al., 1998b; Putten et al., 1999; Huanxin et al., 2000; Ruelas-Inzunza and Paez-Osuna, 2000; Richardson et al., 2001; Yap et al., 2003; Madkour, 2005; Pearce and Mann, 2006; Protasowicki et al., 2008), taxonomy (Ruelas-Inzunza and Paez-Osuna, 2000) and ontogenic age (Richardson et al., 2001). Pb in bivalve shells has been used to monitor the anthropogenic impact (Labonne et al., 1998; Gillikin et al., 2005b; Madkour, 2005).
- (g) **Boron** is concentrated in the inorganic portions of bivalve shells and enriched in aragonite relative to calcite by a factor of 1.5-2 (Furst et al., 1976). Apparently, environmental factors mainly control the B distribution in bivalve shells (Roopnarine et al., 1998). Especially, there is a correlation between B content in shells and salinity of the water in which the shells grew (Furst et al., 1976; Roopnarine et al., 1998). A correlation with temperature was not confirmed (Rucker and Valentine, 1961).
- (h) **Copper.** The concentrations of Cu in the bivalves follows the equation  $Y = aX^b$ , where X stands for the concentration of Cu in the organism and Y represents the concentration of Cu in the environment (Amiard et al., 1987). Cu is many times higher concentrated in soft tissues than in shells (Carriker et al., 1980;

Huanxin et al., 2000), but the Cu concentration of shells exhibits a significant relationship with ambient Cu levels and can therefore be applied monitor the Cu contamination of the environment (Carriker et al., 1980; Prakash et al., 1994; Richardson et al., 2001; Ravera et al., 2003; Madkour, 2005; Dunca et al., 2008). Cu concentration in bivalve shells is not correlated with shell growth (Frazier, 1975) temperature (Rucker and Valentine 1961) or salinity (Rucker and Valentine 1961), but shows a decreasing trend with increasing ontogenetic age (Fang and Shen 1984; Richardson et al., 2001; Ravera et al., 2003). In addition, physiology also effects the Cu distribution in bivalve shells (Amiard et al., 1987; Gundacker, 1999; Huanxin et al., 2000).

- (i) **Zinc.** The Zn concentration of shells is lower than that of the soft parts, but higher than the Cu level of the ambient water (Carriker et al., 1982; Prakash et al., 1994; Huanxin et al., 2000; Yap et al., 2003; Takesue et al., 2007). The content of Zn in bivalve shells reflect the compositon of the ambient water (Bertine and Goldberg, 1972; Carriker et al., 1980; Prakash et al., 1994; Puente et al., 1996; Klerks and Fraleigh, 1997; Richardson et al., 2001; Yap et al., 2003; Madkour, 2005; Pearce and Mann, 2006; Dunca et al., 2008; Protasowicki et al., 2008). Frazier (1975) showed that Zn is not correlated with shell growth. However, internal biological mechanisms determine the Zn level (Fang and Shen, 1984; Amiard et al., 1987; Gundacker, 1999; Huanxin et al., 2000). There is no consensus on the effect of ontogenetic age on Zn. Fang and Shen (1984), Richardson et al. (2001) and Hedouin et al. (2006) found that the amounts of Zn of bivalves decreases with age, while Carriker et al. (1982) proposed that the concentration of Zn increases slightly with age (oysters).
- (j) **Iron.** Fe is also more concentrated in soft tissues than in shells (Frazier, 1975; Carriker et al., 1980). Effects controlling the Fe distribution in bivalves are the ambient environment (Bertine and Goldberg, 1972; Carriker et al., 1980; Jimenez-Berrocoso et al., 2004; Madkour, 2005; Takesue et al., 2007), taxonomy (Pilkey and Goodell, 1963), growth rate (Harriss, 1965), ontogenetic age (Carriker et al., 1982; Fang and Shen, 1984; Ravera et al., 2003; Protasowicki et al., 2008), physiological mechanisms (Fang and Shen 1984) and diagenesis (Jimenez-Berrocoso et al., 2004). It is worth to point out that

there is no agreement on how exactly ontogenic age affects the Fe incorporation in bivalve shells.

- (k) **Chromium.** Cr content in bivalve shells is higher than in soft tissues, but significantly lower than in sediments (Huanxin et al., 2000; Giusti and Zhang, 2002; Hedouin et al., 2006). Cr concentration in shells may reflect the composition of the water in which they lived (Bertine and Goldberg, 1972; Protasowicki et al., 2008). Except for the environmental effects, the concentration of Cr in bivalve shells is also affected by physiology (Hedouin et al., 2006; Protasowicki et al., 2008).
- (l) **Silver.** The bioaccumulation of Ag is more pronounced in the shells of the bivalves than soft tissues (Giusti and Zhang, 2002). The Ag concentration of bivalve shells is reported to reflect Ag composition of the ambient water (Bertine and Goldberg, 1972).
- (m) **Aluminum.** Al levels, however, are concentrated in soft tissues of bivalves. Various authors found a strong relationship between the Al levels in shells and the environment (Prakash et al., 1994; Protasowicki et al., 2008). However, Ravera et al. (2003) proposed that the metal concentration in mussels does not reflect the metal concentrations of the water in which they lived.
- (n) **Cadmium.** Cd is more enriched in soft tissues of bivalves than in shells (Prakash et al., 1994). However, Huanxin et al. (2000) and Yap et al. (2003) found that Cd is concentrated in oyster shells and explained this finding by the easy substitution of Ca by Cd. In soft tissues, Cd concentration exhibits a correlation with Fe and Zn (Ruelas-Inzunza and Paez-Osuna, 2000). In bivalve shells, the concentration of Cd in the organic matrix is 6.5 times as high as that in the carbonate crystal (Lingard et al., 1992). The effects controlling the Cd distribution in bivalve shell are physiology (Prakash et al., 1994; Mubiana et al., 2005) and environmental variations (Carriker et al., 1980; Huanxin et al., 2000; Ruelas-Inzunza and Paez-Osuna, 2000; Yap et al., 2003; Lares et al., 2005; Madkour, 2005; Pearce and Mann, 2006; Dunca et al., 2008; Protasowicki et al., 2008). Therefore, Cd seems to be a good proxy of metal

contamination of the water (Prakash et al., 1994). Furthermore, Cd does not exhibit ontogenetic trends (Protasowicki et al., 2008).

- (o) **Cobalt.** Co is reportedly higher concentrated in shells than in soft parts (Hedouin et al., 2006). The Co concentration and shell size followed an inverse power function (Hedouin et al., 2006). In addition, the Co concentration is also determined by biological controls (Mubiana et al., 2005).
- (p) **Nickel.** Ni in bivalves is mainly concentrated in the (nacreous) shell layer, but not in soft tissues (Puente et al., 1996). The Ni concentration of bivalve shells clearly shows seasonal variations (Ruelas-Inzunza and Paez-Osuna, 2000). There is no clear relationship between Ni in the shells and in sediments (Madkour, 2005), but Ni contents of bivalves can be used as a biomonitor for metal pollution (Puente et al., 1996) and evaluation of the Ni bioavailability (Ruelas-Inzunza and Paez-Osuna, 2000; Protasowicki et al., 2008). According to experiments conducted by Klerks and Fraleigh (1997), the shells adsorb 'dissolved' Nickel, but also adsorb much particulate nickel.
- (q) **Vanadium.** Most of V is bound to the periostracum of the shell by the surface sorption. Only very little V is found in the bivalves (Miramand et al., 1980). V contents of bivalve shells show spatial and temporal changes, but no ontogenetic trends (Protasowicki et al., 2008)
- (r) **Rubidium.** Shell Rb concentrations of bivalves are good proxies of the Rb composition of the water (Bertine and Goldberg, 1972; Richardson et al., 2007). No apparent periodicity of Rb levels was observed in bivalve shells (Richardson et al., 2007).
- (s) **Mercury.** The Hg concentration in bivalve shells is controlled by physiological effects (Mubiana et al., 2005), but not by ontogeny (Protasowicki et al., 2008). Shell Hg levels seem to be a good monitor of environmental Hg contamination (Bertine and Goldberg, 1972; Mubiana et al., 2005).

- (t) **Selenium.** The concentration of Se was found strongly dependent on the ambient selenium concentration in sea water (Fowler and Benayoun, 1976). Se concentrations in the shells of bivalves may reflect the composition of the water in which they lived (Bertine and Goldberg, 1972).
- (u) **Sulfur.** S concentrations in calcitic bivalve shells are enriched near the margins of the calcite prisms and control the elongation of the crystals along with Mg (Rosenberg, et al., 2001). High S levels coincide with high levels of metabolic activity. The amount of S decreases with age (Fang and Shen, 1984; Rosenberg et al., 2001). S in aragonite bivalve shells can trace anthropological effects on the environment (Dunca et al., 2008).
- (v) **Fluorine.** High fluorine concentrations in mollusks shells were proposed to record the annual spawning period. Although the overall pattern of fluorine was quite different to that of strontium, the peaks of F coincided with those of Sr in the spawning line (Coote and Trompeter, 1993, 1995).

## 1.2 Species and study locality

### 1.2.1 *Arctica islandica*

*Arctica islandica* belongs to the family *Arctidae*, order *Veneroida*, class *Bivalvia*. It is a slow-growing (Thompson et al., 1980a; Kennish et al., 1994) and long-lived species (Thompson et al., 1980a; Schöne et al., 2005a; Wanamaker Jr et al., 2008), distributed commonly in much of the continental shelves of the North Atlantic (Dahlgren et al., 2000). *Arctica* lives shallow buried in sand and mud mixtures. It is a suspension feeder and feeds on phytoplankton. The favourable temperature of *Arctica* is from zero to 19°C. *Arctica* grows continuously throughout its life. The growth rate of *Arctica islandica* is related to age (relatively rapid growth for the first 15 years and slow-down thereafter), population density and geographical locality (including climatic and environmental conditions) (Witbaard & Duineveld, 1990; Witbaard et al., 1999). The predators of *Arctica* are the Atlantic Cod (*Gadus morhua*, Brey et al., 1990), the Atlantic wolffish (*Anarhichas lupus*, Hawkins & Angus, 1986) and seabirds.



*Arctica* is dioecious, i.e. females are larger than males. Age and size of maturity show a wide range and may be dependent on environmental conditions. On average, maturity is attained at the age of 9.38 years (Thompson et al., 1980b). The time of the spawning of *Arctica islandica* differs from south to north from May/June to August (Jones, 1980; Mann, 1982; 1985; Rowell et al., 1990; Fritz, 1991). During the main spawning phase, a dark band of the annual increments is secreted (Jones, 1980).

Taylor (1976) and Tschischka et al. (2000) proposed that the decrease of PO<sub>2</sub> in the mantle cavity water of the *Arctica islandica* causes a decrease of the heart rate and the closure of the shells. During the severe oxygen deficiency, *Arctica islandica* can burrow into the sediment and reduce its metabolism to less than 1% of aerobic rates, which is the lowest rate found in marine invertebrates so far (Oeschger, 1990). Abele (2002) showed that *A. islandica* reduces the heart rate to as little as 10% of routine activity during anaerobiosis. Also, under very severely conditions, *A. islandica* can create a self-induced anaerobiosis to reduce its glycogen consumption in order to withstand adverse environmental conditions. Oeschger and Storey (1993) showed that the metabolic activity of *A. islandica* when exposed to hydrogen sulphide is the same as that exposed to anoxia.

Thorarinsdottir & Johannesson (1996) and Thorarinsdottir & Einarsson (1996) made studies on *A. islandica* around Iceland (northwest area, north area, and east area). Results indicated that the largest shell length and the greatest relative meat weights for similar sized *A. islandica* specimens were found in the northwest of Iceland. This was attributed to optimal temperature and highest productivity in northwest. The youngest mature male individual in Icelandic waters was 10 years-old, while the youngest mature female was 13 years-old (Thorarinsdottir & Steingrímsson, 2000). Until an age of 23, 50% of *A. islandica* reached sexual maturity (Thorarinsdottir & Jacobson, 2005). Spawning activity appears to occur around the year around Iceland, but it is most intense from June to August (Thorarinsdottir, 2000).

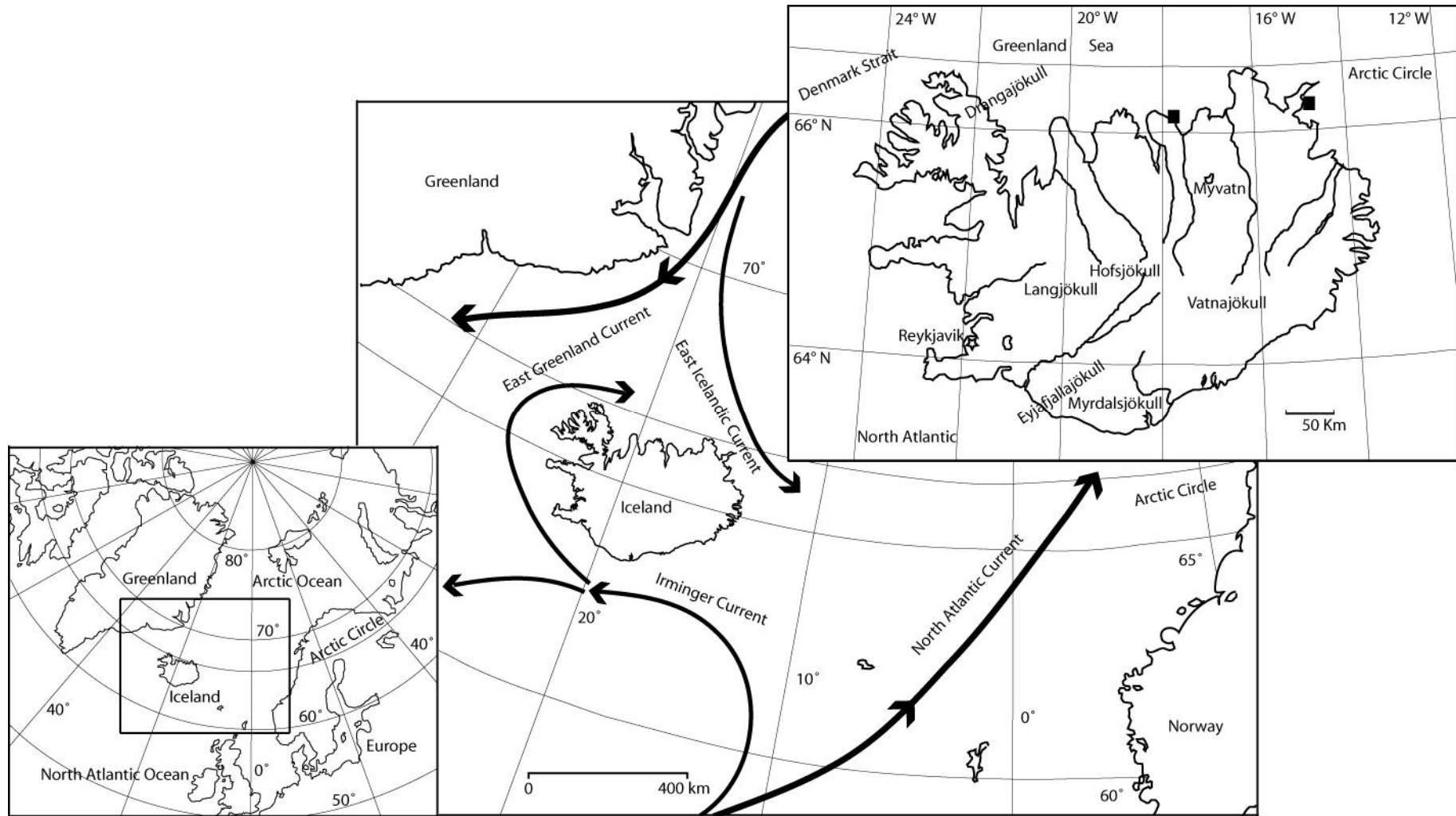


Figure 1.15 Surface current patterns around Iceland and map of Iceland (modified from Masse et al., 2008 and from [http://go.hrw.com/atlas/norm\\_hm/iceland.htm](http://go.hrw.com/atlas/norm_hm/iceland.htm)). Black rectangles indicate the places where specimens of this study were taken from, Langanes in NE Iceland and Flatey in the north. An additional specimen (museum specimen from Kiel University) was probably collected in the east of Iceland.

### ***1.2.2 Iceland***

Iceland is located south of the Arctic Circle between latitudes 63°23'N and 66°32'N and longitudes 13°30'W and 24° 32'W (see Fig. 1.1). Iceland consists of many mountains, deserts glaciers, lakes, rivers and waterfalls. The flora of the island is of arctic-alpine and boreal type (Ogilvie, 1981). The ecosystem of Iceland is very unstable, and the tolerance to changing environmental influences is very limited. Therefore climatic changes can have extreme effects and cause major environmental changes (Stötter & Wilhelm, 1994).

Iceland is situated near the border between warm and cold ocean currents (Fig. 1.1). The Irminger Current (one branch of the warm North Atlantic Drift) encircles the south, west and the north coast. The East Iceland Current (a branch of the cold East Greenland Current) flows in a southerly and southeasterly direction along the east coast. Iceland is just the place where these two currents meet. The weather and climate of most parts of Iceland are characterized by strong winds, frequent precipitation, mild winters and cool summers. Mean temperatures are typically close to 0°C in the winter and not far from 10°C in the summer (Olafsson et al., 2007). Cyclone occurs within the climatological Icelandic Low (IL) and shows a modest seasonal cycle with a winter maximum (Einarsson, 1984; Serreze et al., 1997). The regional patterns of cyclone activity and sea level pressure (SLP) are correlated to changes in high-latitude sea-ice conditions and surface temperatures. During the cold season (Oct-March), IL cyclone intensities are typical for oceanic systems and during the warm season for northern hemisphere values. A positive correlation was also found for the number of Icelandic cyclones with the NAO (North Atlantic Oscillation) index as well as the Icelandic surface pressure and wind speed. For precipitation, the correlation with the cyclones is higher than with the NAO index (Schneieereit et al., 2007).

The biomass of algae is different from site to site in Icelandic waters. A study on two intertidal ecosystems showed that the biomass of algae and the number of different algae species and macrofauna were higher in Sandgerdi near the southwest coast than in Grimsey Island in the north (Espinosa & Guerra-Garcia, 2005). The reason might be the reduction of temperature from south to north. Beare et al. (2000) conducted a survey on zooplankton in May and June during 1960-1996. Results showed that the proportion of specimens with an affinity to Atlantic-type water dropped dramatically over the Icelandic Shelf since 1960s, where the proportion of species with Arctic affinities increased markedly northeast of Iceland.

Due to the special position and unstable ecosystem, the Icelandic environment is very sensitive to react even to very minor changes. It is a very unique place to study the environment and climatic changes of Europe as a whole, and a very crucial place to investigate the global change of the past.

### **1.3 Structure of this thesis**

Four main issues will be addressed in the study: (1) organic-bound trace elements in shells of *Arctica islandica* shells and their effect on in-situ measurements such as LA-ICP-MS of shell carbonate, (2) biological effects controlling the shell elemental geochemistry during life, (3) early diagenetic effects and ionic exchanges between the ambient water and the shells as well as (4) a preliminary application of trace elements as environmental and climate proxies. Chapter 2 deals with the distribution of Mg and Sr in organic and carbonate components of the biomineral; Chapter 3 studies lower concentrated trace elements of *A. islandica* shells; Chapter 4 explores how vital effects influence the trace elemental distribution; Chapter 5 addresses the early diagenetic effects on shell trace elemental composition.

## **2 Effect of organic matrices on the determination of the trace element chemistry of *Arctica islandica* shells by ICP-OES and LA-ICP-MS**

This chapter mainly evaluates the effect of organic matrices of *Arctica islandica* shells on the analytical processes of Sr, Mg and Ca by the two most popular destructive analytical methods, Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) and Laser Ablation-Inductively Coupled Plasma – Mass Spectrometry (LA-ICP-MS). Conclusions are summarized afterwards which should be paid attention to before the applications of trace elemental proxies are carried out to explore the paleoenvironmental variations.

This chapter is currently in press: Schöne BR, Zhang Z, Jacob D, Gillikin DP, Tütken T, Garbe-Schönberg D, McConnaughey T and Soldati A 2009. Effect of organic matrices on the determination of the trace element chemistry (Mg, Sr, Mg/Ca, Sr/Ca) of aragonitic bivalve shells (*Arctica islandica*) – comparison of ICP-OES and LA-ICP-MS data. *Geochemical Journal* 43. – Contribution of author of this thesis: sample preparation, data analysis and co-interpretation.

### **2.1 Introduction**

Sr/Ca and Mg/Ca ratios of biogenic carbonates can provide quantifiable data on ambient water temperature during biomineralization. This has been empirically demonstrated for a variety of different organisms including echinoids (Pilkey and Hower, 1960), brachiopods (Lowenstam, 1961), bivalves (Dodd, 1965, 1967), corals (Smith, 1979; Beck et al., 1992, Mitsuguchi et al., 1996), foraminifera (Nürnberg et al., 1996) and ostracods (Corrége, 1993). These studies were also complemented by inorganic precipitation experiments (Kinsman and Holland, 1969; Mucci, 1987). As temperature increases, the Sr/Ca and Mg/Ca ratios of abiogenic aragonites decrease (Kinsman and Holland, 1969; Gaetani and Cohen, 2006), while those of abiogenic calcites increase (Katz, 1973; Mucci, 1987). In addition, strontium in calcite is strongly controlled by precipitation rate (Kinsman and Holland, 1969). The orthorhombic crystal structure of aragonite best accommodates the larger  $\text{Sr}^{2+}$  ion, while the rhombohedral crystal structure of calcite best accommodates the smaller  $\text{Mg}^{2+}$  ion. Therefore, aragonite often contains about 100 times more strontium than does calcite. Nonetheless, Sr/Ca

and Mg/Ca ratios in either polymorph of calcium carbonate can provide serviceable paleothermometers.

However, the Sr/Ca and Mg/Ca ratios of biological carbonates often depart from apparent thermodynamic equilibrium. Mg/Ca ratios of some calcitic (Dodd, 1965; Lorens and Bender, 1977; Freitas et al., 2005; Lorrain et al., 2005; Lazareth et al., 2007) and aragonitic bivalve shells (Takesue and van Geen, 2004) are lower than predicted by thermodynamics. Shell Mg/Ca and Sr/Ca ratios can also vary contradictorily among different species and even among conspecific and contemporaneous specimens from one locality (corals: Cardinal et al., 2001; brachiopods: England et al., 2007; bivalves: Dodd, 1965; Gillikin et al., 2005a; Lorrain et al., 2005; Freitas et al., 2008). Dodd (1965) also reported an inverse relation between temperature and Sr/Ca ratios in the nacreous (aragonitic) shell layer of *Mytilus edulis*, while Gillikin et al. (2005a) observed the opposite in the aragonitic shell of *Saxidomus gigantea*. The ratios of trace elements to calcium show little consistency in the long-lived bivalve mollusk, *Arctica islandica* (Toland et al., 2000). Skeletal Mg and Sr contents sometimes correlate with skeletal growth (corals: de Villiers et al., 1995; bivalves: Swan, 1956; Takesue and van Geen, 2004; Gillikin et al., 2005a; Lorrain et al., 2005), and also with ontogenetic age (Palacios et al., 1994; Freitas et al. 2005). Such findings suggest that non-thermodynamic factors (e.g., an active, protein-mediated removal of Mg from the inorganic carbonate phase during biomineralization) influence the incorporation of trace elements into biogenic carbonates.

It has also been suggested that organics may influence the skeletal metal content, but such relations have not been quantified (Allison, 1996; Nürnberg et al., 1996; Watanabe et al., 2001; Dauphin et al., 2003; Takesue and van Geen, 2004; Foster et al., 2008). Organics occur both within (intracrystalline; Pokroy et al., 2006; Jacob et al., 2008) and between (intercrystalline) CaCO<sub>3</sub> crystals. During shell formation, these organic components mediate biomineralization (Lowenstam, 1981; Veis, 2003). The main biopolymers of bivalve mollusks are water-insoluble structural proteins ( $\beta$ -chitin), water-soluble polyanionic proteins and silk-like proteins (Levi-Kalisman et al., 2001; Sudo et al., 2001). Some of these proteins can be enriched in certain trace elements, foremost magnesium (Cowan, 1991). Nürnberg et al. (1996) estimated that up to 5 % of Mg in bulk foraminiferan tests may be associated with proteins rather than with calcite. Likewise, Watanabe et al. (2001) showed that up to 40 % of the magnesium in coral aragonite is not lattice-bound. Trace elements released from organic

components during sample preparation or measurement may then increase estimates of Mg concentrations and Mg/Ca ratios of biominerals. The metal contents of bivalve organs and tissues have been examined (e.g., Bustamante and Miramand, 2004), but no study has yet quantified the Mg and Sr content of isolated shell organic matrices and CaCO<sub>3</sub> phases.

Analytical techniques such as laser ablation – inductively coupled plasma – mass spectrometry (LA-ICP-MS) and ion microprobe are now widely used to study the skeletal composition in situ. LA-ICP-MS especially offers undeniable advantages with respect to spatial sampling resolution and sample throughput. But combining results for CaCO<sub>3</sub> crystals and organic matrices potentially complicates interpretations (Watanabe et al., 2001). Only a few studies have compared results from LA-ICP-MS with wet chemical techniques that separate the organic and inorganic phases (Rosenheim et al., 2005; Gillikin et al., 2005a).

This chapter quantifies the organic matrix and CaCO<sub>3</sub> fractions in shells of *A. islandica* and presents the Mg, Sr and Ca concentrations and Mg/Ca and Sr/Ca ratios for both phases, using wet chemical techniques combined with inductively coupled plasma – optical emission spectrometry (ICP-OES). It compares these results with LA-ICP-MS analyses. The benefits of special pretreatment methods and mathematical data corrections are then addressed. The results improve our understanding of trace element chemistry of biogenic skeletons, and can pave the road toward better climate and environmental proxies.

## **2.2 Material and methods**

Eight specimens of *Arctica islandica* were used in the present study (Table 2.1). The bivalves were collected alive by dredging from ca. 25 to 55 m of water depth northwest of Iceland and from the North Sea (Table 2.1). Soft tissues were removed, and ligament and periostracum were physically abraded. The outer ca. 1000 µm of each valve were also mechanically removed to eliminate adhering sediment and shell materials possibly altered by early diagenesis. The valves were then ultrasonically rinsed with de-ionized water.

### ***2.2.1 Sample preparation for chemical analyses of different shell components***

Five specimens were chosen (ICE06-A1 to A5; Table 2.1) for the analysis of Mg, Sr and Ca contents of the different shell components, i.e. the insoluble organic matrix (IOM), the soluble organics (SOM = sugars, proteins etc.) and the inorganic calcium carbonate component

(CaCO<sub>3</sub>). The shells were ultrasonically rinsed multiple times in millipore (18.2 MΩ) water, dried and weighed. Both valves of specimens ICE06-A1 and ICE06-A2 and one valve of each of the remaining specimens (whole, uncrushed valves) were gently dissolved in 5 % ultrapure HNO<sub>3</sub> over several days before centrifuging the solution at 3700 RPM for 30 min. We used a diluted acid to ensure that water-insoluble organics (IOM) were not dissolved and could be analyzed separately. Then, the IOM of each solution was extracted and rinsed multiple times in millipore water, air-dried and weighed. The IOM of one valve of specimens ICE06-A1 and ICE06-A2 were mounted on glass slides and their chemical composition determined by means of LA-ICP-MS (Table 2.1). The other IOM samples were completely digested in a 1:1 mixture of 30 vol% ultrapure H<sub>2</sub>O<sub>2</sub> plus 65 vol% ultrapure HNO<sub>3</sub> at 90°C (Bellotto and Miekeley, 2007).

After extraction of the IOM, the remaining fluid consisted of SOM and dissolved CaCO<sub>3</sub>. For two specimens (Table 2.1), the SOM with molecular sizes larger than 3kDa (SOMlp) was separated from SOM with proteins smaller than 3kDa (SOMsp) plus dissolved CaCO<sub>3</sub> by ultrafiltration. To achieve this, the fluids were centrifuged at 3,700 RPM for more than 90 min in Vivispin filters (Sartorius, 20 ml). Additionally, the solutions containing SOMlp were also rinsed in millipore water. In order to prevent precipitation of trace elements, we added 1 ml 65 % ultrapure HNO<sub>3</sub> to each of the dissolved shell components (IOM, SOM+CaCO<sub>3</sub>, SOMlp, SOMsp+CaCO<sub>3</sub>). All wet fractions were weighed and then analyzed in a Spectro Ciros Vision ICP-OES at the University of Mainz.

### ***2.2.2 Preparation of shell cross-sections for analyses of crystal fabric and chemistry***

One valve of each of the remaining three specimens was mounted on a plexiglass cube and a quick-drying epoxy resin applied to the outer and inner valve surface along the axis of maximum growth (Table 2.1). On that axis, one or two ca. three-millimeter-thick sections were cut from each valve with a Buehler low-speed saw. The cross-sectioned slabs were mounted on glass slides, ground on glass plates with 800 and 1200 grit powder and finally polished on a Buehler G-cloth with 1 μm Al<sub>2</sub>O<sub>3</sub> powder. Prior to the analyses, all samples were ultrasonically rinsed in millipore water.



Shell ID	Locality	Cross-sectioned slabs	LA-ICP-MS		ICP-OES (triplicate analyses)					SEM	Biochemical staining
			Whole biomineral	IOM	Whole biomineral	IOM	CaCO <sub>3</sub> +SOM	CaCO <sub>3</sub> +SOM <sub>sp</sub>	SOM <sub>lp</sub>		
DBG13.2-A1	North Sea	2	9							X	
WH241-597-A1	North Sea	1	52								X
HM-Fla86-A1	Iceland	2	319		19						
ICE06-A1	Iceland			3		1	1	1	1		
ICE06-A2	Iceland			2		1	1	1	1		
ICE06-A3	Iceland					1	1				
ICE06-A4	Iceland					1	1				
ICE06-A5	Iceland					1	1				

Table 2.1. List of *Arctica islandica* specimens used in the present study. IOM = water-insoluble organic matrix, SOM = water-soluble organic matrix, SOM<sub>sp</sub> = molecular sizes smaller than 3kDa, SOM<sub>lp</sub> = molecular sizes larger than 3kDa.

### ***2.2.3 Chemical analyses of shell cross-sections (LA-ICP-MS and ICP-OES)***

One polished cross-section of each of the three specimens HM-Fla86-A1, WH241-597-A1R and DBG13.2-A1 was analyzed by LA-ICP-MS (Table 2.1). Laser ablation spots in the outer shell layer measured 50  $\mu\text{m}$  in diameter in specimens HM-Fla86-A1 and DBG13.2-A1, and 100  $\mu\text{m}$  in WH241-597-A1R. Specimens DBG13.2-A1 and WH241-597-A1R were used to study the intra-annual (seasonal) variations of trace elements. Therefore, only the youth portions of these shells were sampled, i.e. the shell portions that formed when the bivalves grew at the fastest rates. Each laser spot represented a shell portion that formed within days or a few weeks. In order to analyze the average trace element content, however, a shell portion near the ventral margin of specimen HM-Fla86-A1 was analyzed. The centers of individual laser spots were only approx. 3.45  $\mu\text{m}$  apart from each other and formed a line of measurements (Fig. 2.4). The lifespan of this bivalve exceeded 200 years, and the average annual increment width at the ventral margin was narrower than the diameter of a laser spot. Thus, each LA-ICP-MS sample from this specimen represented at least one year.

From the other polished slab of specimen HM-Fla86-A1, aragonite powder was milled under a binocular microscope from the same shell portion that was analyzed by LA-ICP-MS (Table 2.1). We employed a cylindrical diamond drill bit (1 mm diameter, Komet/Gebr. Brasseler GmbH & Co. KG model no. 835 104 010) mounted on a Rexim Minimo drill. Spatial milling resolution parallel to the growth lines was about 55  $\mu\text{m}$ . Each sample swath (55  $\mu\text{m}$  x 1.8 mm) stretched from near the outer shell surface and toward the inside of the shell (approximately 2.8 mm away from the original outer shell surface; it should be noted that the LA-ICP-MS samples were located approx. 1.5 to 2 mm away from the original outer shell surface, i.e. ca. in the middle of the OES-sample swath; Fig. 2.4). Each of the shell powder samples weighed ca. 150 to 400  $\mu\text{g}$ . Powder samples were completely dissolved in 1 ml 70 vol% ultrapure  $\text{HNO}_3$  and visually confirmed to be without residue before dilution in 4 ml millipore water. These samples were analyzed in a Spectro CIROS<sup>CCD</sup> SOP ICP-OES at the University of Kiel.

### ***2.2.4 Biochemical staining and analysis of the shell crystal fabric***

In order to identify the distribution of organic components of the shell and study the crystal fabric (Fig. 2.3), the remaining polished slab of specimen DBG13.1-A2 and the cross-section of WH241-597-A1R that was previously used for LA-ICP-MS were immersed in Mutvei's solution for 25 and 2 min, respectively, at 37-40  $^\circ\text{C}$  under constant stirring (Schöne et al.,

2005b). Immediately afterward, the etched sections were rinsed in de-ionized water and allowed to air-dry. Mutvei's solution consists of 0.5 vol% acetic acid, 12.5 vol% glutardialdehyde and ca. 5 g Alcian Blue per liter solution. The acid gently etches the carbonate portions, while the glutardialdehyde preserves the organic matrix in three dimensions. Simultaneously, the Alcian Blue stains organic components deeply blue. Mutvei's solution is adequate for resolving shell internal growth structures. For scanning electron microscopy (SEM; Hitachi S 4300) the etched shell section of DBG13.1-A2 was sputter-coated with a 30 Å gold layer.

### ***2.2.5 LA-ICP-MS analyses***

Solid shell material (Figs. 2.1, 2 and 4; Table 2.2) and IOM (Table 2.2) were analyzed for Mg, by measurement of the isotope  $^{24}\text{Mg}$ , and Sr as  $^{88}\text{Sr}$  by LA-ICP-MS at the University of Mainz. Ablation was achieved with a New Wave Research UP-213 Nd:YAG laser ablation system (New Wave Research), using a pulse rate of 4-10 Hz, a pulse energy of ~0.3 mJ per spot, and 50 and 100  $\mu\text{m}$  spot diameters with Ar (or a He/Ar mixture) as ablation gas. Analyses were performed on an Agilent 7500ce ICP-MS coupled to the UP-213 platform (one point per peak and 10 ms dwell time) following methods described in Jacob (2006). SRM NIST SRM 612 was used as the external standard (Pearce et al., 1997), and the U.S. Geological Survey glass standard BCR-2G was measured to monitor accuracy and instrumental drift.  $^{43}\text{Ca}$  was used as the internal standard with Ca concentrations measured by ICP-OES. Detection limits generally range between 0.001 and 0.5 ppm for these elements. Relative standard deviations (based on repeated measurements of the external standard) were 7.2 % for Mg and 7.8 % for Sr.

	Whole biomineral	<i>Calculated whole biomineral</i>	IOM	CaCO <sub>3</sub> +SOM	<i>Calculated CaCO<sub>3</sub>+SOM</i>	CaCO <sub>3</sub> +SOM <sub>sp</sub>	SOM <sub>ip</sub>
Rel. abundance (wt%) →	100	100	0.46	99.54	99.54	91.95	7.59
Shell ID	HM-Fla86-A1	<i>ICE06-A1...5</i>	ICE06-A1...5	ICE06-A1...5	<i>ICE06-A1+2</i>	ICE06-A1+2	ICE06-A1+2
N	19	5	5	5	2	2	2
Mg (ppm)	99 ± 25	68 ± 19	130 ± 152	68 ± 19	77 ± 20	78 ± 19	63 ± 37
ICP-OES Sr (ppm)	860 ± 128	1,060 ± 170	10 ± 8	1,064 ± 171	820 ± 373	799 ± 435	1,051 ± 370
Ca (%)	35.72 ± 3.47	35.88 ± 2.82	0.22 ± 0.15	36.02 ± 2.82	36.51 ± 1.98	37.01 ± 2.91	30.18 ± 9.64
Mg/Ca (mmol/mol)	0.46 ± 0.10	0.31 ± 0.09	100.42 ± 67.96	0.31 ± 0.09	0.35 ± 0.11	0.35 ± 0.11	0.34 ± 0.10
Sr/Ca (mmol/mol)	1.10 ± 0.07	1.35 ± 0.22	1.99 ± 0.47	1.35 ± 0.22	1.03 ± 0.41	0.99 ± 0.46	1.59 ± 0.05
Shell ID	HM-Fla86-A1		ICE06-A1+2	<i>ICE06-A1+2</i>			
N	319		2	2			
Mg (ppm)	106 ± 26		134 ± 121	21,757 ± 19,669			
LA-ICP-MS Sr (ppm)	864 ± 59		7 ± 1	1,137 ± 133			
Ca (%)	35.72		0.22	35.72			
Mg/Ca (mmol/mol)	0.47 ± 0.11		100.52 ± 90.63	100.44 ± 90.80			
Sr/Ca (mmol/mol)	1.07 ± 0.06		1.50 ± 0.20	1.46 ± 0.17			

Table 2.2. Results of chemical analyses of different shell components by means of LA-ICP-MS and ICP-OES. The chemistry and trace element to calcium ratios of the IOM deviated significantly from that of the other shell components such as CaCO<sub>3</sub> and SOM (last four columns from left). Results on IOM chemistry revealed by ICP-OES and LA-ICP-MS were statistically invariant (Wilcoxon t-statistics: p>0.05) if a Ca value of 0.22 wt% (given by ICP-OES) was assumed for the internal standard for LA-ICP-MS. However, if it remained unperceived that the laser coincidentally hit a shell portion with pure IOM, LA-ICP-MS would return unrealistically high Mg and Sr values. For meaning of abbreviations see captions of Table 2.1. Errors are given as standard deviation (1σ).

### **2.2.6 ICP-OES analyses**

Dissolved shell samples of five specimens (ICE06-A1 to A5; Table 2.2) were analyzed with a Spectro CIROS Vision ICP-OES system at the University of Mainz and sample HM-Fla86-A1 with a Spectro CIROS<sup>CCD</sup> SOP ICP-OES at the University of Kiel (Table 2.2). We followed the techniques described by Schrag (1999) and de Villiers et al. (2002). Relative standard deviations (triplicate measurements of each sample) were 0.99 % for Ca, 1.39 % for Mg, and 1.34 % for Sr; accuracy for these elements was better than 0.5 %. Mixed standard solutions were prepared from single element standards of Mg, Sr and Ca in proportions to reflect those observed in bivalve shells and organic components.

## **2.3 Results**

According to our findings, shells of *Arctica islandica* consist, on average, of 99.54 wt% calcium carbonate (CaCO<sub>3</sub>) and water-soluble organic matrix (SOM), and  $0.46 \pm 0.19$  wt% water-insoluble organic matrices (IOM) (Table 2.2). In what follows, CaCO<sub>3</sub>+SOM+IOM are referred to as ‘whole biomineral’.

### **2.3.1 Distribution of organics across the shell of *Arctica islandica***

As shown by the sample immersed in Mutvei’s solution (Fig. 2.1), growth lines were deeply stained by Alcian Blue while the portions between major growth lines were mottled in lighter blue colors. Notably, craters produced by laser ablation and the immediate adjacency of these spots were not stained (Fig. 2.1). SEM images of the etched outer shell layer of *A. islandica* revealed a cross-acicular crystal fabric between major growth lines (Fig. 2.3). Toward the annual growth lines, however, the size of these crystals gradually decreased and the amount of insoluble organics increased (Fig. 2.3). Even smaller, more etch-resistant, tightly packed crystals (irregular simple prisms) were observed at the growth lines (Fig. 2.3).

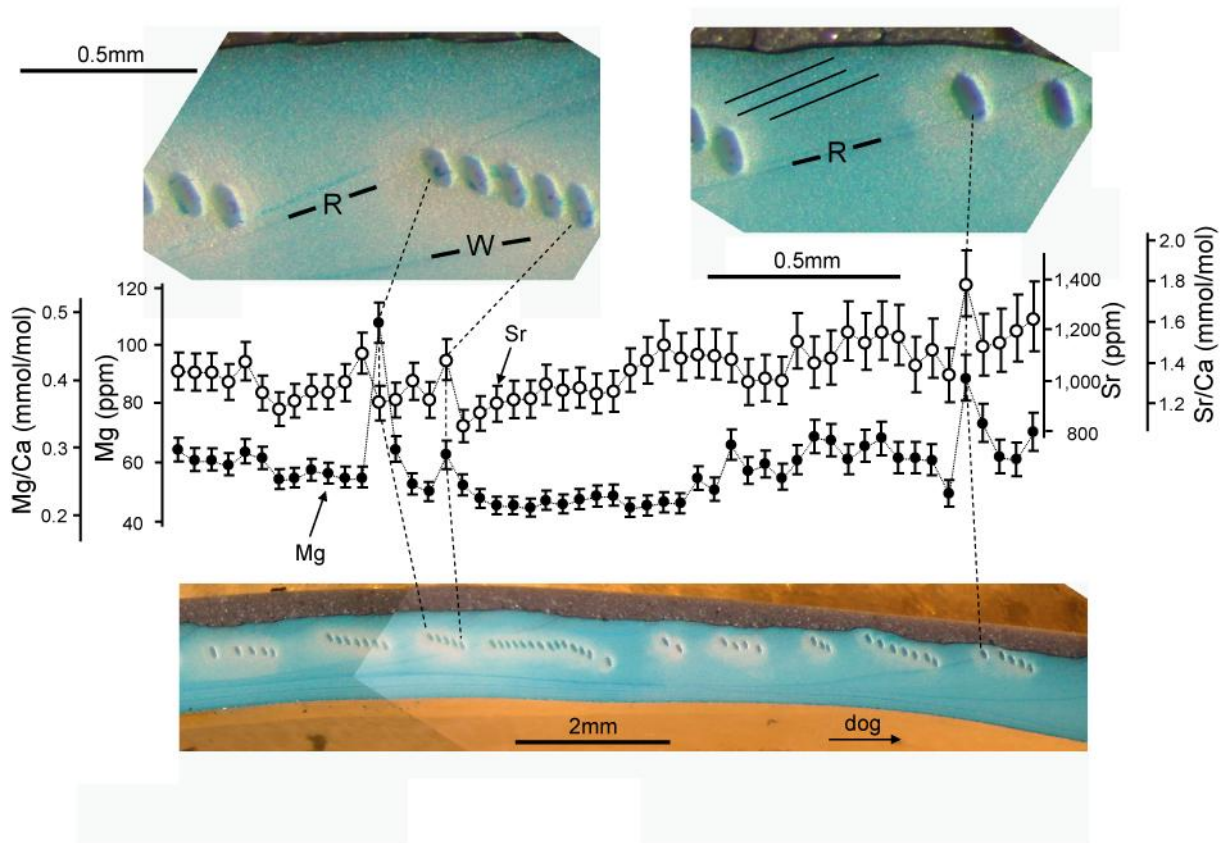


Figure 2.1 Seasonal strontium and magnesium concentration and Mg/Ca and Sr/Ca ratios in cross-sectioned shell slab of *Arctica islandica* (specimen WH241-597-A1; Table 2.1). Data were obtained by LA-ICP-MS. Spots measured 100 $\mu$ m (long axis) in the direction of growth. Strongly enriched Mg content was observed at reproduction (“R”) lines that formed during warm summer temperatures. These Mg peaks stood out significantly from values measured between consecutive reproduction lines. A slightly higher Mg value also occurred at the “W” line that formed during January/February. The Mg curve had a saw-toothed appearance, whilst the Sr values formed a relatively smooth curve with far less outstanding peaks at “R” and “W” lines. After sampling, the shells were immersed in Mutvei’s solution that stained portions with a higher organic content deep blue and those with less organics light blue. Note de-colored craters around laser ablation spots. dog = direction of growth. Error bars in standard deviation units ( $1\sigma$ ).

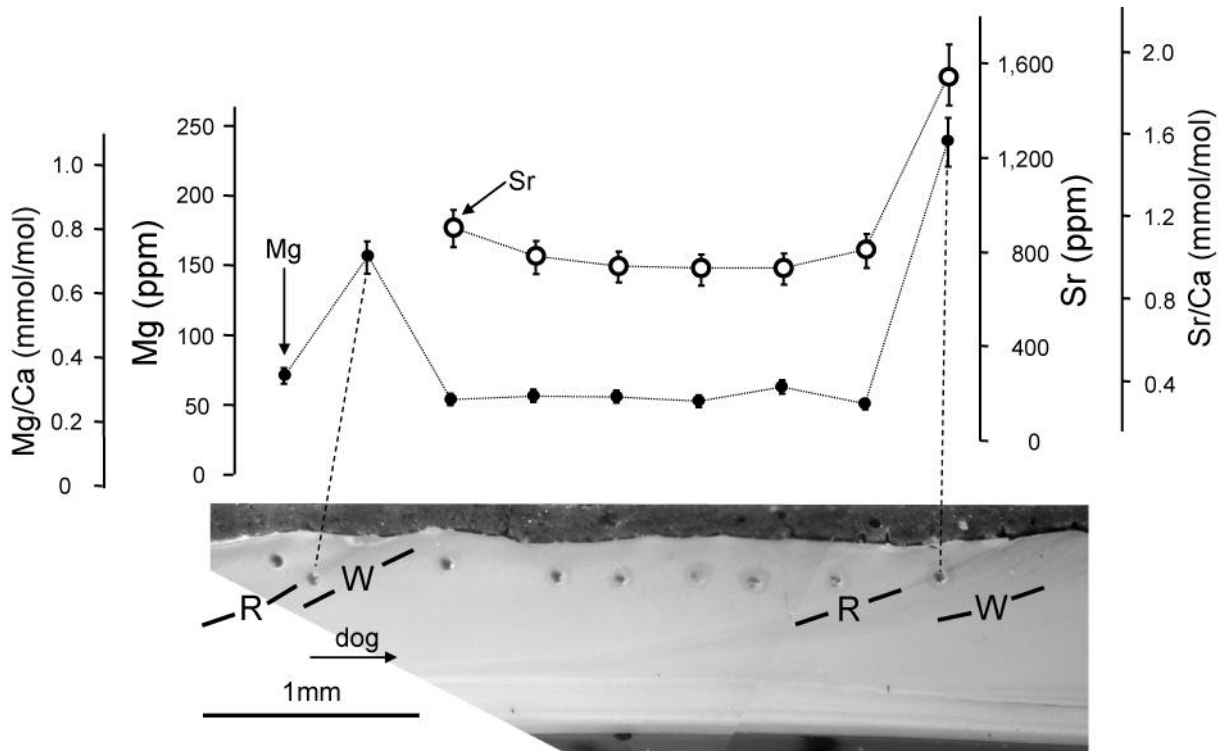


Figure 2.2. Seasonal strontium and magnesium concentration and Mg/Ca and Sr/Ca ratios in cross-sectioned shell slab of *Arctica islandica* (specimen DBG1.2-A1; Table 2.1). See description in caption of Fig.2.1. dog = direction of growth. Error bars in standard deviation units ( $1\sigma$ ). LA spot size is 50 $\mu$ m in diameter.

### 2.3.2 Small-scale (LA-ICP-MS) element variation across the shell of *Arctica islandica*

Magnesium and strontium concentrations in the outer layer of *A. islandica* shells determined by LA-ICP-MS exhibited seasonal oscillations with sharp Mg excursions (Figs. 2.1 and 2). Highest magnesium values of 106 and 238 ppm ( $\mu$ g g<sup>-1</sup>) and strontium values of 1387 and 1466 ppm (Figs. 2.1 and 2) were observed near the major growth lines (“R”) of two different specimens. These values corresponded to Mg/Ca ratios of 0.49 and 1.10 mmol/mol and Sr/Ca ratios of 1.78 and 1.88 mmol/mol. Major growth lines (= reproduction lines; Jones, 1980) form about one month after the hottest part of summer (Fig. 2.1; Schöne et al., 2005b). A second magnesium peak occurred at growth lines denoted as “W” for winter (Fig. 2.1). These lines were laid down during ca. January/February (Schöne et al., 2005d). Lowest magnesium (45 and 55 ppm = Mg/Ca of 0.21 and 0.26 mmol/mol) and strontium (845 and 740 ppm = Sr/Ca of 1.08 and 0.95 mmol/mol; Figs.2.1 and 2) levels, however, occurred in shell portions that formed during the coldest season of the year, i.e. shortly after the “W” lines. Whereas strontium data formed relatively smooth curves, the magnesium curves demonstrated a saw-toothed appearance with two to three times higher values in samples that comprise major growth lines than samples from shell portions between two consecutive lines (Figs. 2.1 and 2).

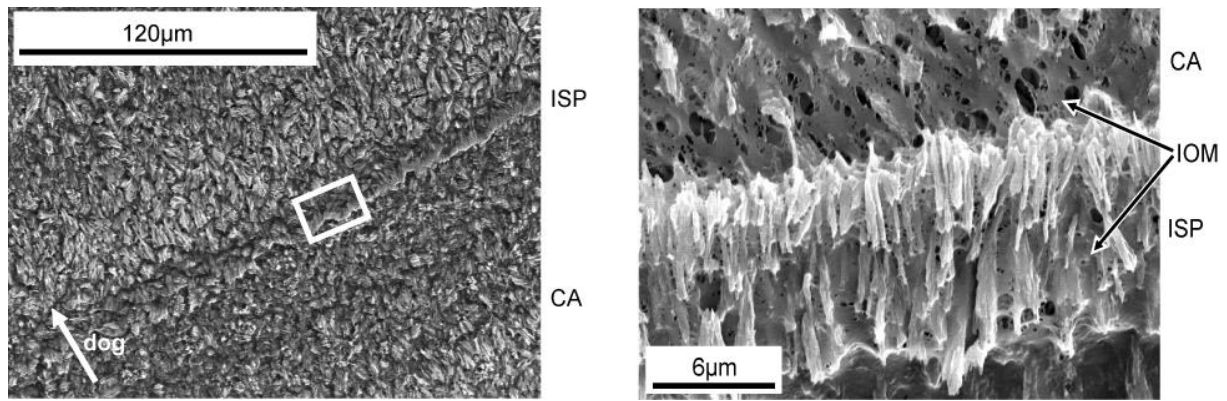


Figure 2.3 Seasonal strontium and magnesium concentration and Mg/Ca and Sr/Ca ratios in cross-sectioned shell slab of *Arctica islandica* (specimen DBG1.2-A1; Table 2.1). See description in caption of Fig. 1. dog = direction of growth. Error bars in standard deviation units ( $1\sigma$ ). LA spot size is 50µm in diameter.

### 2.3.3 Element analyses of *Arctica islandica* shell by LA-ICP-MS and ICP-OES

Contemporaneously deposited shell portions analyzed with different analytical techniques revealed statistically indistinguishable results for Mg and Sr as well as Mg/Ca and Sr/Ca ratios (Wilcoxon t-statistics:  $p > 0.05$ ) (Table 2.2; Fig. 2.4). According to ICP-OES analysis, the average Ca concentration in these shell portions was 35.72 wt%. This value was used as the internal standard for LA-ICP-MS.

### 2.3.4 Compound-specific chemical analyses by ICP-OES and LA-ICP-MS

Average ICP-OES-derived magnesium concentrations of the IOM (130 ppm; specimens ICE06-A1 to A5) can be up to two times as high as the whole biomineral or the soluble components (68 ppm; Table 2.2). However, strontium and calcium contents were much lower in the IOM compared to the biomineral (10 ppm and 0.22 wt%, respectively; Table 2.2). No statistical difference was found between Mg and Sr values or the Mg/Ca and Sr/Ca ratios of the IOM determined by ICP-OES and LA-ICP-MS if a Ca value of 0.22 wt% (= the Ca content in the IOM measured with ICP-OES) was used as the internal standard (Table 2.2).



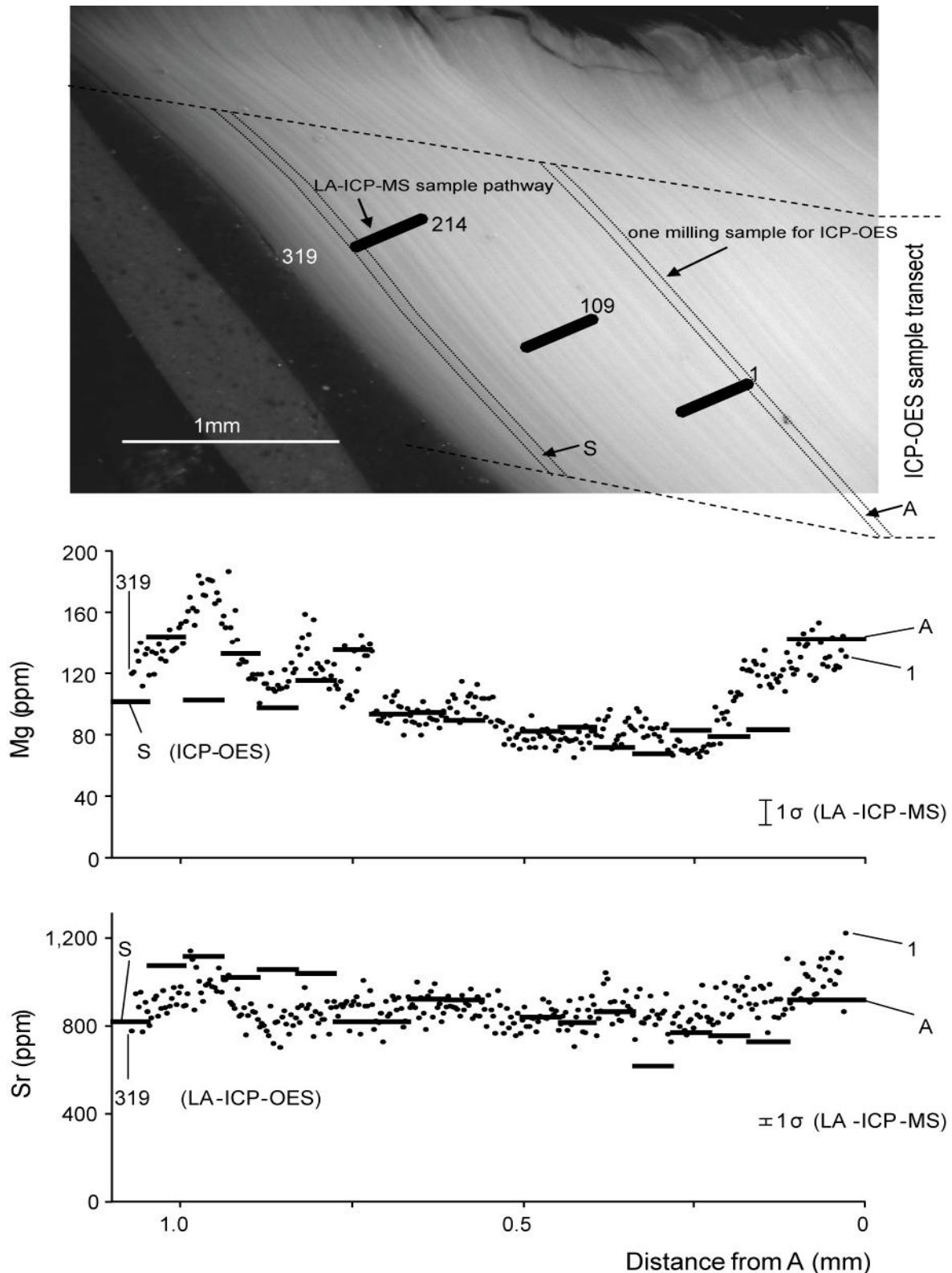


Figure 2.4 Shell portion near the commissure of a more than 200 year-old specimen of *Arctica islandica* (specimen HM-Fla86-A1; Table 2.2) sampled by ICP-OES and LA-ICP-MS. Distinct lines are annual growth lines. LA spot size is 50 $\mu$ m in diameter. Distance between LA spot centers was 3.45 $\mu$ m. Mg and Sr concentrations measured by both analytical techniques were statistically indistinguishable (Wilcoxon t-statistics:  $p > 0.05$ ). Error bars in standard deviation units ( $1\sigma$ ). Error bars for ICP-OES results are smaller than size of labels.

Separation of soluble organics with large (SOM<sub>lp</sub>) and small proteins plus CaCO<sub>3</sub> (CaCO<sub>3</sub>+SOM<sub>sp</sub>) was not successful (Table 2.2). As demonstrated by a Ca concentration of 30.18 wt%, the SOM<sub>lp</sub> component still contained large amounts of dissolved CaCO<sub>3</sub>. Overall, water-soluble organics (SOM<sub>lp</sub> and CaCO<sub>3</sub>+SOM<sub>sp</sub>) were largely within the same range of magnesium and strontium concentrations as the whole biomineral (Table 2.2).

### 2.3.5 Soluble organics: relative abundance and Ca concentration

The relative abundance of SOM in shells of *A. islandica* can be computed as follows. According to ICP-OES, the whole biomineral contained 35.72 wt% Ca (Table 2.2). This value represents the sum of the relative abundances of the three main components of the shell, CaCO<sub>3</sub>, IOM and SOM, and their respective Ca contents.

$$(1) \quad 35.72 \text{ wt\%} = \frac{a}{100} \cdot A + \frac{b}{100} \cdot B + \frac{c}{100} \cdot C,$$

where a, b and c are the relative abundance in wt% of the IOM, CaCO<sub>3</sub> and SOM, respectively, and a = 0.46 wt%, and c = 100 wt%-a-b. A, B and C denote the Ca content (wt%) of the IOM, CaCO<sub>3</sub> and SOM, respectively, and A=0.22 wt% Ca (given by ICP-OES) and B=40.04 wt% Ca (stoichiometrical value for abiogenic CaCO<sub>3</sub>). We can rewrite equation 1 as follows:

(2)

$$35.72 \text{ wt\% Ca} = \frac{0.46 \text{ wt\%}}{100} \cdot 0.22 \text{ wt\% Ca} + \frac{b}{100} \cdot 40.04 \text{ wt\% Ca} + \frac{100 \text{ wt\%} - 0.46 \text{ wt\%} - b}{100} \cdot C$$

and solve equation 2 for C

$$(3) \quad C = \frac{35.72 \text{ wt\% Ca} - 0.0046 \text{ wt\%} \cdot 0.22 \text{ wt\% Ca} - \frac{b}{100} \cdot 40.04 \text{ wt\% Ca}}{\frac{100 \text{ wt\%} - 0.46 \text{ wt\%} - b}{100}}$$

According to the graphic representation of equation 3 (Fig. 2.5), the minimum SOM (c) and maximum possible CaCO<sub>3</sub> content (b) of the whole biomineral is 10.33 wt% and 89.21 wt%, respectively. At this value, the SOM contains no Ca (C=0 wt% Ca). With increasing Ca concentration in the SOM, the relative abundance of SOM in the biomineral increases and, correspondingly, the CaCO<sub>3</sub> content of the mixture decreases. The maximum possible Ca

level of the SOM (C=35.88 wt% Ca) is attained when the entire hypothetical ‘biomineral’ consists only of IOM and SOM (c=99.54 wt%).

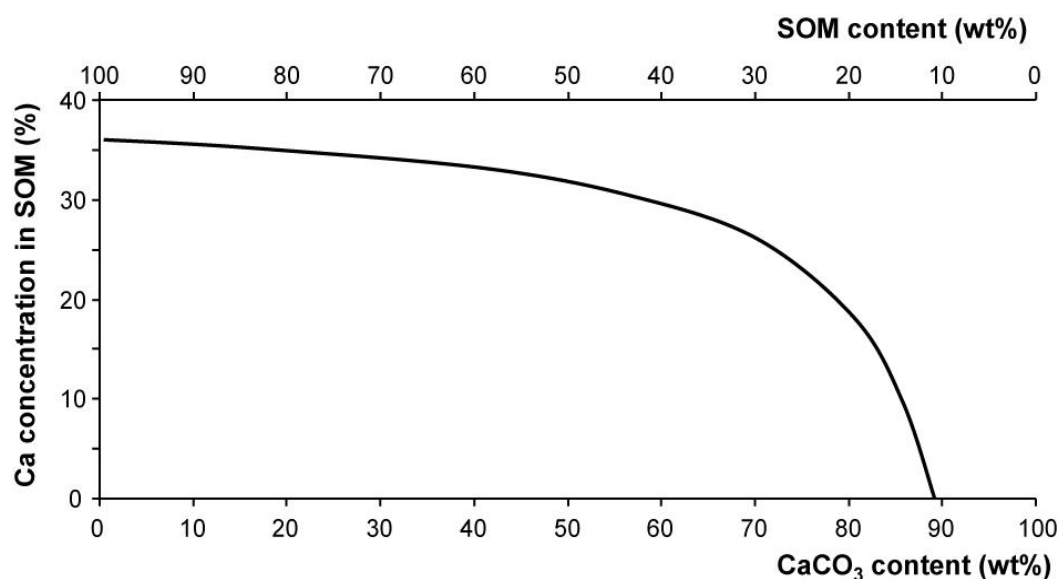


Figure 2.5 Model to estimate the relative abundance of soluble organics in shells of *Arctica islandica*. The Ca content of the whole biomineral was determined by ICP-OES and equals 35.72 wt% (Table 2). This value limits the pure CaCO<sub>3</sub> content to 89.21 wt%. Accordingly, the minimum amount of soluble organics equals 10.33 wt% (100 wt% - 0.46 wt% IOM - 89.21 wt% CaCO<sub>3</sub>). In this case, the SOM would contain no Ca. With increasing amounts of Ca in the SOM, the relative abundance of the SOM in the biomineral increases and the CaCO<sub>3</sub> content decreases. The hypothetical extreme is where the ‘biomineral’ is CaCO<sub>3</sub>-free and consists only of organics, i.e. 0.46 wt% IOM and 99.54 wt% SOM.

## 2.4 Discussion

### 2.4.1 Chemical composition of the insoluble organic matrix

The results of this study demonstrate that considerable amounts of magnesium in shells of *Arctica islandica* are organic-bound (IOM) rather than crystal-bound. In addition, the IOM has a different elemental composition than the inorganic carbonate phase plus soluble organics. On average, the insoluble organic matrix can contain nearly twice as much Mg as the whole biomineral or the soluble components (CaCO<sub>3</sub>+SOM), but ca. 99 % less strontium and calcium (Table 2.2). Likewise, the Mg/Ca ratios of the IOM can be up to 200 times higher than the whole biomineral, and the Sr/Ca ratios two times higher (Table 2.2).

Similar findings have been reported for corals (e.g., Amiel et al., 1973; Sinclair et al., 1998; Fallon et al., 1999), foraminifera (Nürnberg et al., 1996), and most recently the bivalve

mollusk *Corbula amurensis* (Takesue et al., 2008). For example, Allison (1996) observed significantly larger amounts of magnesium in organic-rich portions of coral skeletons and suggested that these metals are complexed by proteins (Mitterer, 1978). Watanabe et al. (2001) conducted several different pretreatment procedures to remove the organic components and adhering metals of coral skeletons. They concluded that 40 % of the total skeletal magnesium is absorbed by organic components or crystal surfaces. The amount of non-lattice-bound magnesium in coral skeletons reported by Watanabe et al. (2001) compares well to our findings.

Comparable studies on bivalve shells are still scarce. However, Takesue and van Geen (2004) found lower Mg levels in subfossil *Protothaca staminea* shells than in modern samples of the same species and concluded that the Mg-rich organic matrix had degraded during diagenesis (Curtis and Krinsley, 1965; Brand and Morrison, 1987). Similarly, Gillikin et al. (2005b) noted lower Mg/Ca ratios in subfossil *Mercenaria* spp. analyzed with LA-ICP-MS. It is noteworthy, that none of the existing studies differentiated between insoluble and soluble organics.

Most recently, Foster et al. (2008) analyzed shells of *Arctica islandica* by means of Synchrotron X-ray Absorption Near Edge Spectroscopy (XANES) and concluded that Mg is not substituted into the aragonite of *A. islandica*, but is exclusively hosted by a disordered phase such as organic components or nanoparticles. According to these authors, Mg does not appear to be a useful paleoenvironmental proxy at all. However, Mg contents in shells of *A. islandica* approach those of current synchrotron methods. Most Mg levels of shells analyzed in the present study remained between 68 and 99 ppm. Therefore, the conclusions drawn by Foster et al. (2008) seem debatable and require further study.

The affinity of Mg for the IOM may result from the existence of organic molecules (metalloproteins, metal complexes etc.) with magnesium-binding capacities (Gómez-Ariza et al., 2004; Gotliv et al., 2005). Actually, Mg is one of the most abundant cofactors of metalloproteins (Dudev and Lim, 2001) and occurs at negatively charged sites of aspartic and glutamic-rich polypeptides (Dudev et al., 1999). These two amino acids belong to the most common constituents of glycoproteins of the molluscan extrapallial fluid (EPF) and play an active role in promoting and modulating shell mineral growth (Sikes et al., 1998; Gotliv et al., 2003). Later during biomineralization, these acidic macromolecules mainly become part of

the intracrystalline matrix (Addadi et al., 1991) while other framework building insoluble components are mainly preserved as intercrystalline matrix. Furthermore, similar proteins sequester and remove metal ions from metabolic pathways. This is necessary because excessive amounts of such ions (mainly derived from food, Chapman et al., 2003) can have adverse effects on organisms. Different metal cation detoxification systems have therefore been developed in organisms (Viarengo and Nott, 1993). A common detoxification system is based upon soluble ligands, i.e. metal-binding proteins such as metallothioneins or phosphoproteins (Margoshes and Vallee, 1957; Noel-Lambot, 1976; Marsh and Sass, 1985). These proteins may become part of the EPF.

Some proportions of Mg may merely co-occur with the IOM. During biomineralization, Mg is largely excluded from aragonite crystals and is therefore enriched in the peripheral fluids surrounding the crystals. In this case, Mg does not necessarily bind to the IOM, but may be adhesively associated with the IOM (or occur as a Mg enriched Mg-Ca-CO<sub>3</sub> surface coating on the carbonate crystals). However, the use of a low-concentration acid (HNO<sub>3</sub>) in the present study to dissolve the biominerals assured that adhesively bound Mg was removed from the IOM prior to the analyses.

#### ***2.4.2 IOM and magnesium distribution across shells of *Arctica islandica****

As demonstrated by geochemical staining experiments (immersion in Mutvei's solution, Fig. 2.1) and SEM analyses (Fig. 2.3), organic components, especially the IOM, are not homogeneously distributed across the shells, but are strongly enriched near major growth lines. Mutvei's solution stained these lines dark blue indicating the presence of large amounts of organic molecules. In addition, SEM analyses revealed that significantly smaller crystals occur near such growth lines compared to other portions of the outer shell layer. Because each crystal is embedded in an organic sheet of IOM, shell portions with smaller crystals contain relatively larger amounts of intercrystalline organics. During biomineralization, these organic matrices provide the structural framework (Clark, 1980) and control the material properties of the biominerals (Mann, 1983; Simkiss and Wilbur, 1989; Crenshaw, 1990; Watabe et al., 1993). Between major growth lines, however, a cross-acicular crystal fabric with larger crystal sizes prevailed, and the amount of insoluble organics in the respective shell portions was much smaller. Immersion of such shell portions in Mutvei's solution resulted in a mottled fabric consisting of large, light blue (probably intracrystalline organics) stained crystals and scanty intercrystalline organics (darker blue).

### ***2.4.3 Pitfalls of element analyses of *Arctica islandica* shells by LA-ICP-MS***

If the precise Ca concentration of the analyzed material is known, LA-ICP-MS returns trace element values that are statistically indistinguishable from those measured by ICP-OES. For example, using the observed Ca concentration of 0.22 wt% (Table 2.2) as an internal standard, LA-ICP-MS and ICP-OES returned similar Mg and Sr data for the IOM (Table 2.2). This finding also suggests that potential interferences by organics on mass 24 have only minor effects on the Mg measurements. If organic molecules would influence the data, LA-ICP-MS values for the Mg concentration were significantly different from those measured by ICP-OES. Likewise, a direct comparison of similar shell portions analyzed by LA-ICP-MS and ICP-OES revealed statistically indistinguishable trace element concentrations (Table 2.2). It should be noted that the analyzed samples came from an ontogenetically old shell portion. Low calcification rates in such shell portions resulted in narrow annual increments. Each sample taken from such locations represented more than a year of shell growth precluding the analysis of seasonal variations of trace elements. Given the relatively low sampling resolution, the distribution of the IOM (and the Ca level; here: 35.72 wt%) in such shell portions can be considered to be nearly homogeneous.

However, fast-growing youth portions of bivalve shells can be analyzed with sub-annual resolution by LA-ICP-MS. In such shell portions, the Mg concentration (and Mg/Ca ratios) near major growth lines (= larger amounts of IOM) were two- to threefold higher than in neighboring shell portions with lower IOM content (Figs. 2.1 and 2). A likely explanation for the overestimation of the Mg concentration of shell portions enriched in IOM is a significant Mg contribution from the larger amounts of IOM present. In most existing LA-ICP-MS studies of biogenic hard parts, the small-scale heterogeneous distribution of the IOM across the shell and its chemical composition has received little attention. Instead of determining the precise Ca concentration of each shell portion designated for element analyses by means of LA-ICP-MS, it is often standard practice to measure the Ca concentration of the entire shell and use this value as the internal standard. However, the IOM to CaCO<sub>3</sub> ratio across the shell is highly variable, particularly in youth portions of the shell, and shell portions containing more IOM evidently contain less CaCO<sub>3</sub> and, thus, have a lower Ca content. It should be noted that it is currently very difficult to determine the Ca concentration of the sample volume ablated for LA-ICP-MS, because the sample volume excited during Ca measurements with the electron microprobe is much smaller. In spots, in which the amount of IOM exceeds 0.46 wt% of the total sample weight, this can result in a

significant overestimation of the Mg and Sr concentration, whereas lower than average amounts can result in an underestimation of these elements. This will be demonstrated by a hypothetical model (Fig. 2.6).

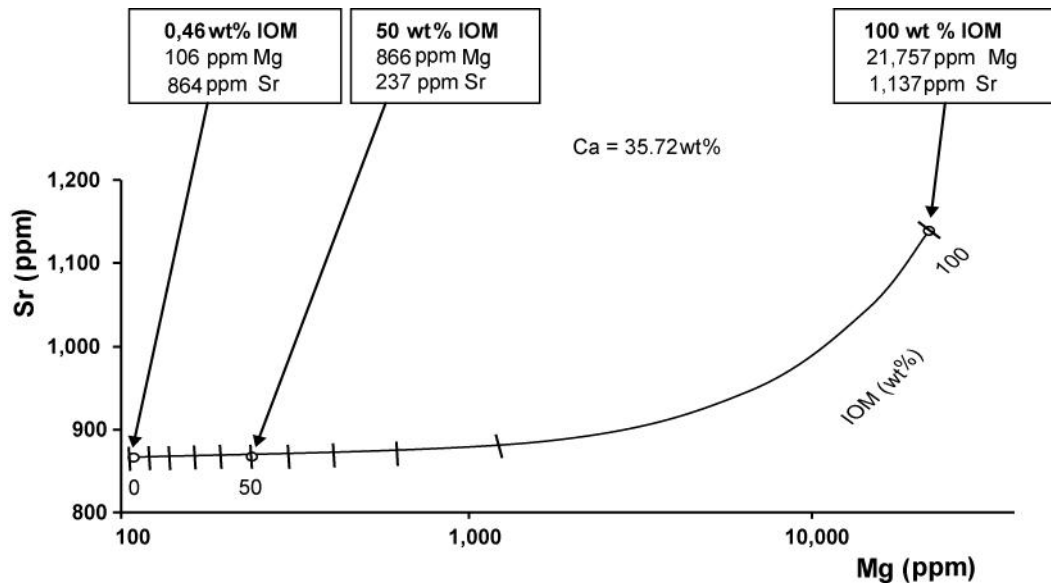


Figure 2.6 Hypothetical model of the potential error of trace element chemistry induced by spatially variable amounts of IOM. Here, it is assumed that the Ca concentration of the sample spot analyzed by means of LA-ICP-MS is not known precisely and an internal standard value of 35.72 wt% Ca (given by ICP-OES analysis) is erroneously assumed. If the laser hit a shell portion without IOM, all Mg and Sr would come from the CaCO<sub>3</sub> + SOM fraction. However, assuming that the laser coincidentally hit a shell portion containing pure IOM, this would result in a significant overestimation of the Mg and Sr concentrations of 21,761 ppm and 1,155 ppm, respectively. A mixing model for these two end members, 0 wt% and 100 wt% IOM, provides a means to estimating the potential error induced by different amounts of IOM in the biomineral.

In this model, we use a Ca content of 35.72 wt% as internal standard value (Ca concentration given by multiple ICP-OES measurements; Table 2.2) for all calculations. In the two extreme cases (a) ablation of an IOM-free shell portion and (b) coincidental ablation of a purely organic spot, the Mg and Sr concentrations will be underestimated (a) or overestimated (b), respectively. In case (b), an unchanged internal standard value that does not take into account the admixture of virtually Ca-free, but Mg- and Sr-rich IOM would result in an overestimation of the actual Mg and Sr concentrations (Mg=134 ppm; Sr=7 ppm) by 16,139 % and 16,400 %, respectively (Mg=21,761 ppm; Sr=1,155 ppm; Table 2). Mixing calculation between these two extremes, 0 wt% and 100 wt% IOM, provides a means to estimate the potential error induced by different amounts of IOM in the biomineral (Fig. 2.6). For example, if a shell portion containing 50 wt% insoluble organics is ablated, apparent Mg and Sr values would be as high as 237 and 866 ppm, respectively. These hypothetical

considerations demonstrate that a local enrichment of IOM can lead to a significant overestimation of the Mg level of the sampled shell portion if the actual Ca concentration is not precisely determined. Precisely how the IOM concentration varies across the shell on  $\mu\text{m}$ - and nm-scales remains to be studied.

#### 2.4.4 Trace element to calcium ratios of *Arctica islandica* shells

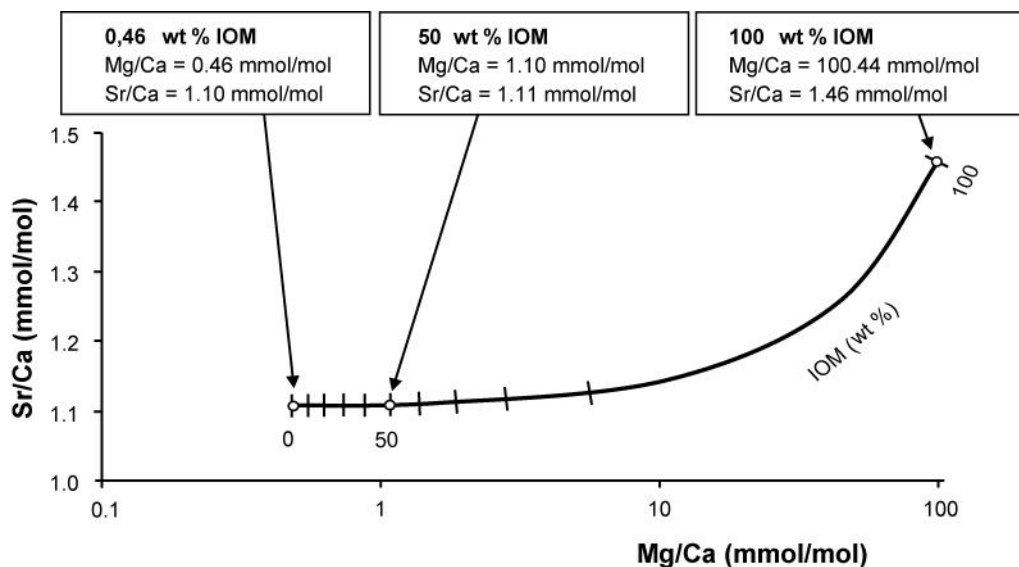


Figure 2.7. Model of IOM-induced changes of the trace element to calcium ratios of a biomineral. Because of the different element chemistry of the IOM (Table 2), Mg/Ca and Sr/Ca ratios increase with increasing relative abundance of IOM in the biomineral.

So far, our analyses have demonstrated that element concentrations of homogeneous shell portions and isolated IOM measured by ICP-OES and LA-ICP-MS agree well. Furthermore, the Mg/Ca and Sr/Ca ratios calculated from the respective ICP-OES and LA-ICP-MS data were statistically identical (Table 2.2). However, as shown in Figures 1 and 2, high-resolution LA-ICP-MS analysis of fast-growing youth portions of the shells not only resulted in higher Mg values near major, IOM-enriched growth lines, but also in elevated Mg/Ca ratios. This is likely to be due to the different chemical composition of the IOM and the  $\text{CaCO}_3$ +SOM components. In the model in Figure 7, the effect of increasing amounts of IOM on the Mg/Ca and Sr/Ca ratios is simulated. If the analyzed shell portions consist of pure IOM, Mg/Ca and Sr/Ca ratios are ca. 100.44 and 1.46 mmol/mol, respectively. However, in an assumed extreme case that the analyzed shell portions consist of a 1:1 mixture (by weight) of IOM and  $\text{CaCO}_3$ +SOM, the Mg/Ca and Sr/Ca ratios will amount to 1.10 and 1.11 mmol/mol, respectively. For the calculation, we assumed that the  $\text{CaCO}_3$  to SOM ratio remained



unchanged and that SOM was Ca-free. Therefore, the relative abundance of CaCO<sub>3</sub> amounts to 44.81 wt%, and the Ca concentration will be 44.81 wt% x 40.04 wt% Ca / 100=17.94 wt% (for calculation of the SOM abundance and Ca content in SOM see next section). For comparison, the average shell (0.46 wt% IOM) returned Mg/Ca ratios of 0.46 mol/mol and Sr/Ca ratios of 1.10 mmol/mol. This model suggests that shell portions enriched in IOM can have higher Mg/Ca and – if the IOM content is large enough – higher Sr/Ca ratios. Trace element to calcium ratios of shell portions enriched in IOM may not be useful as paleothermometers, because the relationship between temperature and Mg/Ca and Sr/Ca ratios has only been verified for CaCO<sub>3</sub>, but not for the IOM.

#### **2.4.5 Soluble organics (SOM)**

It is currently very difficult to ascertain the trace elemental composition of the soluble organics or the precise relative abundance of SOM<sub>lp</sub> and SOM<sub>sp</sub> in the whole biomineral. Our approach to separate the soluble organics by molecular size was not successful, because the SOM<sub>lp</sub> fraction apparently still contained large amounts of dissolved CaCO<sub>3</sub> (Table 2). Preliminary data, however, suggest that the chemical composition of the SOM does not vary as much as the IOM from the inorganic shell fraction. Other available techniques to separate the SOM from the CaCO<sub>3</sub> involve cationic ion exchange resin (Albeck et al., 1996). However, these methods involve non-ultrapure agents and are thus inappropriate to determine the trace element composition of the SOM.

It is noteworthy that the relative abundance of SOM in the shells of *A. islandica* was unexpectedly high in comparison to previous reports. Kawaguchi and Watanabe (1993) found only 2.6 wt% IOM+SOM in *Crassostrea virginica* shells. However, these data came from the prismatic shell layer which may contain considerably less organics than the crystal fabrics of *A. islandica*. Typically, the prismatic shell layer is only very weakly stained by Mutvei's solution indicating low amounts of organics (Schöne et al. 2005a). Further studies are required to confirm the amounts of SOM in *A. islandica* shells.

#### **2.4.6 Implications for geochemical analyses of bivalve shells**

As demonstrated here, an indiscriminate use of analytical techniques may produce imprecise data on trace element concentrations of bivalve shells. Strontium levels of bivalve shells can be determined with sufficient precision and accuracy by both ICP-OES and LA-ICP-MS techniques. However, Mg concentrations are severely overestimated by LA-ICP-MS, because

(1) the IOM is enriched in Mg and (2) the internal standard value assumed for the whole biomineral may not be appropriate to each shell portion; some shell portions such those as near major growth lines can be significantly enriched in IOM and, thus, depleted in Ca compared to the whole biomineral. Without proper sample pretreatment or mathematical modeling, high-resolution LA-ICP-MS-derived Mg concentrations cannot be compared with ICP-OES data. Potential sample pretreatment procedures can include removal of the IOM by oxidative cleaning (see Takesue and van Geen, 2004). However, some of the intracrystalline organic matrix may be insoluble as well and thus difficult to remove (e.g., by roasting; Gaffrey et al., 1991), without dissolving the shell (compare Watanabe et al., 2001). Alternatively, the surplus of Mg contained in the IOM can be mathematically corrected for. If the average IOM content and its artifactual Mg concentration were known, its contribution to the Mg concentrations of the whole biomineral could be calculated and subtracted.

The use of trace element to calcium ratios as faithful paleothermometers, however, does not require absolute element concentrations. Even if the exact Ca-concentration of the ablated material is not known due to the heterogeneous nature of the material, Mg and Sr values will change proportionally, and Mg/Ca or Sr/Ca ratios will not be fractionated. However, existing studies (inorganic precipitation experiments) have only confirmed a temperature effect on the Mg/Ca and Sr/Ca ratios of the  $\text{CaCO}_3$ , while such effects on the IOM have never been studied. Given the different chemical composition of the IOM, shell portions enriched in IOM will not only show higher Mg, Sr and Ca concentrations, but also higher Mg/Ca and Sr/Ca ratios than the  $\text{CaCO}_3$ +SOM fraction. This is applicable to both the ICP-OES and LA-ICP-MS techniques. However, wet analytical techniques permit the separation of the different shell components prior to the analysis, whereas a reliable removal of the IOM from the biomineral prior to LA-ICP-MS analysis is not currently established. During LA-ICP-MS analysis the entire shell material is ablated, i.e. IOM, SOM and  $\text{CaCO}_3$ . Therefore, shell portions with higher relative IOM abundance will return elevated Mg/Ca and Sr/Ca ratios. Without removal of the IOM prior to the analysis, Mg/Ca and Sr/Ca ratios of shell portions with higher IOM content cannot be used as paleothermometers.

## **2.5 Summary and conclusions**

The insoluble organic matrix of *Arctica islandica* shells is significantly enriched in magnesium and depleted in strontium and calcium in comparison with the inorganic carbonate

fraction and soluble organics. Although the average relative abundance of the IOM barely exceeds 0.5% by weight, its chemical composition can significantly increase estimates of the Mg content of the shell if measured by LA-ICP-MS. This overestimation is related to the heterogeneous distribution (on  $\mu\text{m}$ - and  $\text{nm}$ -scales) of the IOM across the shells. It is currently still very difficult to determine the Ca concentration (used as internal standard) of the exact same volume that is ablated for LA-ICP-MS. Thus, Mg concentrations of shell portions with higher than average IOM content, such as major growth lines, are prone to be overestimated by LA-ICP-MS. Removal of the IOM prior to the chemical analysis or mathematical correction for the IOM-derived magnesium concentrations is strongly advised.

For paleoenvironmental reconstructions, however, it is necessary to determine the element to calcium ratios of the  $\text{CaCO}_3$  component. Existing studies (inorganic precipitation experiments) have only demonstrated a temperature effect on the Mg/Ca and Sr/Ca ratios of the  $\text{CaCO}_3$ , but not of the IOM. Without removal of the IOM prior to the analysis, Mg/Ca and Sr/Ca ratios of shell portions enriched in IOM cannot be used as paleothermometers. Because it is currently not possible to remove the IOM prior to LA-ICP-MS analysis, we recommend the use of wet chemical techniques such as ICP-OES at the expense of lower sampling resolution.

The trace metal chemistry of soluble organics in biominerals still requires further study. Our preliminary approach only focused on molecular sizes of the SOM above and below 3kDa and separation of these two phases from dissolved  $\text{CaCO}_3$  was incomplete. Chromatographic separation methods (Mazon et al., 1990) may help to determine the Mg content in different molecule size classes as well as the relative abundance of each different SOM class in the whole biomineral. Such data may help to define mathematical models that can eliminate the potential overestimation of Mg by means of LA-ICP-MS.

Similar influences of organic proteins on chemical estimates are also expected for other metals that are often bound to organic molecules such as Co, Fe, Mn, Mo, Ni, Se and Zn (e.g., Lochmüller et al., 1974; Gómez-Ariza et al., 2004). Further studies should therefore isolate different organic components of the shell and determine their amount and chemical composition by wet chemical analyses.



### 3 Effect of organic matrices on trace elemental compositions of *Arctica islandica* shells

In Chapter 2, the concentration of Ca, Mg and Sr in organic matrices and shell carbonate were analyzed by means of ICP-OES and LA-ICP-MS. The conclusion was reached that the Mg concentration of organic-rich shell portions of *Arctica islandica* can be significantly overestimated when measured by LA-ICP-MS. In this chapter, the distribution of other trace elements in organic and inorganic components of the shell will be evaluated.

#### 3.1 Introduction

Like Mg and Sr, other trace elemental compositions of bivalve shells were also extensively applied as proxies to reconstruct the environmental and climatic variations of the past. Bertine & Goldberg (1972) proposed the trace elements (Rb, Fe, Co, Sb, Sc, Ag, Cr, Zn, Se and Hg) of shells of mussels and clams might reflect the composition of the waters in which they lived. Frazier (1975) proved that the concentration of Zn, Cu, and Cd in the American oyster (*Crassostrea virginica*) exhibited a gradual increase during the spring and dearly summer, decreases during August-September. Carriker et al (1980) cultured the *Crassostrea virginica* in a natural habitat and two environmentally controlled systems, and confirmed that all the trace element concentrations (Cd, Cu, Fe, Mg, Mn, Sr, and Zn) of the shells were affected by the variation of the environment habitats. Concentrations of the Cu, Pb and Zn of horse mussel *Modiolus modiolus* shells, collected from two sites in the southern North Sea, were determined by LA-ICP-MS (Richardson et al., 2001). All the three metals were significantly elevated in the shells from a dump site compared with the control site. These results suggested that the environmental effects on the elemental distribution of bivalve shells. Intra-shell trace element variations in Santonian inoceramids from Spain were studied Jimenez-Berrocoso et al. (2004). The authors proposed that the saw-toothed intra shell variation of Na/Ca, Ba/Ca, Fe/Ca and Mn/Ca ratios may be mainly related to the periodically changing palaeoenvironmental conditions, such as seawater temperature variation and phytodetritus rainfall. Madkour (2005) conducted a research on determining the Fe, Mn, Zn, Cu, Pb, Ni and Cd contents of the Giant clam, *Tridacna maxima*, and sediments collected from clean and contaminated coastal sites of the Egyptian Red Sea. The levels of most metals in the giant clam shells and sediments were higher in the anthropogenic sites than in the uncontaminated sites. Shells of the pod razor (*Ensis arcuatus*) shell from 13 locations around the west coast of mainland Britain have been investigated by LA-ICP-MS for a range of trace metals including

Zn, Cd, Pb, U, Ba, Sr and Mg (Pearce and Mann, 2006). The conclusion was that the regional distribution of these metals of the shells, except the anomalies of high U, was consistent with known sources of contamination and patterns of seawater migration around the coast of Britain. Dunca et al. (2008) utilized *Arctica islandica* shells to monitor local climate variations and the influence of human activities on the local environment from Øresund, Kattegat and Skagerrak. The elevated contents of S, N, Cu, Zn, As, Cd and P in shell portions formed during the last century were found to relate to human activities such as mining and industrial development. Protasowicki et al. (2008) investigated the bioaccumulation of the heavy metals (Hg, Pb, Cd, Cu, Zn, Cr, Ni, Fe, Mn, V, Li, Al) in the shells of *Mytilus edulis* collected from 12 stations on the Polish coast of Baltic Sea. No significant differences were detected in metal concentration between different shell lengths, and these shells are confirmed to have the abilities to show spatial and temporal changes in metal bio-availabilities, suggesting a good bio-monitoring archive.

However, the concentration of trace elements of bivalve shells is also influenced by other factors than the environment and climate. These factors can be grouped as follows: species-specific affects (Pilkey and Goodell, 1963; Morrison and Brand, 1986; Ruelas-Inzunza and Paez-Osuna, 2000), growth rate (Harriss, 1965; Carriker, et al., 1982; Carre et al., 2006; Freitas et al., 2008), age/ ontogenetic trends (Carriker et al., 1982; Fang and Shen, 1984; Bourgoïn, 1990; Carriker et al., 1991; Richardson et al., 2001), metabolic activity (Harriss 1965; Rosenberg, et al., 2001), ‘microstructure’ (crystal fabric) of the shells (Carriker et al., 1991; Tanabe et al., 2007), effects of organic matrices (Lingard et al., 1992), other un-known internal physiological regulations (Carriker et al., 1980; Koide et al., 1982; Fischer, 1983; Bourgoïn, 1990; Nolan and Dahlgard, 1991; Almeida et al., 1998b; Gundacker, 1999; Huanxin et al., 2000; Putten et al., 2000; Ravera et al., 2003; Yap et al., 2003; Gillikin, 2005; Gillikin et al., 2005a; Carroll and Romanek, 2008), and diagenesis (Brand and Morrison, 1987; Wassenaar et al., 1988; Elorza and Garcia-Garmilla, 1996, 1998; Jimenez-Berrocoso et al., 2004; Mansour, 2004). Therefore, before any applications of trace elements of bivalve shells as proxies to explore the environmental and climatic information of the past are carried out, the influences of all the above factors should be fully evaluated and subtracted. In fact, such an evaluation has rarely been conducted (see examples in the paragraph further above), because the mechanism of the secretion of the shells is still not fully understood. More concretely, while the organic matrices in the shells play a dominating role in the process of

bio-mineralization, the effect of these organic matrices on the geochemical composition of the bivalve shells is not fully comprehended.

In this chapter the main focus is to explore the effects of the organic matrices of bivalve shells on the trace elemental distributions of various trace elements. Two problems will be addressed: (1) how the trace elements are distributed in insoluble organic matrices (IOM) and shell carbonate with soluble organic matrices; (2) how the trace elements are distributed in the primary and secondary layer of the bivalve shells; Both ICP-OES and LA-ICP-MS analytical methods are also applied here.

## **3.2 Material and methods**

### ***3.2.1 Specimens and analytical strategy***

A total of nine specimens of *Arctica islandica* were used in the study. Five specimens, (ICE06-4.2-A1 to ICE06-4.2-A5) were collected alive in 2006 from ca. 25 to 55m water depth northwest of Iceland. The other four were also collected alive from different localities around Iceland: the specimen, M071868-A3R, collected in July 1868, is the oldest ever reported bivalve shell (Schöne, et al., 2005a); HM-Fla86-A1L was taken in summer 1986 from about 30m of water depth near Flatey Island (N66°09.53'55, W17°51.36"); Specimens Möller-A9L and Möller-A5L were collected in November 2003 in the same place near the coast in about 30-40m depth (N66°16', W14°55.20').

The five ICE06-4.2 specimens were utilized to determine the trace elemental compositions of two different shell portions, insoluble organic matrices (IOM) and shell carbonate with soluble matrices, by means of ICP-OES. It was expected to gain insight on the properties of different trace elements and the effects of IOM on the distributions of these elements in the whole shell.

The remaining four shells were measured at both primary layer and secondary layer by means of LA-ICP-MS. Only three parts of the primary layer were analyzed, namely umbonal shell portion, middle shell portion and tip portion of the shell, while the secondary layer was investigated in the umbonal shell portion near the cardinal tooth including most of the shell annual increments (see the sketch in Fig. 3.1). The purpose of analyzing different shell layers

was to pursue the traits of trace element composition of the two different layers and the traits of the variation of trace element composition over lifetime of the animal.

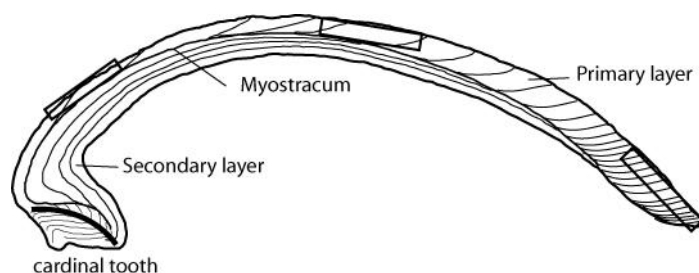


Figure 3.1 Sketch of cross-section of *Arctica islandica*. The rectangles are the shell portions in the primary layer analyzed by LA-ICP-MS (from left to right: umbo, middle, tip portion), the bold line in the cardinal tooth indicates the position analyzed by LA-ICP-MS (secondary layer).

### 3.2.2 Analyses of shell portions by ICP-OES

One valve of each the five ICE06-4.2-A shells was firstly physically cleaned with a diamond coated hand drill to eliminate adhering sediment and organics. Then, the shells were rinsed in tap water and subsequently ultrasonically cleaned in MQ water, dried and weighed. Afterwards, all the shells were crushed in the opal mortar into powder (particle diameter is about 16 $\mu$ m), 25-46g of shell powder was then dissolved in enough 5 wt% ultrapure HNO<sub>3</sub> at room temperature for several days. All the solutions (including the insoluble material) were centrifuged (Thermo scientific Heraeus instruments, Megafuge 1.0) at 3700 RPM for 30 min. Then, the IOM of each sample was extracted and ultrasonically rinsed in MQ water, air-dried and weighed. After that, the IOM of the five ICE06-4.2-A shells were dissolved in 30 wt% ultrapure H<sub>2</sub>O<sub>2</sub>, and the solutions were put in the oven at 60°C for several hours. Finally, the soluble solutions were separated, and 1 ml 69 vol% ultrapure HNO<sub>3</sub> was added to prevent precipitation of trace elements.

The IOM and the remaining solution (which includes shell carbonate and soluble organic matrices), derived from the five ICE06-4.2A shells, were determined by ICP-OES at University of Mainz. The type of the instrument is SPECTRO CIROS VISION SOP ICP-OES (Spectro Analytical Instruments, Kleve, Germany). A standard Fassel-type torch, and a SeaSpray TM nebulizer (Glass Expansion, Australia) with a Cinnabar TM cyclonic spraychamber (Glass Expansion) were used. The sample uptake speed of the SeaSpray TM nebulizer is 200 $\mu$ l min<sup>-1</sup>. The protocol for all the analyses was made according to Schrag (1999) and de Velliers (2002). The ICP was maintained by a 27 MHz solid state RF generator with the power 1400 W. The flows of Plasma, Auxiliary, and Carrier Gas are 12.00, 1.00, and



0.70 L min<sup>-1</sup> respectively. The sample wash-out time was set 80s to remove the previous sample effects and maintain the equilibrium of the introduction system with the new sample. The net measuring time was set 24 s including all the five phases' integration time in order to cover all the signals. Five replicates measurements per sample were chosen to get good reproducibility. The analytical precision is about 1.5% RSD.

### ***3.2.3 Analyses of shell layers by LA-ICP-MS***

Only one valve of each specimen was utilized for the analyses. In order to protect the fragile shells from breaking, a quick-drying metal epoxy resin was applied to both surfaces of the valves before cutting. Then, two adjacent three-millimeter-thick sections were cut from the shell by means of a Buehler low-speed along the axis of maximum growth. After that, all the sections were mounted on glass slides with the same epoxy, ground with 800 and 1200 SiC grit, and polished with 1 µm Al<sub>2</sub>O<sub>3</sub> powder. Lastly, the sections were ultrasonically cleaned with de-ionized water and air-dried.

For the sclerochronological analysis, one section of each specimen was taken to immerse into Mutvei's solution (Schöne, et al., 2005b) for 20 min at 37-40°C. Then the etched section was rinsed with MQ water and air-dried. Afterward, the surface of the etched section was digitized with a Nikon Coolpix 995 camera attached to a stereomicroscope (Leica Wild M2C). All the images got from the stereomicroscope-camera system were imported into the software Panopea. In this program, the growth patterns of the specimens were analyzed and annual increments were measured (Fig. 3.2 ).

The primary layers of the four specimens were analyzed by the magnetic sector analyzer LA-ICP-MS at Frankfurt University. The device is the Element II combined with a UV-213 Nd:YAG laser (New Wave, USA). The pulse repetition rate of the laser was set 10 Hz. The diameter of the crater was 100µm. During laser ablation, Helium gas was used to carry the sample joining the argon carrier gas in a speed of 0.2 liter per minute. <sup>43</sup>Ca had been used as an internal standard and NIST612 was an external standard. The laser signal was optimized using the standard-NIST612 while monitoring Ce for sensitivity and Th/ThO for oxide production (below 1.5%). The analyses were carried out in the mode of discrete craters. The precision of the results is 5-10% RSD.

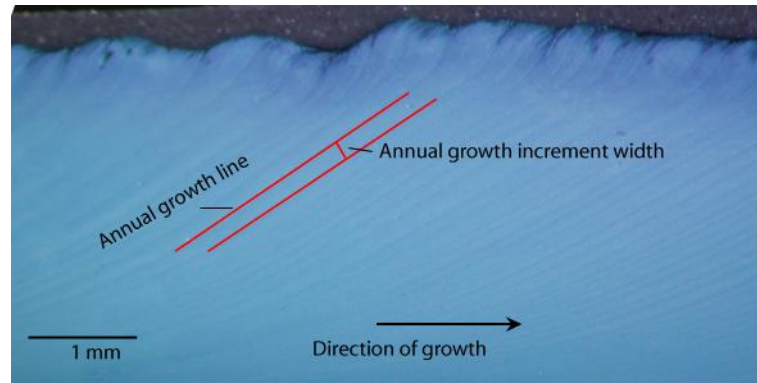


Figure 3.2 Stained section of HM-Fla86-A1L tip part by Mutivei' solution shows the sclerochronological analysis of grow patterns.

The four secondary shell layers were analyzed by a quadrupole analyzer LA-ICP-MS machine at the University of Mainz (showed in Fig. 3.3). The instrument at Mainz is an Agilent 7500ce ICP-MS connecting the same laser system as at Frankfurt (a UV-213 Nd: YAG laser from New Wave, USA). The RF power was also set 1200W. The plasma, auxiliary and sample carrying argon gas flowed in the speed of 15, ~1.0, and 1.2 liters per minute, respectively. The laser moving forward speed was  $10\mu\text{m/s}$ . The dwell time was 10s, and only one point per peak was chosen for signal integration. The secondary shell layers were analyzed not in discrete craters mode but in continuous lines mode. Other laser parameters were adjusted the same as those at Frankfurt. The precision is also between 5 to 10% RSD.

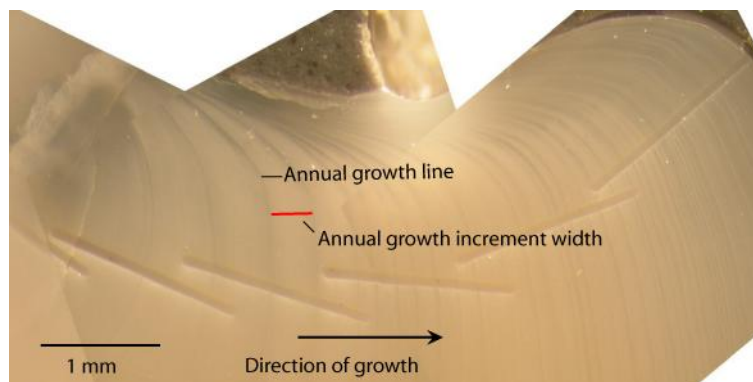


Figure 3.3 Hinge plate of Möller-A9L presents the analytical lines of LA-ICP-MS in the scan-line module.

### **3.3 Results**

#### ***3.3.1 Geochemistry of shell components***

26 elements were investigated in these analyses, only 19 elements concentrations were above the detection limits of the machine (Fig. 3.4). The results show that the concentrations of trace elements in IOM are apparent different from the shell carbonate: (1) most trace elements only occurred in IOM. Only Li, B, Na, Mg, Fe, Sr, Sb, and Ba are detected in shell carbonate; Li was only found in the carbonate fraction; (2) Ba is about evenly distributed in both inorganic and organic shell portions; (3) Li and Na are more enriched in shell carbonate, while B, Mg, Al, P, K, Fe and Sb are higher concentrated in IOM. Because Ca concentration in IOM is much lower than in the rest of shell carbonate, Me/Ca ratios of most of the elements in the Figure 3.2 are very high.

#### ***3.3.2 Geochemistry of the different shell layers***

120 samples (craters) were analysed in the primary shell layer, and 762 annual increments in the secondary shell layer. According to Figure 3.5, the trace elemental composition of both shell layers differs slightly from each other. Trace elements (B, V, Cr, Mn, Co, Ga, Pb and U) are enriched in the primary layer than in secondary layer (Li, Mg, Zn, Sr, Sn and Ba show the opposite). Three elements show larger difference in both layers. Cr and U are higher in primary layer, while Zn is enriched in secondary layer.

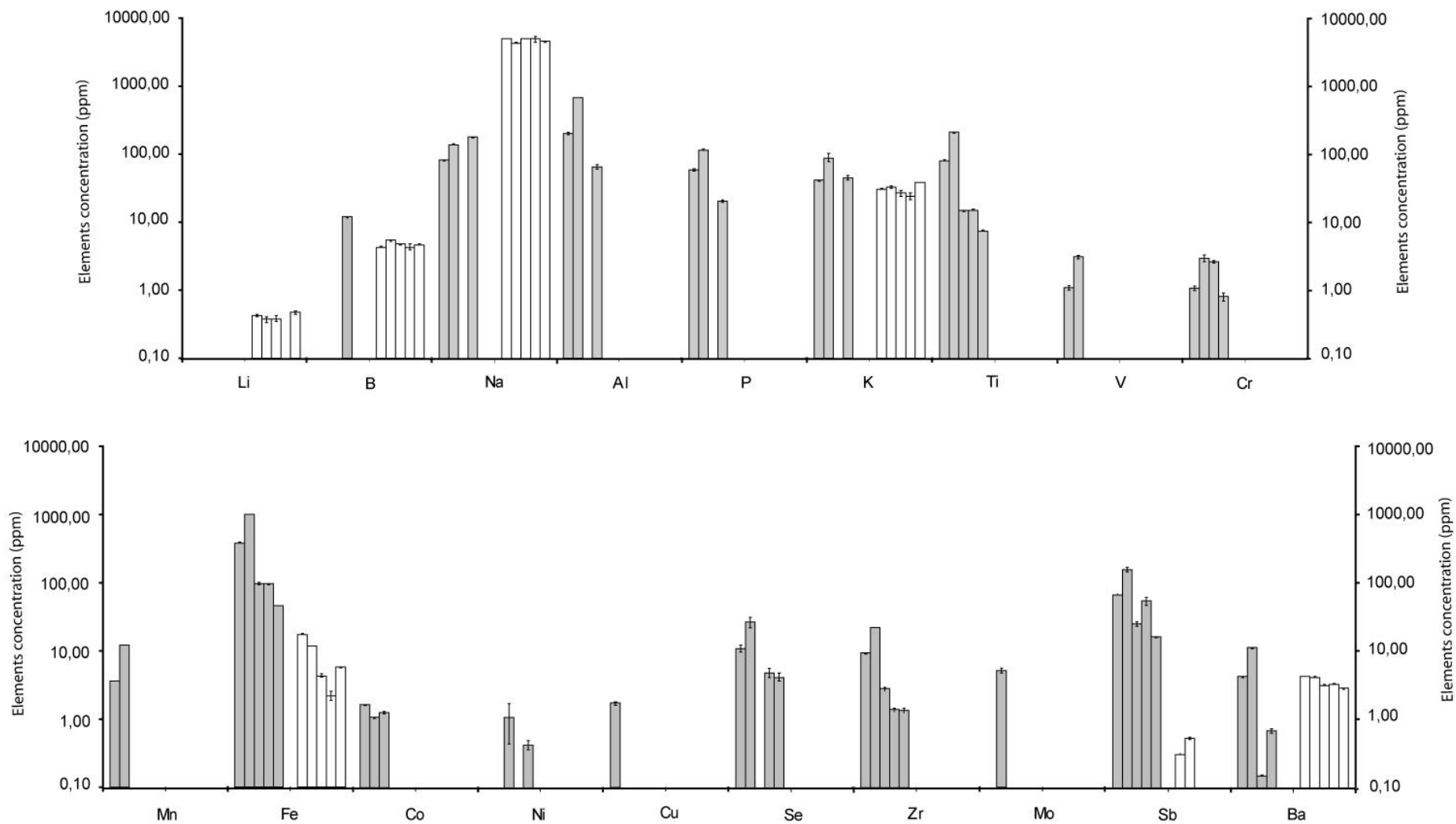


Figure 3.4 Elemental concentrations in the insoluble organic matrix (grey) and in the carbonate phase with soluble organic matrix (white). Each histogram represents an average value from five replicates obtained by ICP-OES from one sample. Error bars = two standard deviations. Empty positions mean that such elements were under the detection limit.

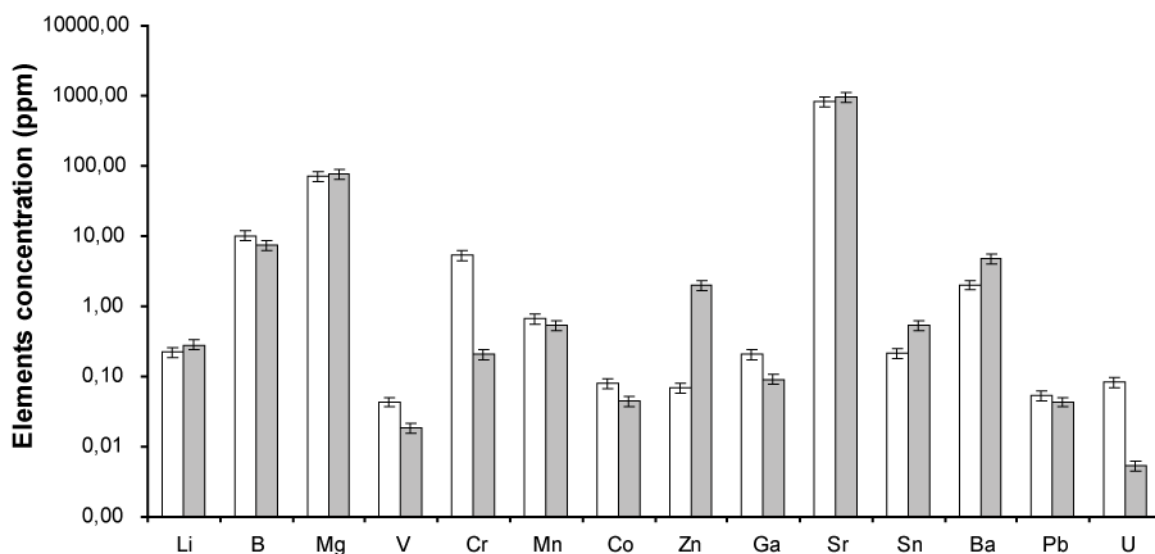


Figure 3.5 Elemental concentrations of shell primary (white) and secondary layers (grey). Data of primary layer represents the average of 120 samples of three shells (Möller-A5R, HM-Fla86-A1L and M071868-A3R), and those of the secondary layer are the average of 762 annual increments of four shells (Möller-A9L, Möller-A5R, HM-Fla86-A1L and M071868-A3R). Error bars = two standard deviations; precision = 8% RSD. All analyses were made by LA-ICP-MS.

### 3.4 Discussion

#### 3.4.1 Difference of trace elemental composition between two shell layers

Until present, only few investigations have been made on the relations among trace element constituents in different shell layers which deposited contemporaneously (Carroll & Romanek, 2008, and the citations therein). According to the results from this study, most elements are detected in similar concentrations in both primary and secondary layers, which might indicate that both layers are formed from solutions with similar chemistry. This was also concluded by Carroll and Romanek (2008) in their study on the chemical traits of two different aragonitic layers of firewater bivalve shells, *Elliptio complanata*.

Yet, there are still differences between the two shell layers in respect to some trace elements. Unlike in the case of the freshwater bivalve shells used by Carroll & Romanek (2008), only Cr and U are more enriched in primary (outer) layer of *Arctica islandica*, whereas Zn is more concentrated in secondary (inner) layer. Four reasons might be responsible for the difference: (1) The primary layer is deposited by outer extrapallial fluid, while the secondary is formed by inner extrapallial fluids (in Figure 3.6, Crenshaw, 1972;

Lorens, 1981; Wilbur & Saleuddin, 1983; Carriker et al., 1991; Carroll & Romanek, 2008). (2) As opposed to the central zone of the mantle (inner extrapallial fluid) intensified metabolic activity was found in the marginal zone of the mantle of molluscs (outer extrapallial fluid) (Crenshaw, 1980). (3) Haugen & Sejrup (1990) had conducted a study on the amino acid composition of shells of *Arctica islandica*. They demonstrated that no significant difference in amino acid composition could be found in the two layers, but the quantity of hydrolyzed amino acid fraction, of which the dominating amino acids are aspartic acid, glycine, alanine and glutamic acid, is different. More hydrolyzed amino acids are contained in the secondary layer than in the primary layer. (4) Difference in shell microtexture could account for the different elemental compositions of the two layers. For example, Carriker et al. (1991) had conducted a study on the elemental concentrations of different microtextural groups of shells of the oyster *Crassostrea virginica*. The diversity in concentration of elements in the prismatic shell portions in contrast to that of the foliated and chalky shell portions was identified. The former shell portion consists of primary shell layer, while the latter are supposed to be deposited from the inner extrapallial fluid and thus belong to the secondary layer. As shown in Fig. 3.6, the two shell layers of *A. islandica* are formed from different epithelia. This may be the reason for the observed difference in elemental concentrations.

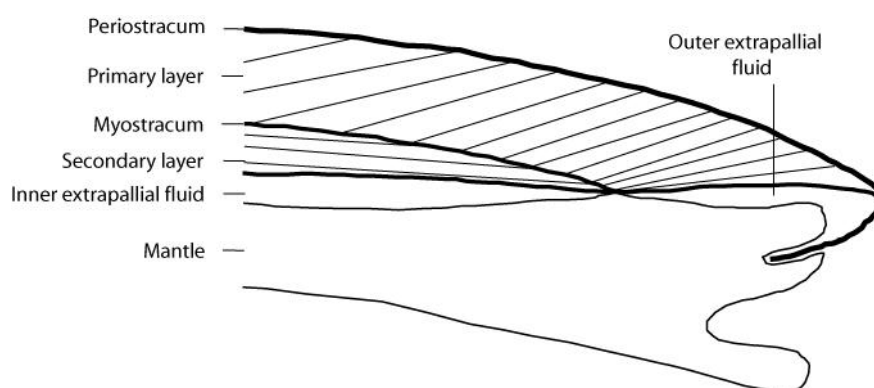


Figure 3.6 Sketch of cross-section of *Arctica islandica* shell (modified from Carroll & Romanek, 2008, and Klünder et al., 2008). The sketch shows that different shell layers of the specimens are deposited from different extrapallial fluids.

### 3.4.2 Correlations between elements Mg and Sr, Ga and Ba

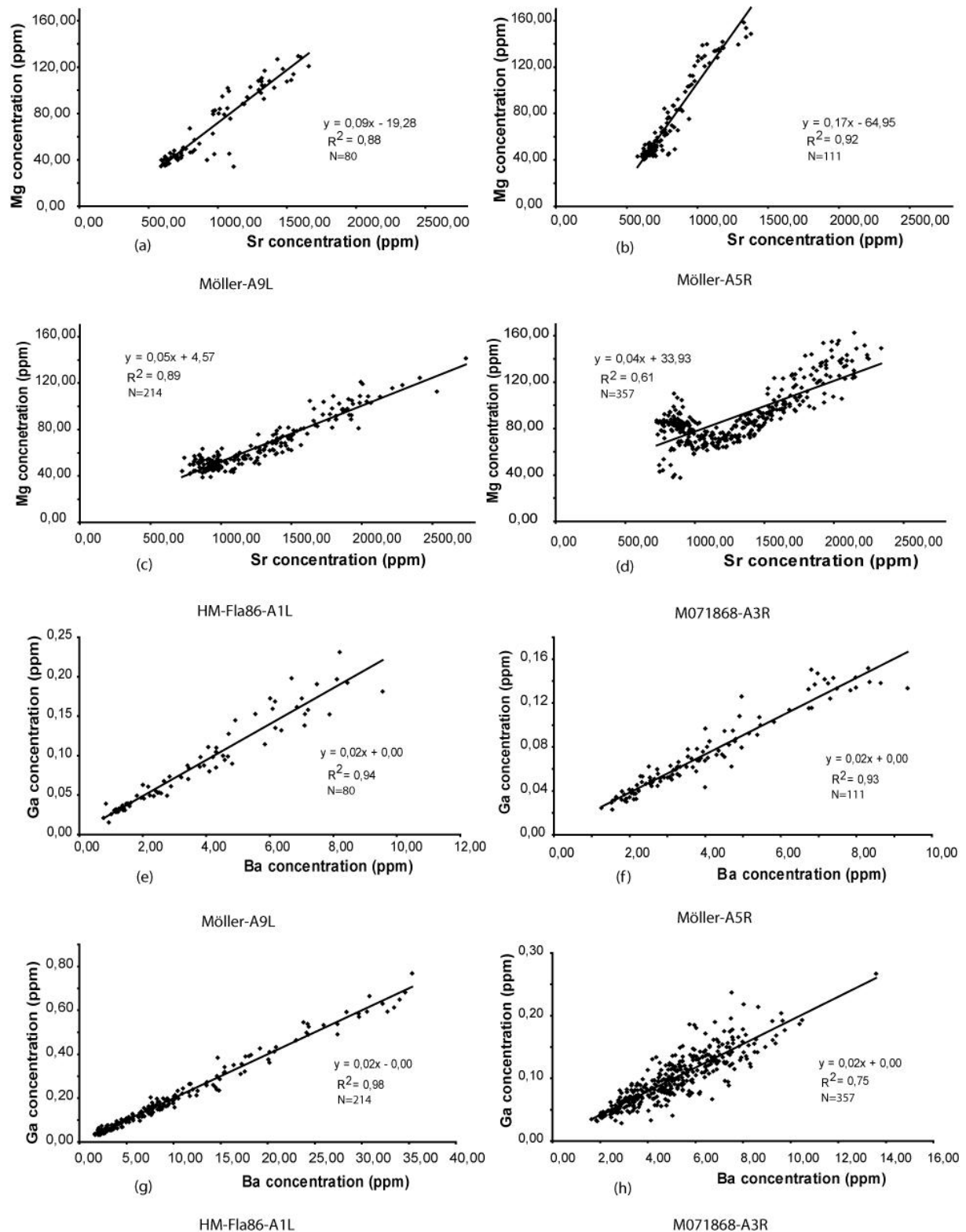


Figure 3.7 Correlations between the concentrations of Sr and Mg, and Ga and Ba in the shell inner layer of *Arctica islandica*. Each data point represents an average of one annual increment.

The Sr concentration of the inner layer varies with that of Mg, while Ga co-varies with Ba. For a check in detail, the concentrations of one element in each pair are plotted against the concentrations of the other. The correlations are depicted clearly in Figure 3.7.

Figure 3.7 shows that the Sr contents are highly correlated with Mg in all four studied specimens of *A. islandica*. While no study has been found that reports this correlation from aragonitic shells, the high correlation of Sr and Mg has been confirmed from the calcitic lattices. Mucci & Morse (1983) reported that the concentration of Sr in abiotic calcite overgrowths is linearly correlated with that of the Mg content. Carpenter et al. (1991) and Carpenter & Lohmann (1992) found that the Sr content of abiotic marine calcite can be calculated using a linear function from the Mg content. In biotic calcite, the high correlation between concentrations of Sr and Mg was also proposed (Carpenter & Lohmann, 1992; Freitas et al., 2006). According to the arguments in Chapter 2, the crystal lattices of aragonite and calcite are different. The bond of Sr-O in aragonite is longer than the Ca-O bond in calcite. More substitutes such as Mg and other ions might be included in the crystal instead of Ca. However, in biogenetic aragonite, Mg ions can be combined with the organic matrix, which has been shown in Chapter 2. This makes the relation among the two elements more complicated. For example, in this research, ICP-OES was also applied on the same specimens, but no correlation between Sr and Mg concentration was found.

The same correlation also applies to Ba and Ga (Fig. 3.7). Notably, the functions of each of the four correlations between Ba and Ga are very similar. Until now, no study has reported such correlation between these element pair. More investigation should be conducted before the reasons behind this are uncovered. It may be possible that Sr and Mg or Ba and Ga combine with similar organic matrices or /and occupy similar amounts of positions in the carbonate lattice.

#### ***3.4.3 Effects of organic matrices on trace elemental distribution***

Shell organic matrices can be divided into two groups: soluble and insoluble. The former are mainly referred to intracrystalline matrices, and the latter is intercrystalline matrices (Addadi & Weiner, 1989; Dauphin, 2002). It is also demonstrated that, during the process of biomineralization, the cells in the mantle epithelium responsible for shell formation first produce the intercrystalline matrices in the extrapallial fluids. These matrices function as frameworks of the shell. The intracrystalline matrices are secreted later during



biomineralization and control the nucleation, formation, and morphology of the carbonate crystals (Weiner & Hood, 1975; Albeck et al., 1996; Belcher et al., 1996; Addadi & Weiner, 1997; Dauphin, 2001; Addadi et al., 2006; Takeuchi, et al., 2008). The soluble organic matrices are mainly composed of acidic macromolecules (Addadi & Weiner, 1989; Dauphin, 2000; 2003; Addadi et al., 2006). Therefore, effects of organic matrices of the shells on trace elemental distribution should be assessed separately in soluble and insoluble organic matrices. In this study, however, it was not possible to separate the soluble organics from the inorganic carbonate component. It was only possible to analyze the IOM and compare the values with data obtained from the fraction carbonate with soluble organics.

As proposed by Dauphin (2001), organic matrices play an important role on the chemical composition of shell carbonate. Therefore the distribution traits of organic matrices of the shell should affect the trace elemental distributions across the shells. As shown in Figure 3.4 many trace elements are more enriched in insoluble organic matrix (intercrystalline matrix) than in soluble organics and inorganic carbonate portion. The contents of Li in the shell appear not to be connected with the insoluble organic matrices. Na ions are more concentrated in the shell carbonate. The controlling factor of the incorporation of trace elements in the organic matrices is still not understood. However, it is now clear that the organic matrices affect the trace elemental distribution both directly and indirectly. Organic molecules could bind the ions directly (such in the case of metalloproteins) and could also control the precipitation of the ions indirectly by their activity.



## 4 Vital effects and trace elemental concentration of *Arctica islandica* shells

### 4.1 Introduction

Vital effects (Urey et al. 1951) and kinetic effects are known to control the chemical composition of bivalve shells (e.g. Swan, 1956; Harriss 1965; Moberly Jr, 1968; Stecher III et al., 1996; Carre et al., 2006; Freitas et al., 2008). Kinetic effects can occur when precipitation (or biomineralization) rates are too high: Precipitates form so rapidly that no equilibrium fractionation can establish. As a result, the geochemical composition of the crystal differs from the medium from which it formed. The two phases are not in equilibrium. Kinetic effects can occur during early ontogenetic stages of animals during which biomineralization rates are significantly faster than during later stages of life. Vital effects are another source for non-equilibrium fractionation. These include, for example, ontogenetic trends and shell growth rates. The latter are controlled by food availability and temperature, while ontogenetic trends may include ageing of enzymes at the semipermeable membranes of the mantle epithelium which control the types of ions that can pass the bilayer and reach the sites of biomineralization.

Studying shell growth patterns in combination with geochemical properties of the shell can thus be potentially very helpful in distinguishing environmental and physiological signals. It should be possible to mathematically eliminate the vital effects on the trace element composition and retain largely environmental signals. Such studies have never been done before with long-lived bivalves. Only few studies have employed such integrated approaches as applied in the present study. For example, Lorrain et al. (2005) demonstrated that the Sr/Ca ratios of *Pecten maximus* (which has a calcitic shell) are strongly controlled by kinetic effects (meaning largely growth rate related effects). For this purpose, Lorrain et al. (2005) measured daily growth increments and total daily surface growth in conjunction with Sr/Ca ratios.

In the present study, the approach by Lorrain et al. (2005) is applied to shells of *Arctica islandica*. It is tested if trace elements are controlled by vital effects. Is correction for vital effects sufficient to extract the environmental signals from the trace elemental composition? Results of this study may help to better understand if environmental signals are preserved in the trace element data of the shells and how such information could potentially be extracted.

## 4.2 Material and methods

Four specimens of *Arctica islandica* have been used in this chapter, namely Möller-A9L, Möller-A5R, HM-Fla86-A1L, and M071868-A3R. The hinge plate (cardinal tooth section) of each specimen was analyzed by LA-ICP-MS (line scans) at the University of Mainz, Germany. The analytical procedure has been described in chapter three. The cross-sections were digitized by means of a Nikon 995 digital camera connected to a stereomicroscope. Growth patterns were measured with the image processing software Panopea®.

## 4.3 Results

### 4.3.1 Variations of elemental concentrations with growth rate

In Figures 4.1 and 4.2 annual increment widths were plotted against 21 different trace elemental concentrations. From these two figures three main findings can be drawn: (1) Different specimens with similar growth rates do not always show similar elemental compositions; (2) When growth rates exceeded 50 $\mu\text{m}/\text{year}$ , no clear relation between shell growth and elemental concentrations could be identified; (3) However, when growth rates were lower than 50 $\mu\text{m}/\text{year}$ , trace elements were strongly linked to shell growth. In turn, these relationships can be classified into four different correlation types: (a) In all studied specimens the concentration of elements B, Mg, Ga, Sr, Ba, Pb and U (“B group”) increase with decreasing growth rates (Fig. 4.1); (b) On contrary, the concentration of elements of the “Li group” (Li, Na, Mn, Cu and Sn) decrease with growth rate in all studied shells (Fig. 4.1); (c) On average, V, Cr, Co, Ni, and Rb (“V group”) show no trend (Fig. 4.2); (d) The concentration of the elements Si, P, Fe and Zn (labeled as “Si group”) present different trends among different specimens (Fig. 4.2).

For elements with the strongest link to growth rate (= biomineralization rate) regression curves were computed. Sr, Mg, B and Ba were fitted with a negative logarithmic function, while logarithmic equations worked best for Na and Mn ratios (Figs. 4.3, 4.4). Because some elements are extremely enriched in the umbonal portions of the shells, the values from the first 15 ontogenetic years were omitted in these analyses.

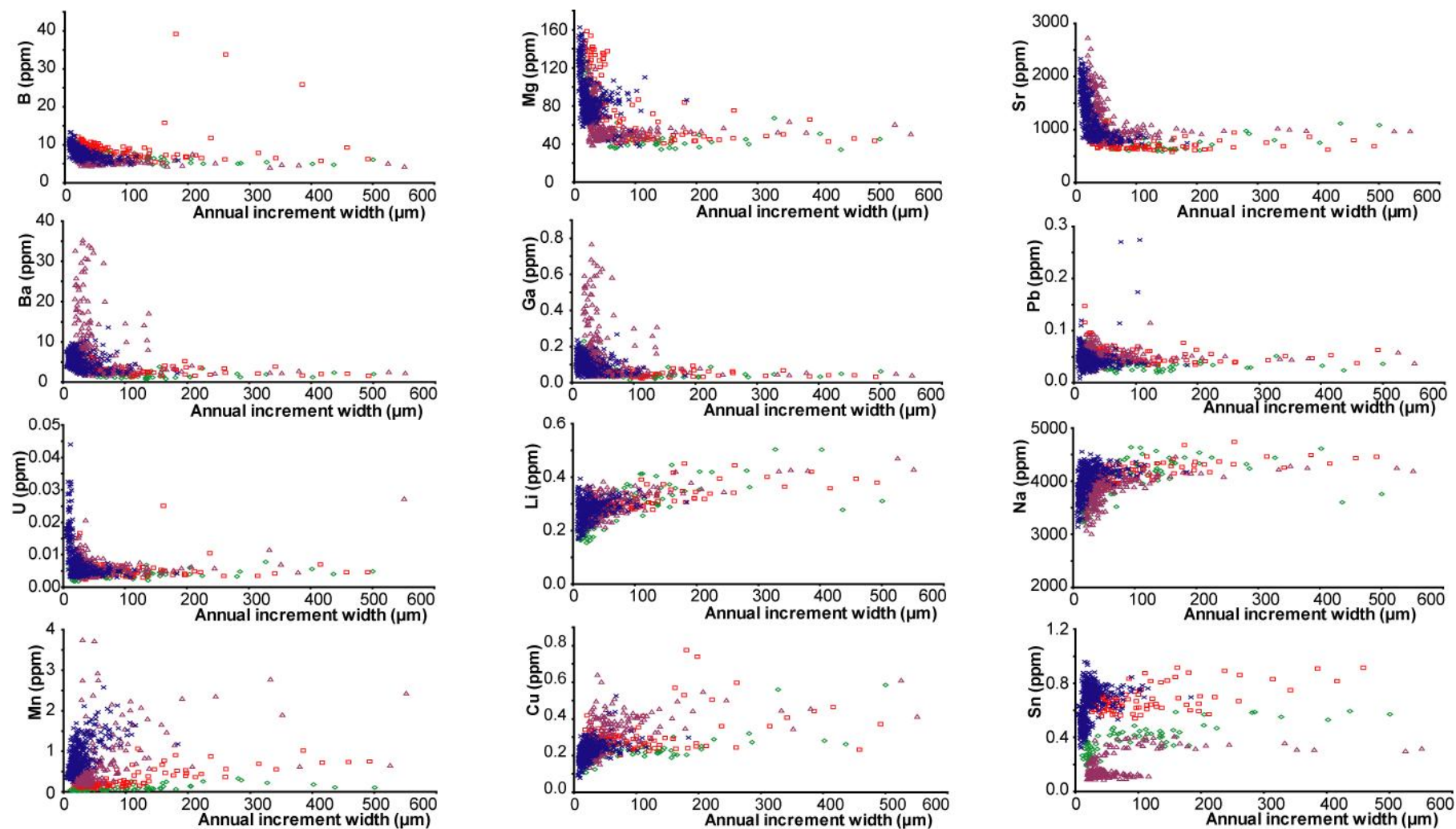


Figure 4.1 Variation of shell elemental concentrations with annual increment width. The concentration of the elements B, Mg, Sr, Ga, Ba, Pb and U is higher in shell portions that formed by slower growth, while the concentration of elements Li, Na, Mn, Cu and Sn show opposite trends. Green diamonds = Möller-A9L; red rectangles = Möller-A5R; violet triangles = HM-Fla86-A1L; blue crosses = M071868-A3R.

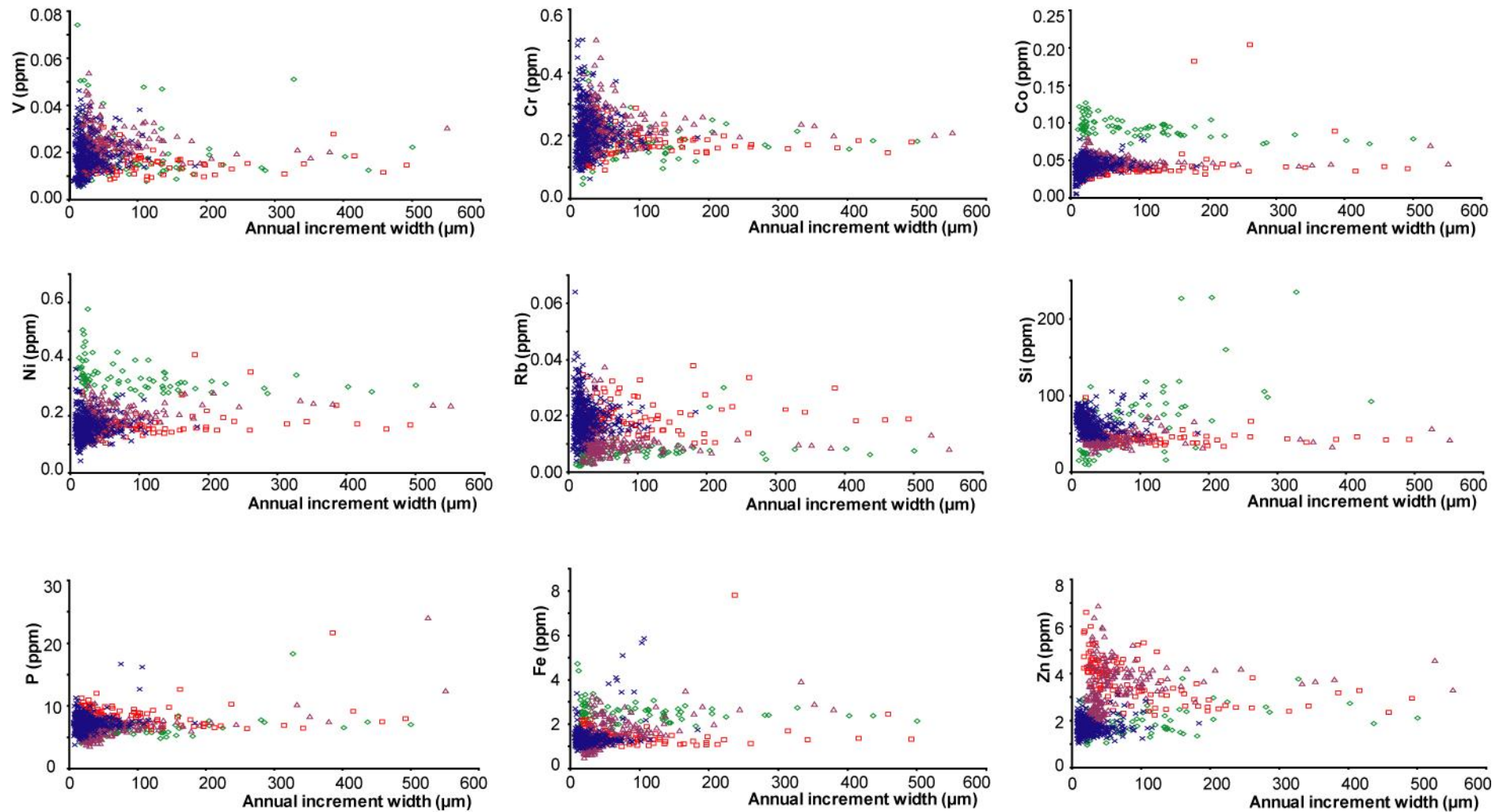


Figure 4.2 Variation of shell elemental concentrations with annual increment width. The concentration of the elements V, Cr, Co, Ni and Rb are apparently not associated with shell growth. However, the concentration of the elements Si, P, Fe and Zn show different trends in different specimens. Green diamonds = Möller-A9L; red rectangles = Möller-A5R; violet triangles = HM-Fla86-A1L; blue crosses = M071868-A3R.

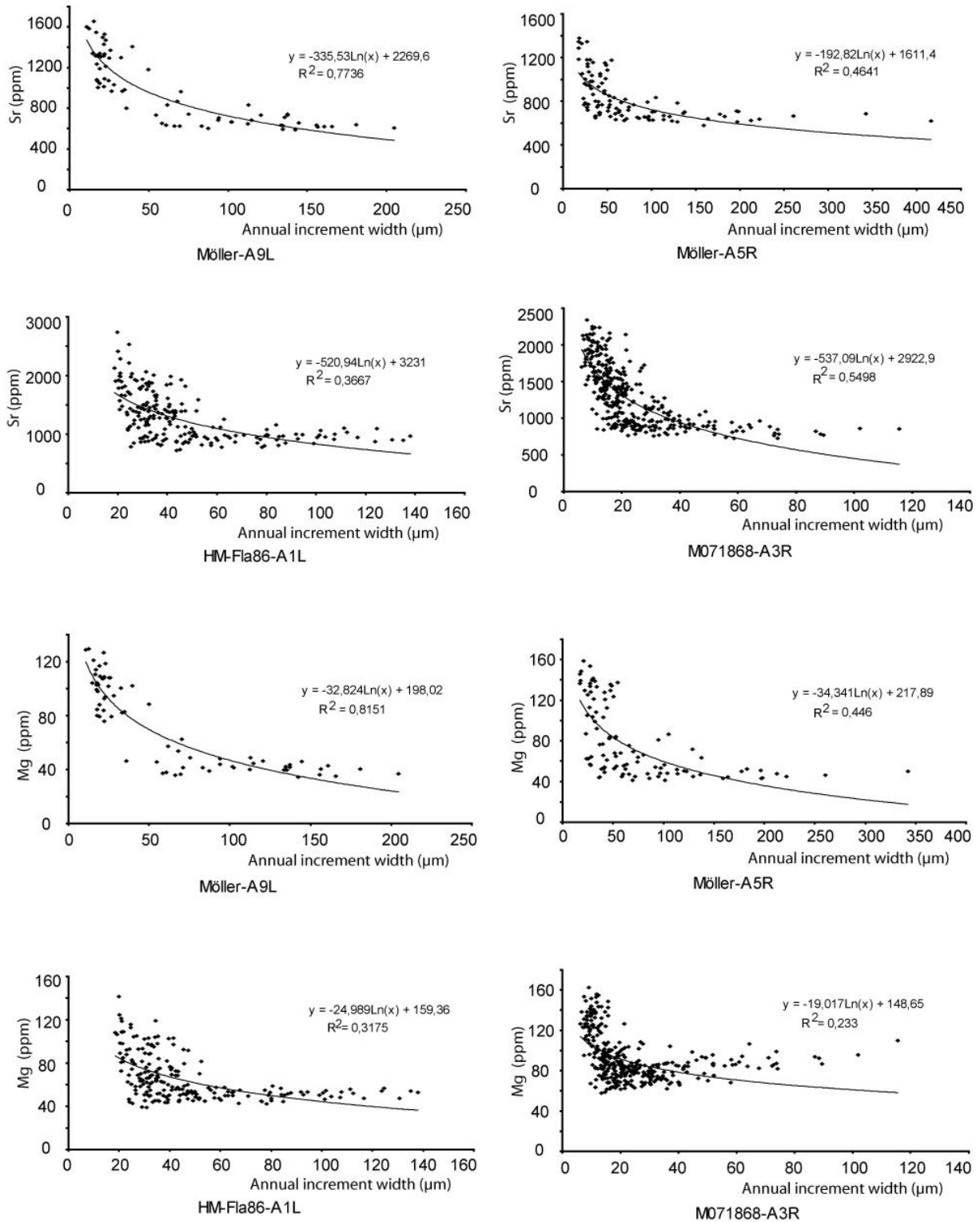


Figure 4.3 Correlations between elemental concentration (Sr and Mg) and annual increment width. Sr and Mg concentrations of shells are highly significantly ( $p < 0.05$ ) and non-linearly (logarithmic fit) correlated with shell growth.

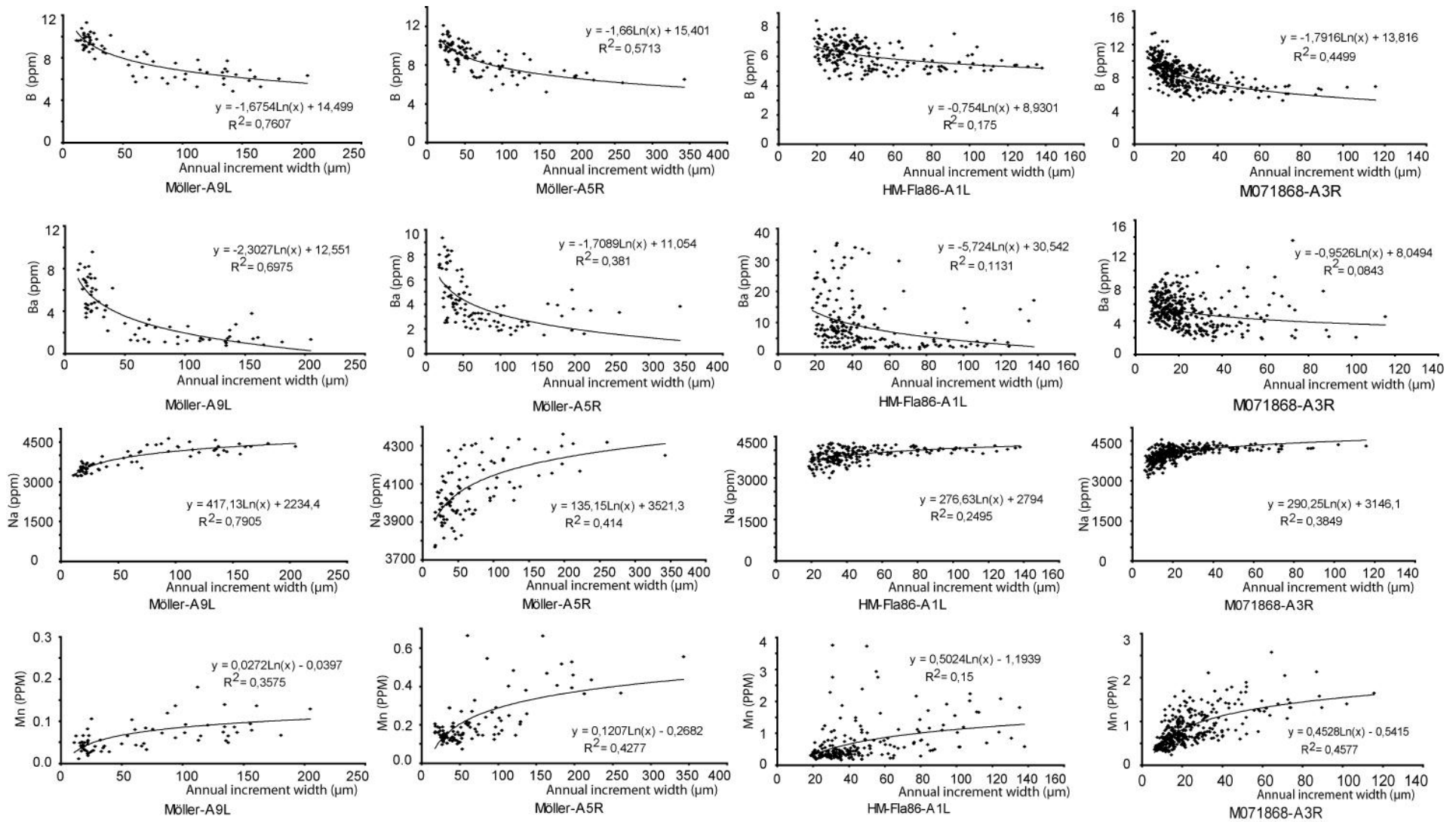


Figure 4.4 Correlations between elemental concentration (B, Ba, Na and Mn) and annual increment width.



### ***4.3.2 Variations of elemental concentrations with ontogenetic age***

When elemental concentrations are plotted against ontogenetic age (Figs. 4.5 to 4.8), two main observations can be made: (1) The trace elements can be classified in four different groups of ontogenetic trends, which are identical as the four groups divided by the trends of elemental concentrations with growth rate (Figs. 4.1 and 4.2): (a) The concentrations of elements B, Mg, Ga, Sr, Ba, Pb and U (“B group”) increase through ontogeny (Fig. 4.5); (b) concentrations of elements of the “Li group” (Li, Na, Mn, Cu and Sn) decrease through ontogeny in all studied shells (Fig. 4.6); (c) V, Cr, Co, Ni, and Rb (“V group”) remain nearly unchanged through lifetime, but show a considerable year-to-year variability (Fig. 4.7); (d) The concentration of the elements Si, P, Fe and Zn (“Si group”) vary inconsistently through lifetime and among different specimens (Fig. 4.8). (2) Ba, Ga and Mn show the largest (absolute) amplitudes.

Regression curves (exponential equations) were computed for metal-to-calcium ratios of the above listed elements and ontogenetic age (Figs. 4.9, 4.10). For Sr/Ca, Mg/Ca, B/Ca, and Ba/Ca values positive exponential functions were used and negative exponentials for Na/Ca and Mn/Ca ratios. Because some elements are extremely enriched in the umbonal portions of the shells, the values from the first 15 ontogenetic years were omitted in these analyses.

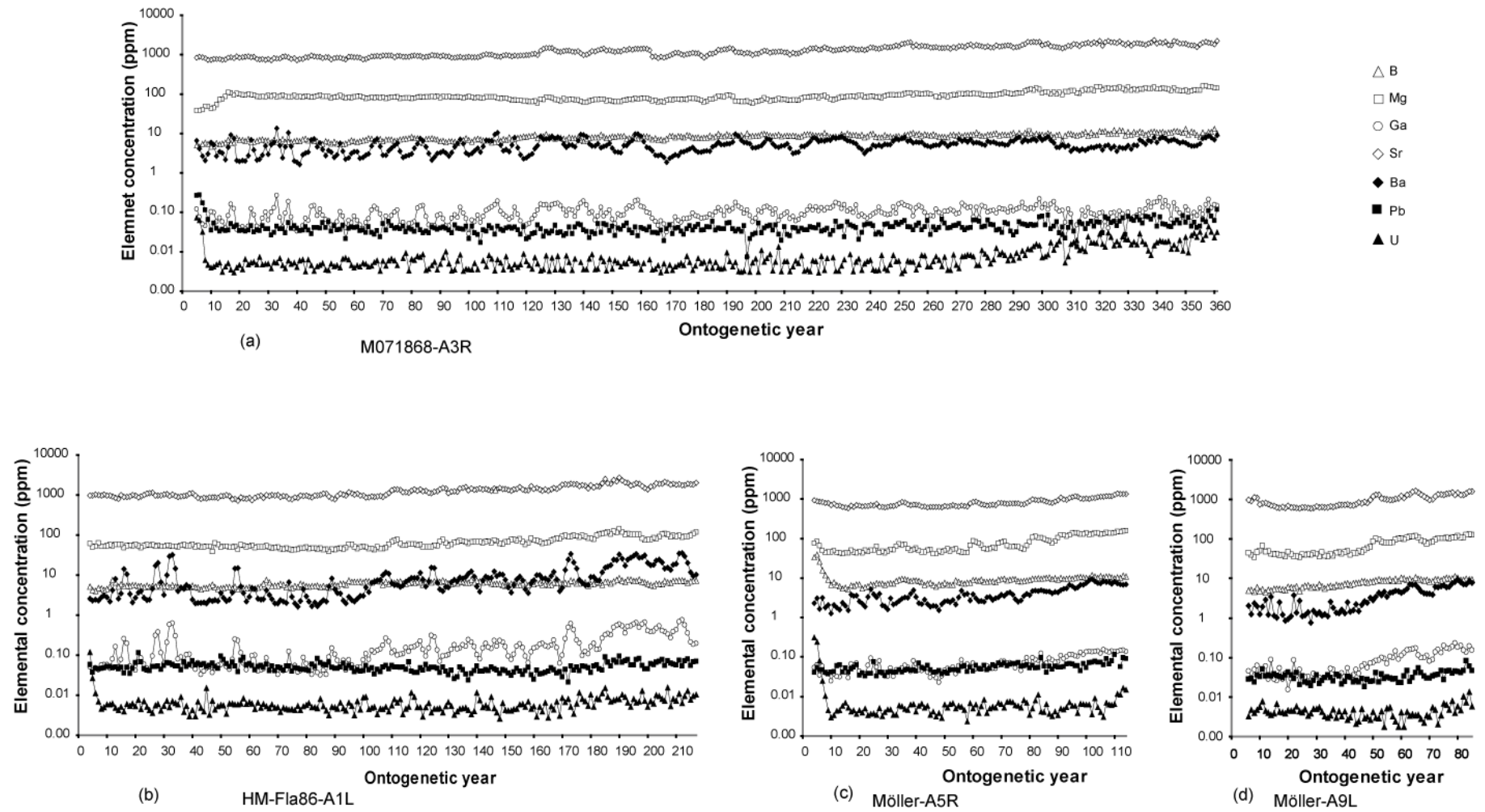


Figure 4.5 Variations of elemental concentrations through ontogeny (I): Elements of the “B group” (B, Mg, Ga, Sr, Ba, Pb, and U) increase through ontogeny. Each data point represents the average of one annual increment.

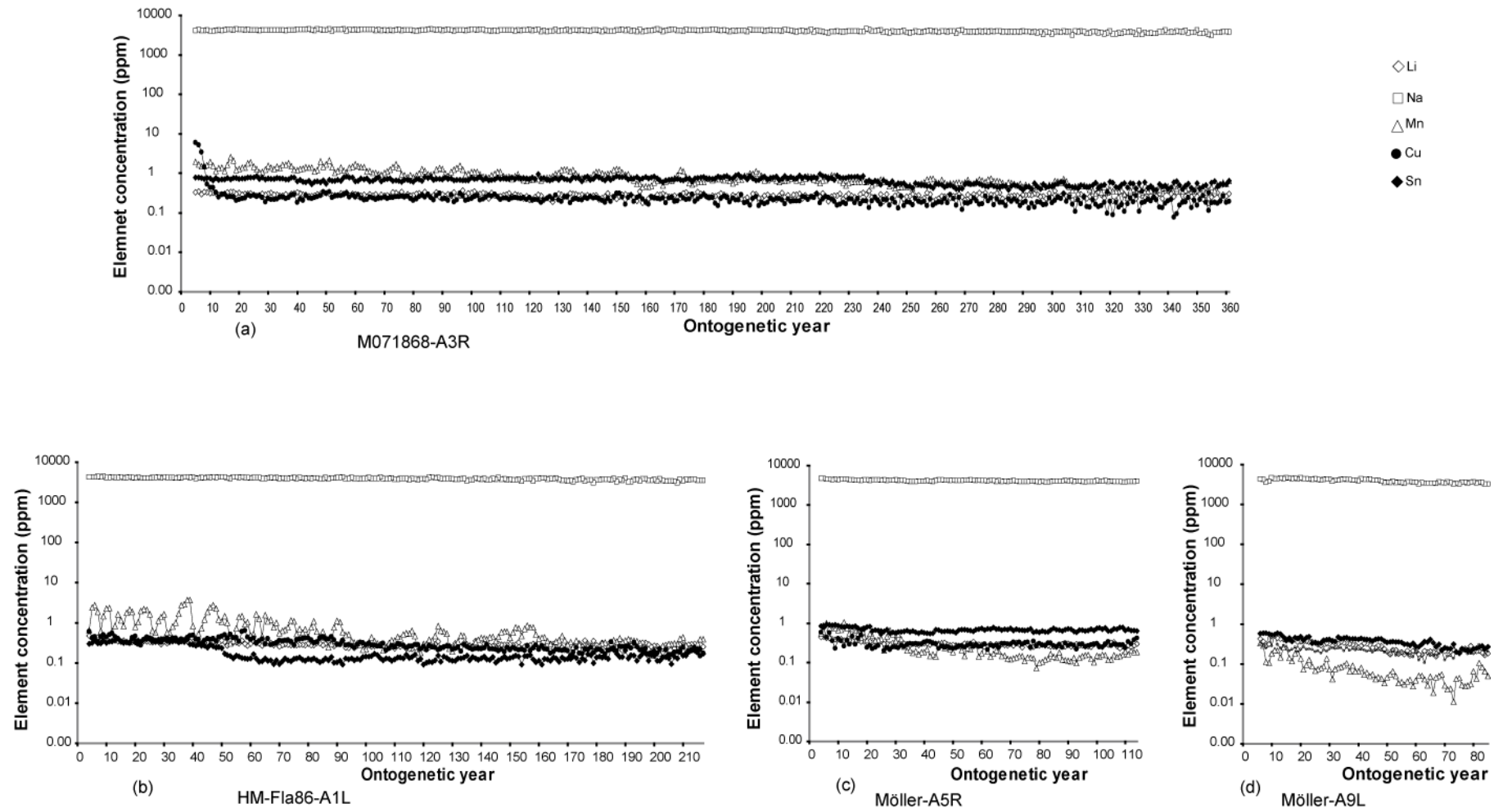


Figure 4.6 Variations of elemental concentrations through ontogeny (II): Elements of the “Li group” Li, Na, Mn, Cu and Sn decrease through ontogeny. Each data point represents the average of one annual increment.

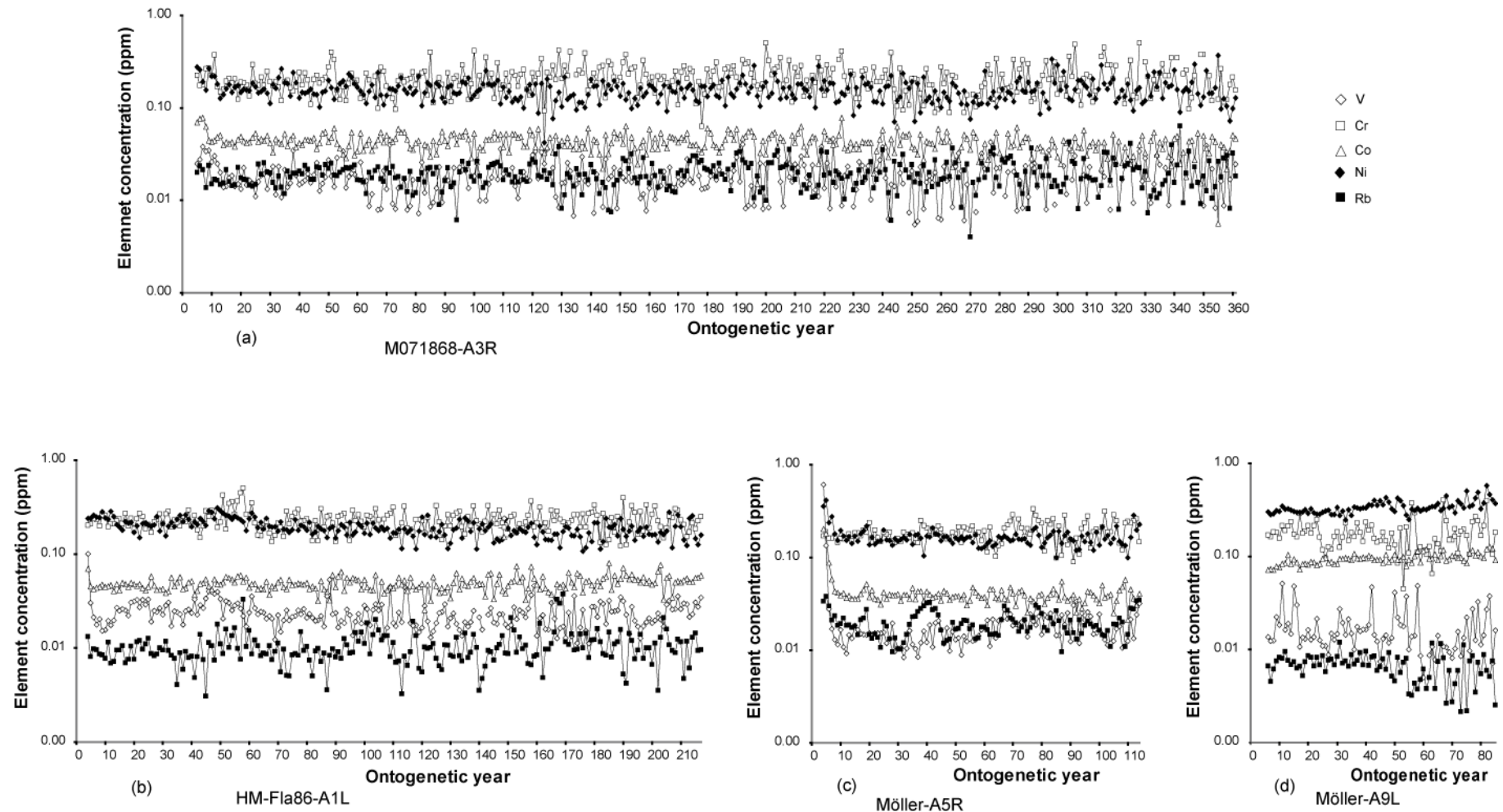


Figure 4.7 Variations of elemental concentrations through ontogeny (III): On average, the elements V, Cr, Co, Ni and Rb (“V group”) remain nearly unchanged through lifetime. However, the year-to-year variability is very large and increases through lifetime. Each data point represents the average of one annual increment.

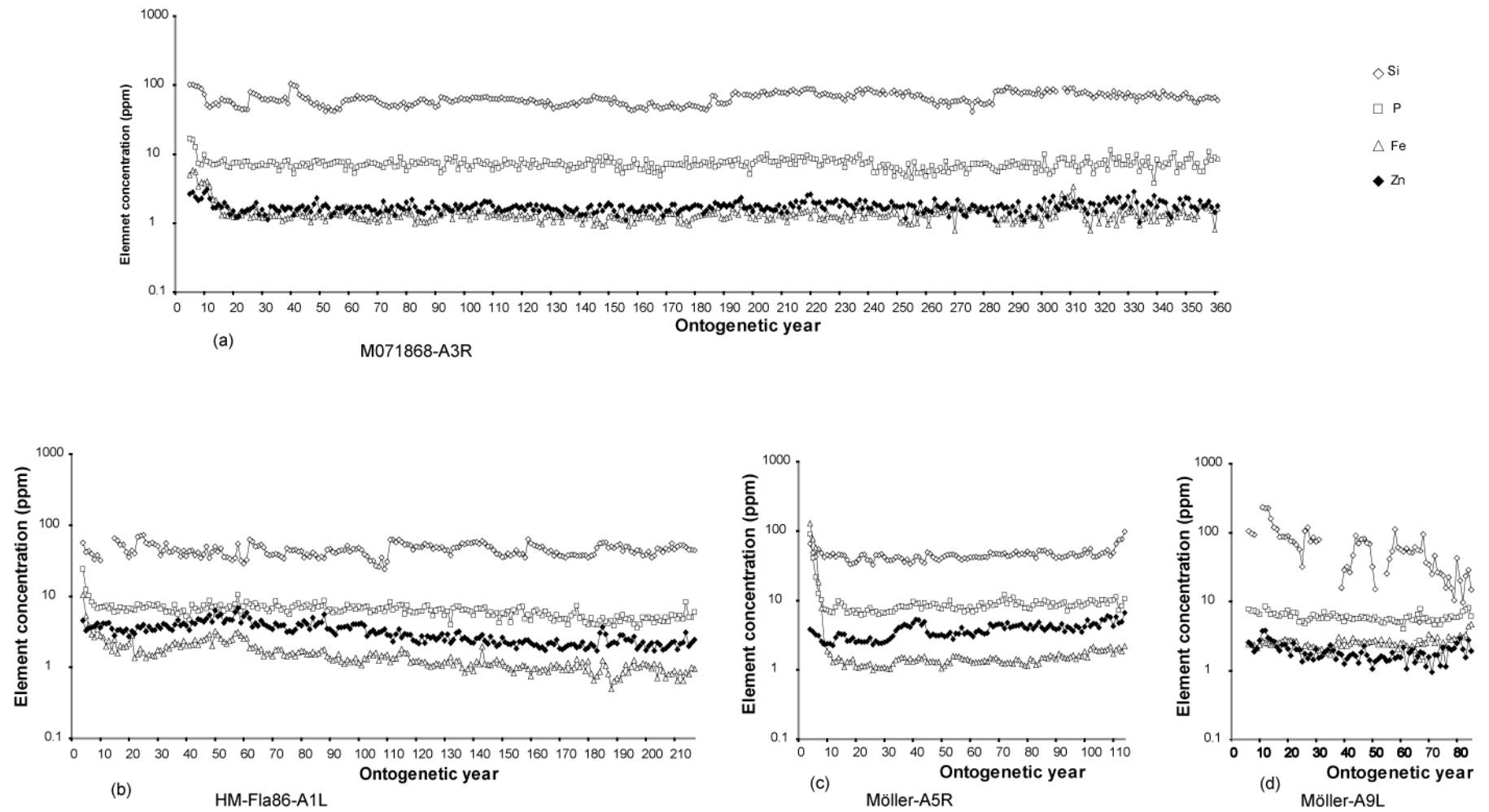


Figure 4.8 Variations of elemental concentrations through ontogeny (IV): The elements Si, P, Fe and Zn (“Si group”) vary inconsistently through lifetime. Each data point represents the average of one annual increment.

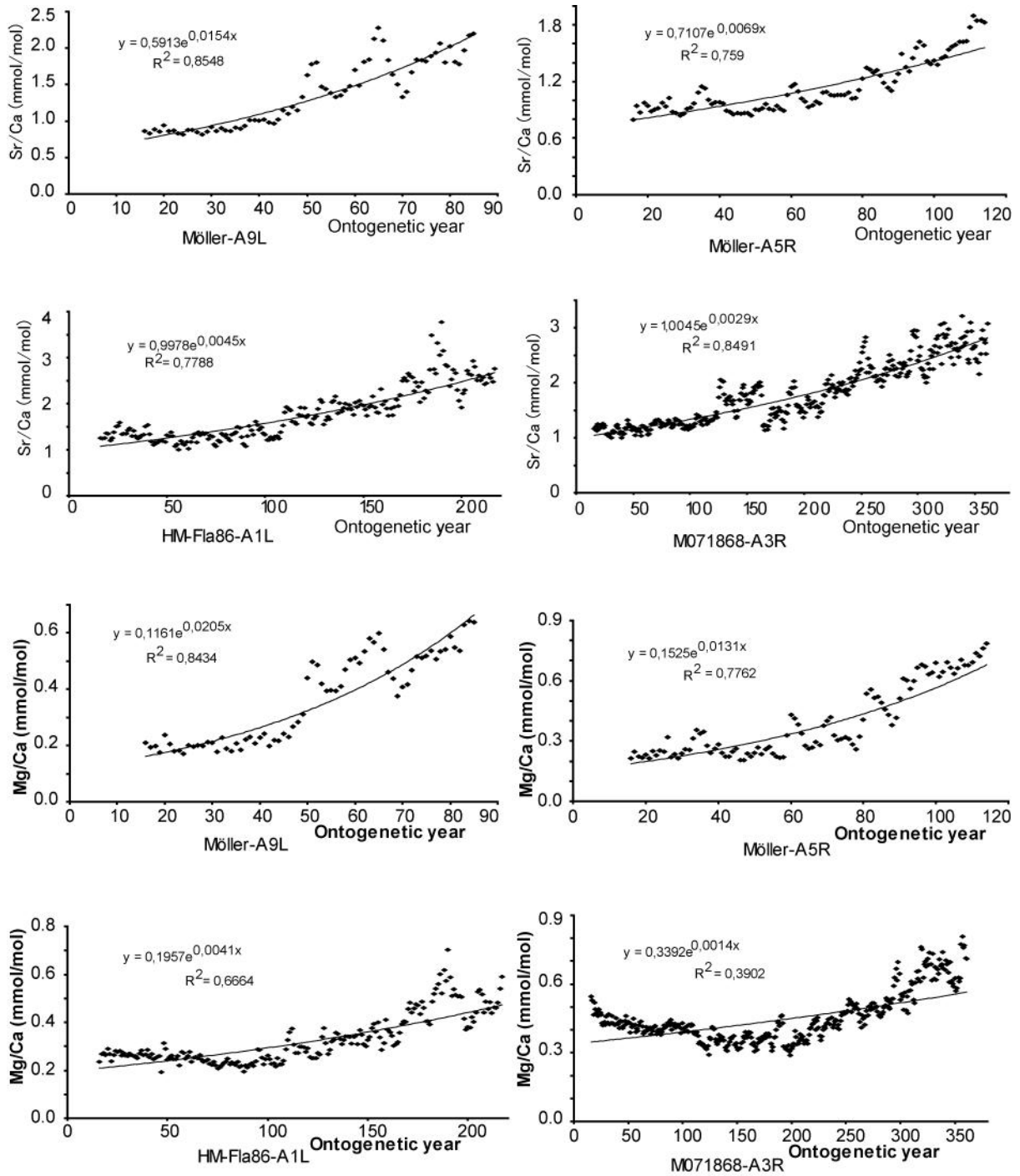


Figure 4.9 Correlations between metal-to-calcium ratios and ontogenetic age. Sr/Ca and Mg/Ca are non-linearly (exponential functions) and highly significantly ( $p < 0.05$ ) correlated with ontogenetic age.

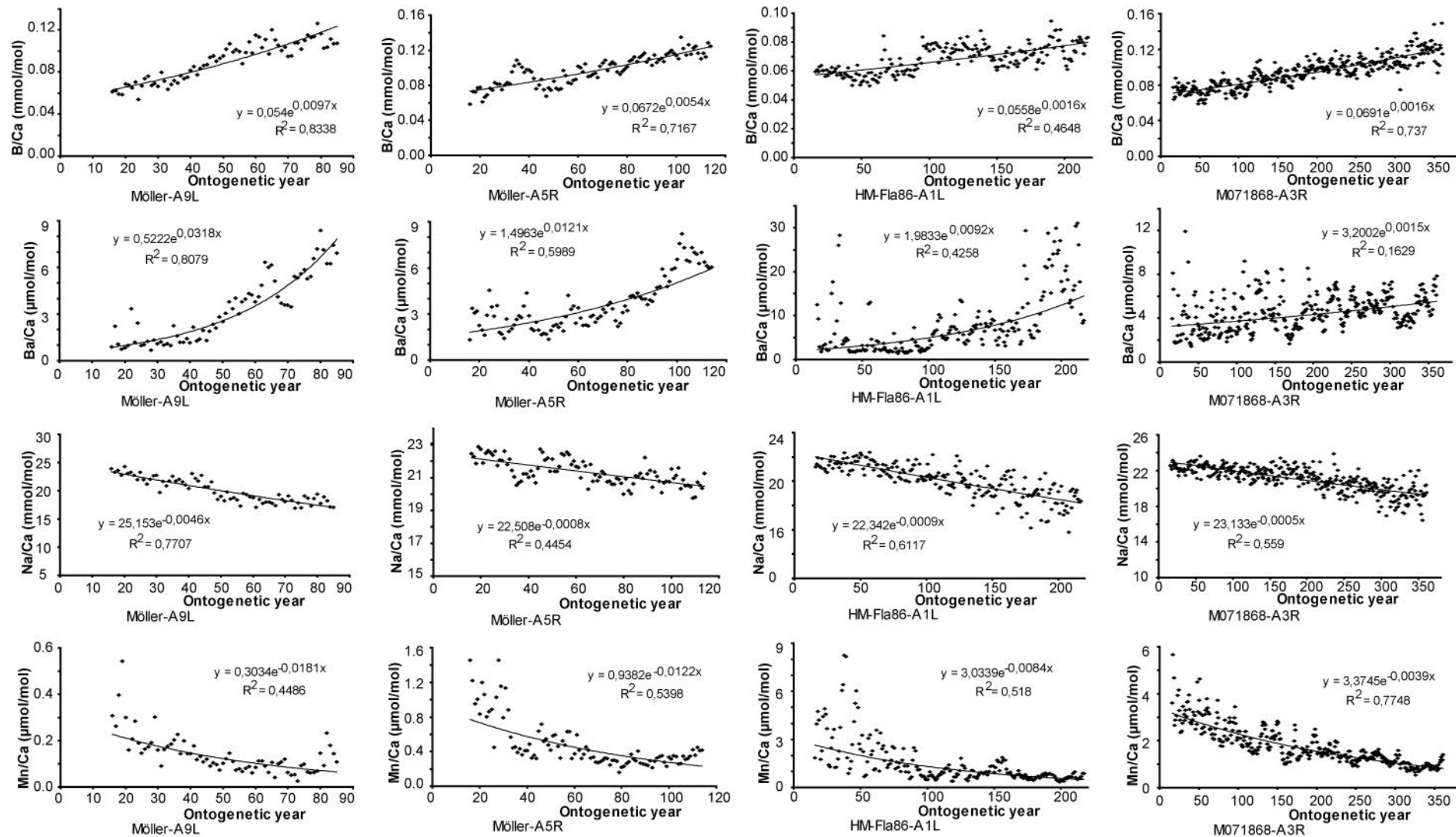


Figure 4.10 Correlations between metal-to-calcium ratios and ontogenetic age. B/Ca, Ba/Ca, Na/Ca, and Mn/Ca non-linearly (exponential functions) and highly significantly ( $p < 0.05$ ) correlated with ontogenetic age.

#### 4.4 Discussion

Fifteen of the 21 measured elements that were analyzed in this study show no relation with age or biomineralization rate, hence they do not seem to be controlled by vital effects. However, Sr, Mg, B, Ba, Na, and Mn were strongly (non-linearly) related to ontogenetic age and annual increment width (Figs. 4.9, 10). This suggests that some kind of vital effects exert a strong control on the concentration of these elements in shells of *Arctica islandica*.

For biogenic calcium carbonate, in particular bivalve mollusk shells, a variety of studies have previously demonstrated that growth rate affects the trace elemental concentrations. Stecher III et al. (1996) proved that the kinetic effects play a more important role than temperature on the incorporation of Sr in shells of *Spisula solidissima*. Takesue & van Geen (2004) and Gillikin et al. (2005a) presented the same phenomenon in two other species, *Protothaca staminea* and *Saxidomus gigantea*. Lorrain et al. (2005) demonstrated the same for the calcitic bivalve shell, *Pecten maximus*. Calcification rate seems to influence both marine and freshwater aragonitic bivalve shells (Carré et al., 2006: marine bivalves, *Mesodesma donacium* and *Chione subrugosa*; Takesue et al., 2008: freshwater bivalve, *Corbula amurensis*).

However, in all of the above mentioned studies only short-lived and fast-growing species were used and the correlation between trace element concentration and growth rate was always positive. In the present study, shells of a long-lived bivalve species were analyzed for the first time and over the entire lifespan of the bivalves. Surprisingly, growth rate and some element concentration were negatively correlated, the complete opposite of any previous results. If only traditional kinetic effects were involved in this process, one would indeed expect a positive correlation between growth rate and element concentration. Faster crystal growth results in stronger element enrichment (e.g., Gaetani & Cohen, 2006). The opposite findings of the present study, however, suggest that other controls are involved. Because the element concentrations were also related to ontogenetic age, it is hypothesized here that some kind of physiological effect that influences the element partitioning between the ambient water and the EPF.

##### 4.4.1 Vital effects: ageing ion channels?

One possible explanation for the observed element trends is physiological ageing. Perhaps, the transport control mechanisms at the semipermeable membranes for such elements work



better in younger shells and prevent these elements reaching the EPF. With increasing ontogenetic ages, such mechanisms may increasingly deteriorate and loose control over active element partitioning. The ion channel model by Carré et al. (2006) is explained below. The  $\text{Ca}^{2+}$  cations are mainly transported from the surrounding medium to the extrapallial fluid (EPF) by diffusion through calcium channels. Calcium channels are aqueous pores formed by proteins in the membrane bi-layer in both the inner and outer mantle epithelium. The force which activates the  $\text{Ca}^{2+}$  diffusion across the epithelium membranes to the EPF is a bioelectric potential generated by local  $\text{Ca}^{2+}$  concentration gradients. Therefore, no energy will be consumed by this process. During the diffusion process, the discrimination of other elemental ions will be reduced while the  $\text{Ca}^{2+}$  concentration gradient across the membrane increases as the result of higher mineralization rate.

#### ***4.4.2 Vital effects: changes in amino acid concentration?***

Addadi & Weiner (1989) pointed out that two kinds of macromolecules (organic matrices) are involved in the process of biomineralization, namely acidic glycoproteins and framework proteins. Acidic glycoproteins are rich in aspartic (Asp) and glutamic (Glu) acids and have a high affinity for ions and charged crystal surfaces. Acidic proteins foster the formation of biominerals (Addadi et al., 2006). Framework macromolecules are more hydrophobic than acidic glycoproteins. Therefore, it is reasonable to assume that the interaction between trace elements and aspartic acid-rich proteins determines the distribution of the trace elements.

Noteworthy, in *A. islandica* the composition of amino acids and the overall amount changes significantly through lifetime (Goodfriend and Weidman, 2001). During youth when fast shell growth is highly important acidic amino acids such as Asp dominate the amino acid composition. However, during later stages of life, structural proteins become more important and, consequently, the amount of less acidic framework proteins increases. This alone would contradict the finding of the present study of increasing element concentrations during life. However, the total amount of amino acids increases during ontogeny as well. And this may provide a second explanation for the observed increase in element concentration at later stages of life.

#### ***4.4.3 Further support for vital effects on trace element concentrations***

Further support for an active control of the elemental concentration in the shells of *A. islandica* comes a comparison of metal-to-calcium ratios in shells and sea water (Fig. 4.11).

Most elements are enriched in shell carbonate in comparison with sea water, except for Na, Mg, and Rb. However, metal-to-calcium ratios show different results. Only Cr/Ca, Ni/Ca, Cu/Ca and Ba/Ca values are in the same range in shells and seawater. Mn/Ca, Fe/Ca, Co/Ca, Zn/Ca, Ga/Ca, Sn/Ca, Pb/Ca and U/Ca values are enriched in the shells are bigger than those in the seawater, while Li/Ca, B/Ca, Na/Ca, Mg/Ca, Si/Ca, P/Ca, V/Ca, Rb/Ca, and Sr/Ca are depleted in shells relative to seawater. Therefore, the *Arctica islandica* shells seem to incorporate elements from the ambient seawater selectively. This finding cannot be explained by kinetic and environmental controls, but suggests that physiological effects are relevant during shell formation.

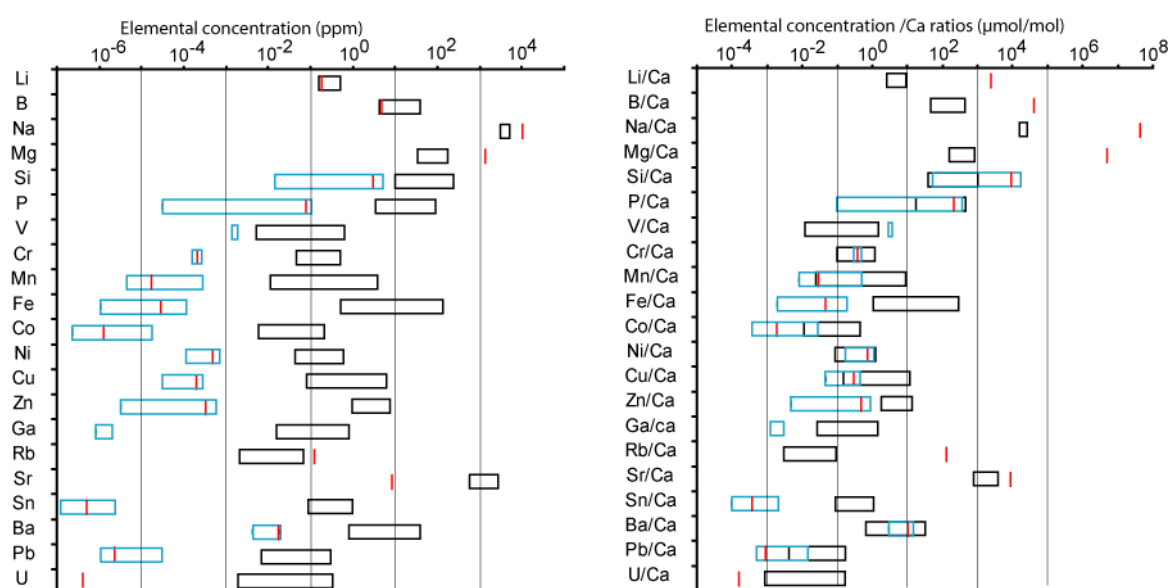


Figure 4.11 Comparison of elemental concentration range of shells with those of normal sea water. The concentration values of sea water were calculated from the original data taken from Bruland & Lohan (2006), Uranium data in sea water came from Turekian (1968). Black rectangles indicate the ranges of the shells, blue rectangles indicate the ranges of seawater. The mean values of sea water are indicated by short red lines.

Similar conclusion have been drawn from results of many other studies, e.g. by Dodd, (1964), Lorens and Bender (1977), Carriker et al. (1980, 1991), Gillikin et al. (2005a) and Strasser et al. (2008). However, it is still not clear what the mechanism is. A variety of studies have suggested that organic matrices play a dominant role on the incorporation of trace elements into bivalve shells (e.g., Albeck et al., 1996; Belcher et al., 1996; Addadi & Weiner, 1997; Dauphin, 2001; Addadi et al., 2006; Takeuchi, et al., 2008; etc).

#### **4.4.5 Environmental controls on trace elemental concentration of *A. islandica* shells**

The environment is believed to control the elemental distributions of shells. As shown above, physiological effects also play a major role in element partitioning between the environment and the shell. Is it possible to eliminate the physiological effects from the data and use the element concentrations (or metal-to-calcium ratios) as environmental proxies?

To explore this hypothesis, the regression curves between element levels and age (Figs. 4.9, 10) were used to detrend the Sr/Ca, Mg/Ca, B/Ca, Ba/Ca, Na/Ca and Mn/Ca time-series. The predicted element concentrations (fitting curves) were removed from the data by computing index values (measured divided by predicted values). Later, the indices were standardized. The standardized data of the four specimens of *A. islandica* were combined into single time-series for each element, smoothed with a five-year moving average and then compared with different instrumental recordings including solar irradiance (Lean et al., 1995), sun spot number (<ftp://ftp.ngdc.noaa.gov/>), North Atlantic Oscillation (NAO, Luterbacher et al., 2002), number of hurricanes in the North Atlantic (Nyberg et al., 2007), precipitation data of Europe (Pauling et al., 2006), surface air temperature (three kinds of data set from Luterbacher et al. (2004), Xoplaki et al. (2005), and Briffa et al., (2001), respectively), sea ice (as a proxy measure for marine productivity) (Koch, 1945), Arctic sea atmosphere temperature index (D'Arrigo et al., 2003), sea-level pressure (D'Arrigo et al., 2003) and Arctic sea surface temperature anomaly (Overpeck et al., 1997). Only the highly correlated relationships are showed in Figs. 4.12 to 4.15.

Standardized indices of Sr/Ca ratios were strongly related to the sun spot number, autumn NAO, autumn Europe surface air temperature (SAT) and Arctic sea surface temperature anomaly (TA) (Fig. 4.12). Time intervals with high sun spot numbers seemed to be positively linked with Sr/Ca ratios, but Sr/Ca ratios still exhibited peaks in the Maunder Minimum and Dalton Minimum, when the sun spots were very low. A detailed analysis demonstrated that the Sr/Ca peaks in the Maunder Minimum coincide with the peaks of the same time of autumn NAO and Europe SAT, while the Sr/Ca peak in the Dalton Minimum agreed well with the peak of Arctic sea surface TA at this time.

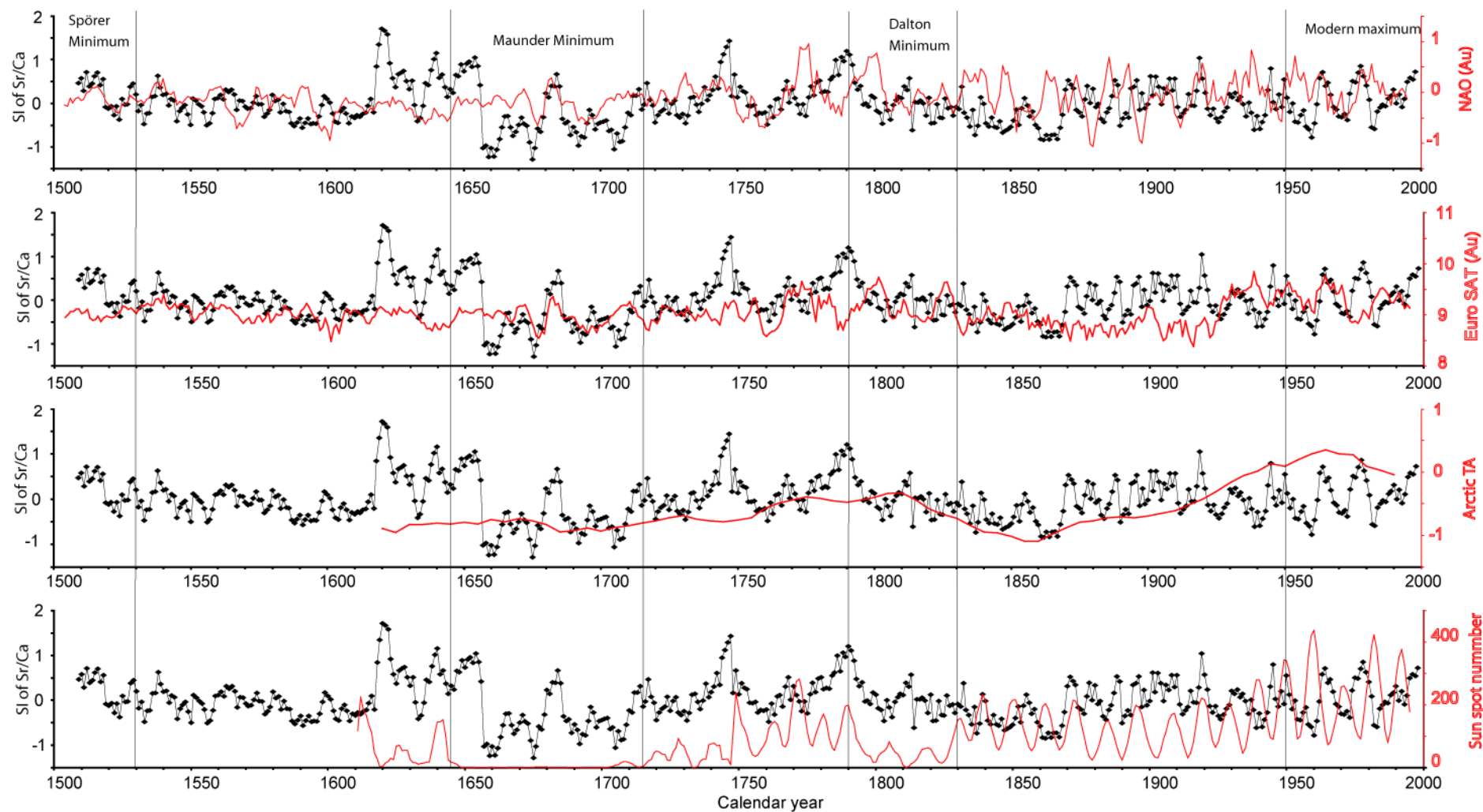


Figure 4.12 Comparison of standardized indices (SI) of Sr/Ca with environmental and climatic parameters. NAO data are from Luterbacher et al. (2002), Europe surface air temperature (SAT) from Luterbacher et al. (2004), Arctic sea surface temperature anomaly (TA) data are from Overpeck et al. (1997), and sun spots numbers are taken from <ftp://ftp.ngdc.noaa.gov/>. Four stages of solar variation are according to Eddy (1976, 1980). Red lines are five-year-moving averages.

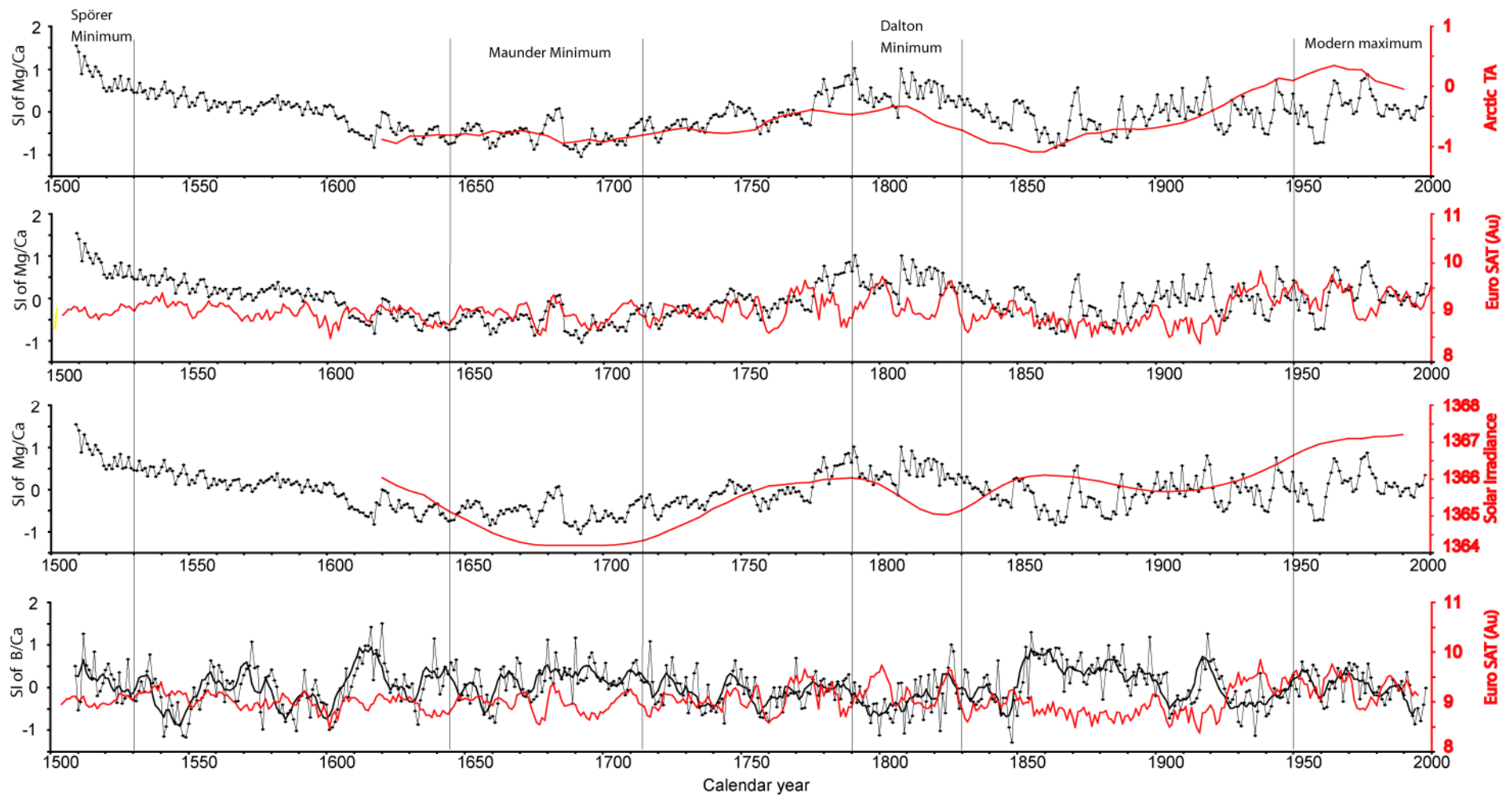


Figure 4.13 Comparison of standardized indices (SI) of Mg/Ca and B/Ca with environmental and climatic parameters. Europe surface air temperature (SAT) data are from Luterbacher et al. (2004), Arctic sea surface temperature anomaly (TA) data are from Overpeck et al. (1997), and sun irradiance data are taken from Lean et al. (1995). Four stages of solar variation are according to Eddy (1976, 1980). Red and black lines are five-year moving averages.

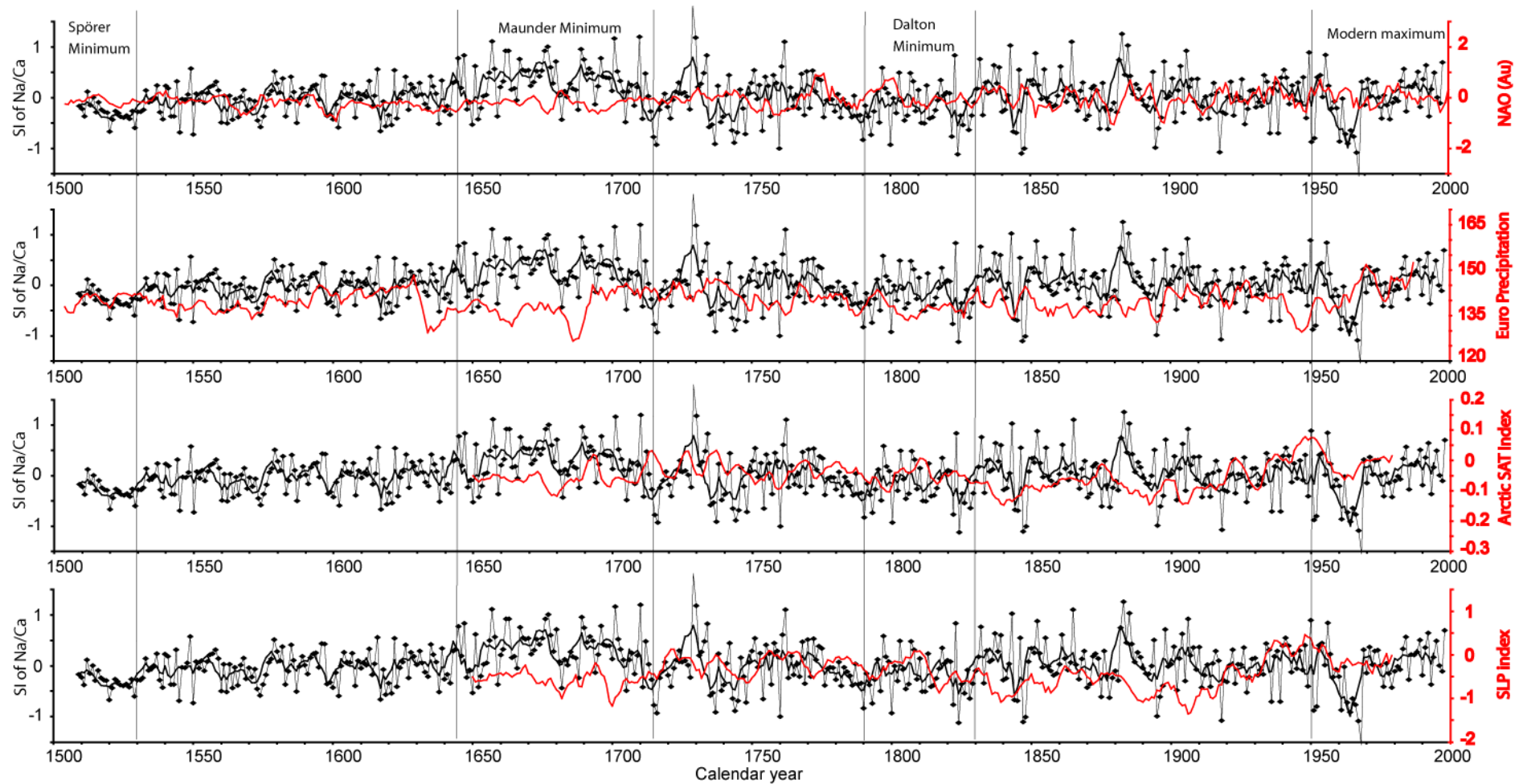


Figure 4.14 Comparison of standardized indices (SI) of Na/Ca with environmental and climatic parameters. NAO data are from Luterbacher et al. (2002), Europe precipitation data are from Pauling et al. (2006). Arctic surface air temperature (SAT) index data are from D’Arrigo et al. (2003), Arctic sea level pressure (SLP) index (data from D’Arrigo et al., 2003). Four stages of solar variation are according to Eddy (1976, 1980). Red and black lines are five-year moving averages.

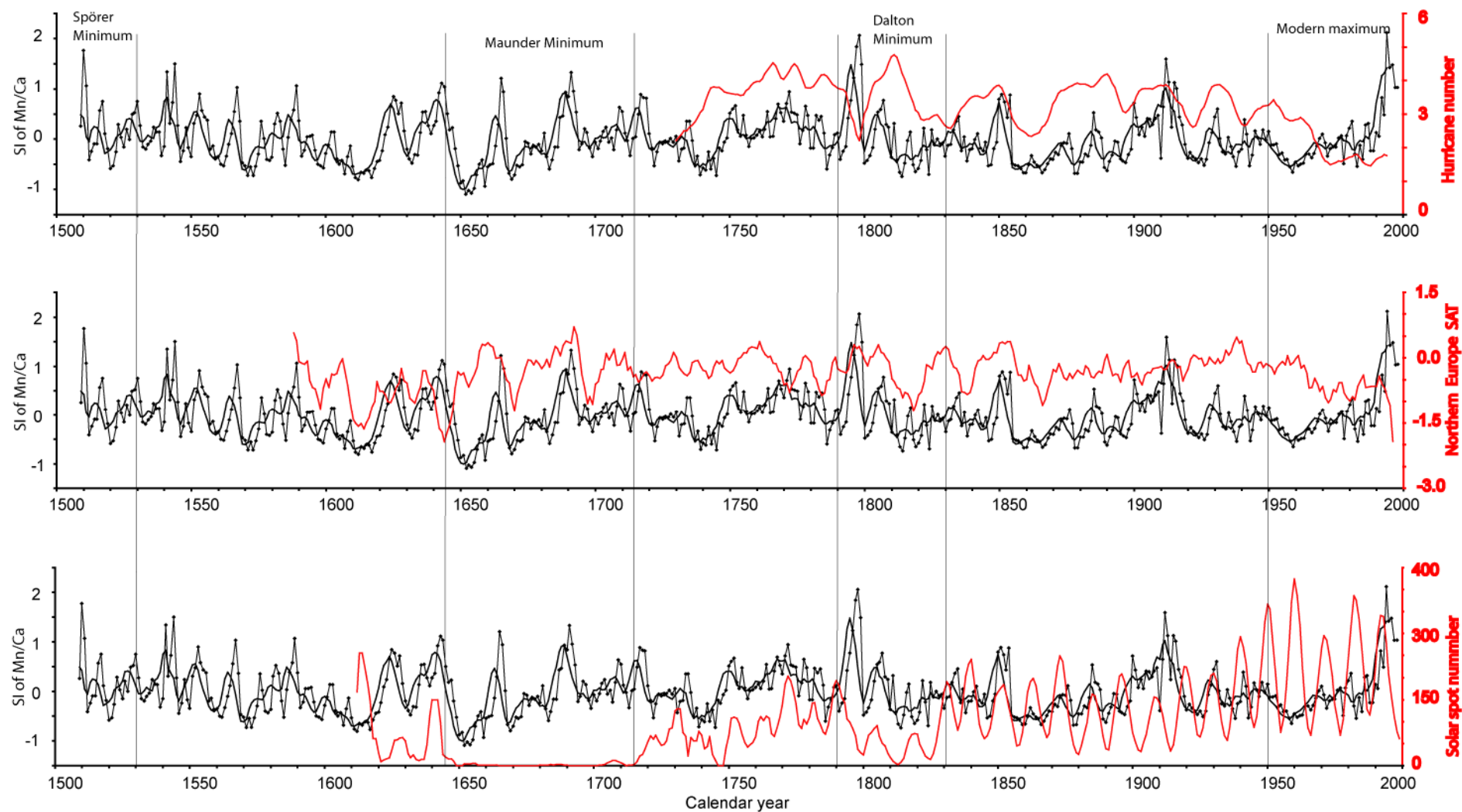


Figure 4.15 Comparison of standardized indices (SI) of Mn/Ca with environmental and climatic parameters. Hurricane numbers of North Atlantic data are from Nyberg et al. (2007), Northern Europe April-September surface air temperature (SAT) data are taken from Briffa et al. (2001), and sun spots numbers are taken from <ftp://ftp.ngdc.noaa.gov/>. Four stages of solar variation are according to Eddy (1976, 1980). Red and black lines are five-year moving averages.

Mg/Ca ratios (Fig. 4.13) were strongly associated with Arctic TA, Europe SAT and Solar variation (irradiance). Only during the Spörer Minimum Mg/Ca ratios were rather high. The variations of autumn Europe SAT demonstrated more similarity with standardized indices of B/Ca than other parameters

Except for the SAT index of Arctic, the standardized indices of Na/Ca showed no distinct relation to temperature (Fig. 4.14). European precipitation and the Arctic sea level pressure index compared well the Na/Ca ratios of the shells, and so did the autumn NAO.

Standardized indices of Mn/Ca were correlated with the number of hurricanes in the North Atlantic, Northern Europe SAT and sun spot numbers (Fig. 4.15).

Further research is certainly required to confirm and corroborate these preliminary findings. However, the combined sclerochronological and geochemical approach clearly indicated that vital effects on the metal-to-calcium ratios can be estimated and mathematically eliminated from the time-series. Afterward their environmental information can be retrieved.



## **5 Early diagenesis of *Arctica islandica* shells – ionic exchanges between water and shell**

### **5.1 Introduction**

As noted in previous chapters and many publications, the trace element composition in bivalve shells is controlled by biological and environmental effects. The latter include the trace element composition of the ambient water (e.g., Odum 1951; 1957a; 1957b; Lorens and Bender, 1980; Almeida et al., 1998a; Richardson et al., 2007; Carroll and Romanek, 2008), water temperature (e.g. Lowenstam, 1954a; 1954b; Palacios et al., 1994; Lazareth et al., 2003; Wanamaker Jr et al., 2008), salinity (e.g. Odum, 1951; 1957; Dodd, 1967; Eisma et al., 1976; Roopnarine et al., 1998), bio-availability of trace elements (e.g., Bourgoin, 1990; Klerks and Fraleigh, 1997; Langlet et al., 2005; Protasowicki et al., 2008), climatic effects (e.g. Frazier, 1975; Clifton et al., 1989; Hendry et al., 2001; Tynan et al., 2008), anthropogenic effects (e.g. Labonne et al., 1998; Gillikin et al., 2005c; Dunca et al., 2008), the chemistry of the sediment (e.g. Swann et al., 1991; Homziak et al., 1993; Madkour, 2005), and some unknown environmental factors (e.g. Gillikin et al., 2008).

However, no study has quantified early diagenetic changes of the trace elemental composition of bivalve shells. After the shells have formed they are largely precluded from the vital activity and can potentially exchange ions with the ambient water. Such ionic exchanges may significantly alter the chemical signature of bivalve shells and may reconstructions of environmental conditions from the shell chemistry impossible.

This chapter reports on ionic exchange experiments between water and shells. It was studied how the chemistry of shells with (fresh, alive or soon after death) and without perisotracum (subfossil) changes through time. In addition, it was tested if such ionic exchanges are stronger near the surface of the shells than deeper inside. Results of this study may help to better understand the environmental information of the trace element record in bivalve shells. Furthermore, the results may help to devise an appropriate sampling strategy for trace elements, e.g., sampling at a constant distance from the outer shell surface.

## 5.2 Material and methods

### 5.2.1 Sample material, preparation and experimental procedure

30 *A. islandica* specimens of different ontogenetic ages were collected alive from ca. 30m water depth in NE Iceland in 2006 and stored in a freezer since then. After thawing the bivalves, soft parts were removed and the valves cleaned in tap water, VE water and MQ water. Then, the shells were ultrasonically rinsed for three minutes in MQ water and dried at room temperature.

For preparation of experiment I, five PE containers (labeled 01 to 05) were filled with one liter MQ water (pH: ca. 6.16). The MQ water of containers 04 and 05 were doted with 2 ppm Mg, Sr, As, Ba and Mn. Then, one valve (Tab. 5.1: L=left, R=right valve) of each of 20 specimens of *A. islandica* (ICE06-6.2-A01 to ICE06-6.2-A20) was selected and the other halves were put aside as controlling valves. The selected valves were split into four groups and sorted by shell sizes so that every group contained about the same spectrum of different shell sizes. For this purpose, shell height, length and width were measured with a digital caliper. The periostracum of two sets of valves (2 x 5 shells) was physically removed with a diamond coated drill bit, ultrasonically rinsed in MQ water and dried from air. Removal of the periostracum should simulate worn, dead shells. Does the shell geochemistry changes quicker in shells without the protecting layer? In addition, it was planned to analyze if the periostracum exchanges ions with the ambient water while protecting the shell carbonate directly underneath. The four sets of valves, two sets containing shells with the periostracum still intact and two sets containing shells without periostracum, were placed in four of the plastic containers. Valves with periostracum were placed in containers 02 and 04, while valves without periostracum were put in containers 03 and 05. Container 01 served as a control container (blank, background).

Experiment II was prepared similar to experiment I, but with MQ water buffered to pH 8 by adding NaOH in order to simulate seawater pH. Both valves of each of the remaining ten specimens (ICE06-6.2-A21 to ICE06-6.2-A25, ICE06-4.2-A101 to ICE06-4.2-A105) were used in this experiment. The valves were distributed among four different containers, so that container 07 and 09 contained shells without periostracum and containers 08 and 10 valves with periostracum. 1 ppm Mg, Sr, As, Ba, Mn and Pb were added to containers 07 and 08. Container 06 was used as control.

Each of the two experiments lasted for 47 days. Table 5.1 gives an overview of the two experiments.

Container	pH at the beginning	Specimens	Solution	Trace Elements	Periostracum	Area (cm <sup>2</sup> )	pH at the end
01	6.16		MQ water				6.16
02	6.16	ICE06-6.2-A11R, 12L, 13L, 14L, 15L	MQ water		with	244.86	7.94
03	6.16	ICE06-6.2-A6R, 7L, 8L, 9L, 10L	MQ water		without	261.73	8.11
04	6.16	ICE06-6.2-A16L, 17L, 18L, 19R, 20L	MQ water	T1	with	269.55	7.98
05	6.16	ICE06-6.2-A1L, 2L, 3R, 4R, 5L	MQ water	T1	without	239.20	8.02
06	7.96		MQ water with NaOH				7.53
07	8.01	ICE06-4.2-A101L, 102L, 103L, 104L, 105L	MQ water with NaOH	T2	without	220.15	8.04
08	7.99	ICE-6.2-A21L, 22L, 23L, 24L, 25L	MQ water with NaOH	T2	with	242.02	8.04
09	8.00	ICE06-4.2-A101R, 102R., 103R, .104R., 105R	MQ water with NaOH		without	220.15	8.27
10	8.04	ICE06-6.2-A21R, 22R, .23R., 24R., 25R	MQ water with NaOH		with	242.02	7.99

Table 5.1 Ionic exchange experimental setup. Containers 01 to 05 were used in experiment I, containers 06 to 10 in experiment II. Containers 01 and 06 were control samples for blank measurements containing MQ water and buffered MQ water, respectively. All other containers contained shells with and without periostracum. T1 = 2ppm Mg, Sr, As, Mn and Ba; T2 = 1ppm Mg, Sr, As, Mn, Ba, and Pb. The values in the “Area” column were computed by summing up the height times width values of each valve in the containers. R = right valve; L = left valve.

After the end of the experiments, 50 ml of the water of each container was acidified to 2 wt% with suprapure nitric acid (69 wt%) and stored for subsequent chemical analyses. All valves were ultrasonically rinsed in MQ water and dried at room temperature. The periostracum was physically removed from shells that still contained this protecting layer and stored for later chemical analyses. Then, all valves were cut along the axis of maximum growth with a Buehler low-speed saw.

Three portions (tip, middle and umbo) of each valve including the 20 control valves from the first experiment were sampled with a diamond-coated drill bit. From each shell portion, four to seven samples were milled in the outer (primary) shell layer sub-parallel to the outer shell surface toward the inside of the shells. Each milling step measured about 200µm.

From each sample, 2.1-2.3 mg of shell powder were dissolved in 1 ml suprapure HNO<sub>3</sub> (69 wt%) and filled with MQ water to about 5 g. In addition, 2.1 to 2.3 mg of each periostracum sample was dissolved in 1 ml suprapure HNO<sub>3</sub> (69 wt%), reacted at a temperature of ca. 60°C for several hours until all particles were dissolved. Then, each sample was filled with MQ water to ca. 5 g and stored for chemical analyses.

### **5.2.2 Chemical analysis**

The chemical composition was measured with an ICP-OES (Spectro Ciros Vision SOP) at the University of Mainz. Because of different matrices, two different analytical methods were used to measure the water samples and the shell carbonate samples. For the water samples, a sequence of X-Spex standard (Horiba Jobin Yvon, X-Spex 1:20, 1:50, 1:100, 1:200, 1:1000, and 1:2000) and one blank were used as calibration standards. For the shell carbonate and periostracum samples, calibration solutions were kept at the same Ca concentration (170 mg/L) as the sample solutions. A sequence of As (1, 10, 20, 40, 80 ppb), Mg (10, 100, 200, 400, 800 ppb), Mn (1, 10, 20, 40, 80 ppb), and Sr (50, 500, 1000, 2000, 4000 ppb) were added to the calibration solutions, respectively, to cover expected element concentrations of the samples. All water samples, some of the shell powder samples and four periostracum samples of two specimens were chosen to be analyzed. The analytical precision of the elements measured were below 1 % RSD for shell powder samples. For water samples the precisions was about 1.5 % RSD, except for samples of container 07 where RSD was 5.6 %.

### **5.3 Results**

After 47 days of experiment duration, the pH of the unbuffered solutions containing shells changed from 6.16 to near 8, whereas the pH of buffered solutions remained approximately constant (Tab. 5.1). Notably, these changes were unaffected by the preservation status of the shells, with or without periostracum. The pH of the control water of experiment I was unchanged, whereas the pH of the buffered control water decreased slightly from 7.96 to 7.53 (Tab. 5.1).

The control water in containers 01 and 06 was investigated after the experiments and results showed that no elements were detected by ICP-OES, except for Na and K in the NaOH-buffered water in container 06.

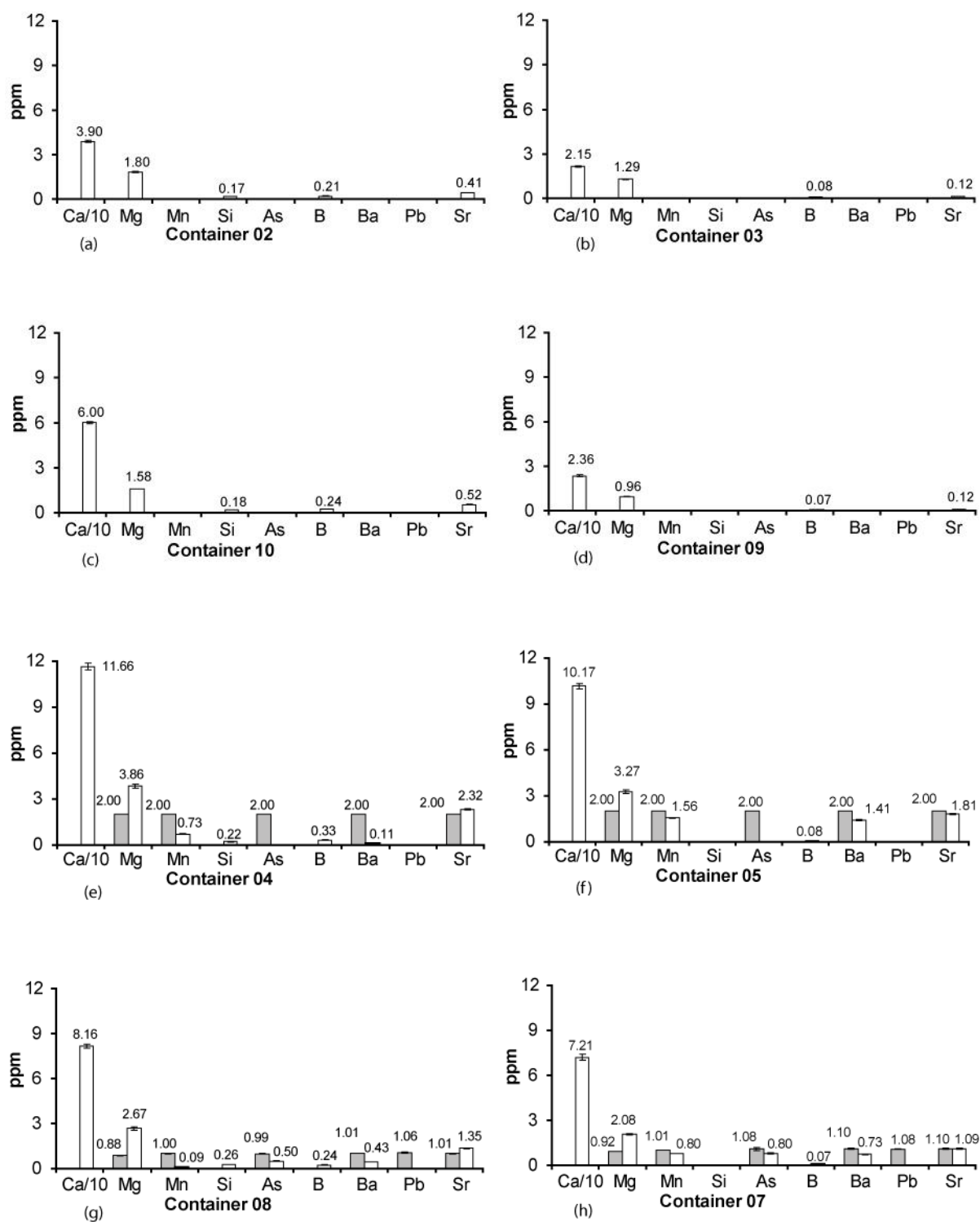


Figure 5.1 Elemental concentrations in the solutions before (grey) and after (white) the experiments. For meaning of container numbers see Table 5.1. Error bars are given in two standard deviations.

### ***5.3.1 Changing element concentrations in non-doted water (containers 02, 03, 09, 10)***

After the experiments, the water in containers 02 and 03 (originally MQ water) contained Ca, Mg, Sr and B (Figs. 5.1a, b). The amounts of these elements were slightly higher in the water of container 02 that contained shells with periostracum. In addition, container 02 showed some amounts of Si.

Similar findings were made in case of the NaOH-buffered water (Figs. 5.1c, d). Element concentrations were about the same as for pure MQ water. However, the Ca levels were significantly higher in the buffered water containing shells with periostracum (Fig. 5.1c).

### ***5.3.2 Changing element concentrations in doted water (containers 04, 05, 07, 08)***

For both experiments with doted water, the net gain in elemental concentration was comparable to that in the experiments starting with MQ water. Mg, Si and B increased by about the same amount in the solutions containing shells with periostracum (Figs. 5.1e, g) and solutions without periostracum (Figs. 5.1f, h). The Sr content increased slightly in the solutions with shells with periostracum (Figs. 5.1e, g). However, in the solutions with shells without periostracum, Sr levels in the water remained unchanged or slightly decreased (Figs. 5.1f, h). Ca concentrations were significantly higher in all experiments. Concentration of Mn, As, Ba and Pb that were added to the initial solutions were significantly lower after the end of the experiments (Figs. 5.1 e-h).

### ***5.3.3 Changing element concentrations in the periostracum***

After the end of the experiment, two chosen periostracum samples of left valves from two specimens that were immersed in a buffered, doted solution containing 1 ppm Mg, Sr, As, Mn and Ba (container 08) were clearly enriched in Pb, As and Mn (Fig. 5.2). The enrichment was stronger in the larger, ontogenetically older shell ICE06-6.2-A24 than in ICE06-6.2-A22. Mg, Sr and Ca, however were nearly identical.

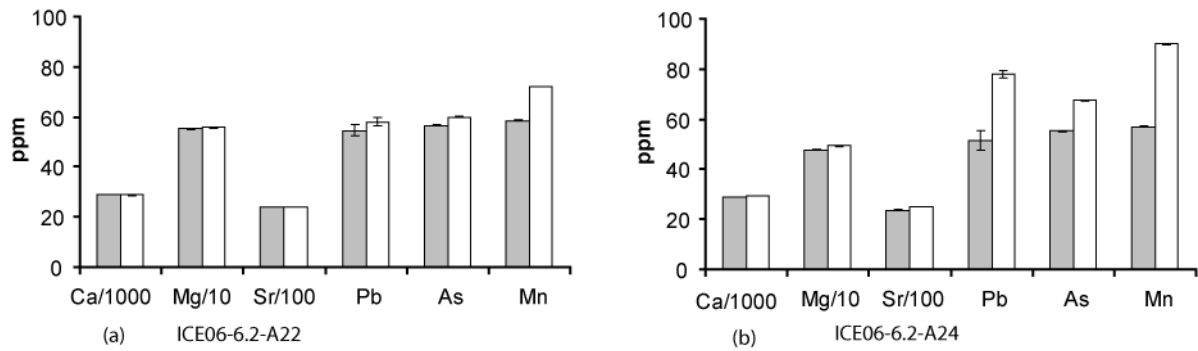


Figure 5.2 Elemental concentrations in the periostracum of specimens ICE06-6.2-A22 (a) and ICE06-6.2-A24 (b). Right valves of the two specimens were immersed in buffered (pH 8) MQ water (grey), while the left valves of both specimens were immersed in dotted (1 ppm Mg, Sr, As, Mn, Ba and Pb), buffered (pH 8) MQ water (white) for 47 days. Error bars are given in two standard deviations.

#### 5.3.4 Changing element concentrations in shell carbonate

The left valve of specimen ICE06-6.2-A20 was chosen to analyze how the element concentration of shell carbonate was affected by exposure to a solution with 2 ppm Mg, Sr, As, Mn and Ba (container 04), while the right valve of this specimen was used as a control sample and not used in the ionic exchange experiments. As and Mn were undetectably small. Therefore, only results for Ca, Mg and Sr are depicted in Fig. 5.3. As seen from the data, the tip portion of the shells was most severely affected by ionic exchanges: Mg and Sr were significantly depleted and Ca enriched in shell portions close to the outer shell surface, while the umbonal and middle portions of the shell remained nearly unchanged. A slight depletion was also observed for Mg in the outer shell layer of the middle and umbonal shell portions, while the concentration of Sr in the outer shell layer of the two portions showed almost no change. Note that the shell carbonate was covered by periostracum during the experiment.

Interestingly, even the valve that was not used in the experiment showed significant variations of all three elements. Ca decreased toward the outer shell surface, while both Sr and Mg strongly increased.

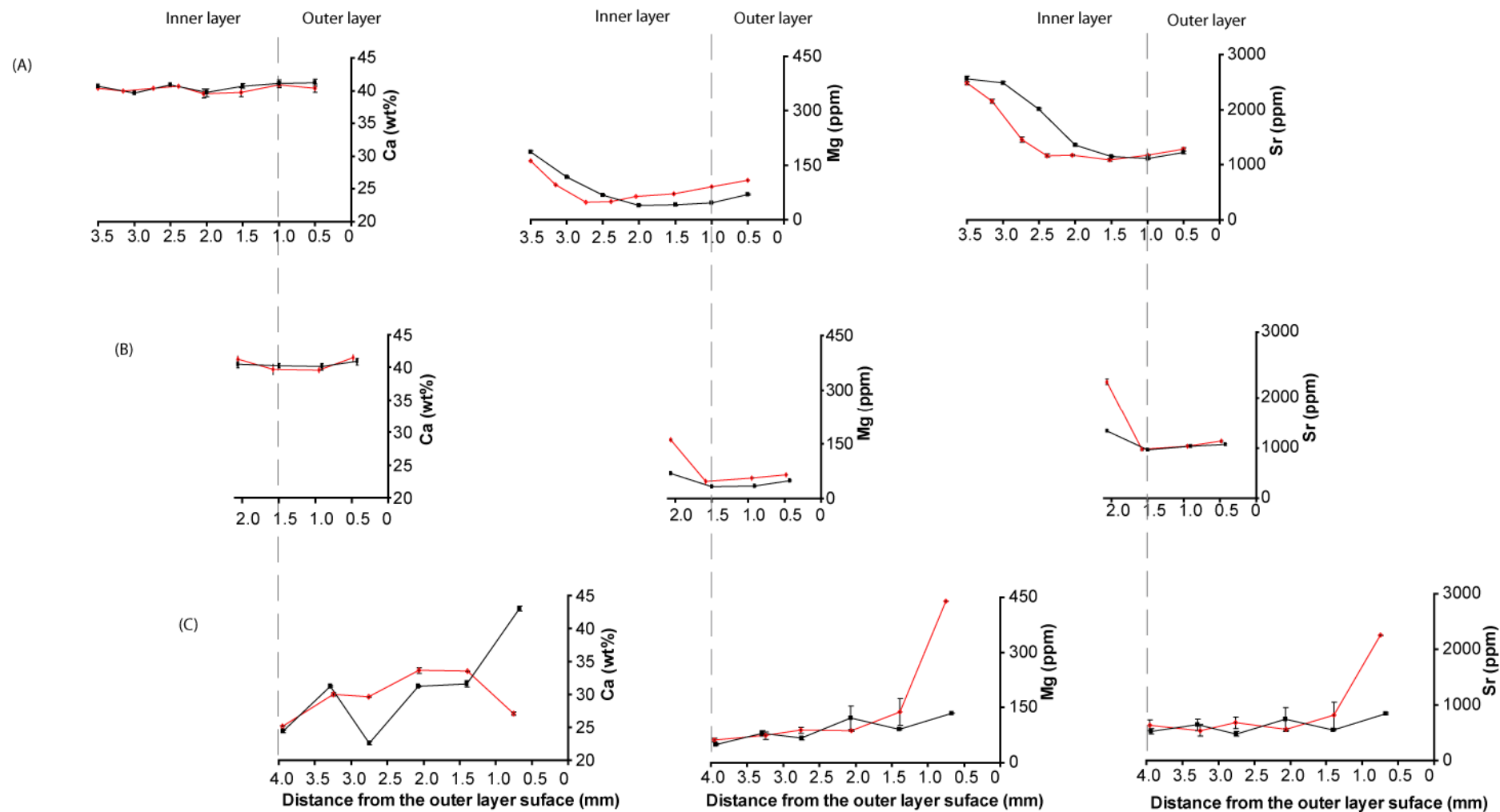


Figure 5.3 Ca, Mg and Sr concentration in shell carbonate of specimen ICE06-6.2-A20 before (black; left valve) and after the immersion in a solution containing 2 ppm Mg, Sr, As, Mn and Ba (red; right valve). Note that the shell surface was covered by periostracum during the experiment. Error bars = two standard deviations. Figure (A) refers to the umbonal part of the shell, Figure (B) and (C) depict middle and tip part of the shell respectively. Error bars are given in two standard deviations.



## 5.4 Discussion

The experiments clearly demonstrated that Ca, Mg and B were leached from the shells to the water, irrespective of the initial pH, irrespective of ionic concentrations in the starting solutions and irrespective of the presence of a periostracum (Fig. 5.1). These findings contradict findings by Carriker et al. (1991) who demonstrated that Mg and Mn increased in concentration near the shell surface during chemical and physical weathering

As seen from Fig. 5.3, Sr and Mg likely come from the carbonate of the tip portions of the shells. These shell portions may be composed of smaller and more numerous aragonite crystals than umbonal and middle shell portions and therefore provide a larger surface area that is in contact with the ambient water. And the umbonal and middle shell portions keep stable Sr concentrations during the experiments in outer shell layer. However, further studies need to investigate the changing crystal fabrics across shells of this species.

Another source for Mg in the water is the periostracum rather. This interpretation agrees with findings presented in chapter 2 suggesting that Mg is bound by metalloproteins.

However, Ca release from the shells was significantly larger in cases with initially doted water (Figs. 5.1e-h). This finding may indicate that Ca was also released from intercrystalline organics. Maybe, Ca of the organics was exchanged against other ions (Pb, As, Mn, Ba) that were available in the ambient solution.

Si leached only from shells with periostracum (Figs. 5.1a, c, e, g). Most likely, the silicate comes from small sediment grains that were trapped in the organic protecting layer.

Sr release from the shells was not observed in experiments with doted starting solutions and shells with periostracum (Figs. 5.1e, g). This may indicate that the periostracum protected the shell carbonate of the outer shell carbonate from extensive leaching. As shown in Figure 5.3, however, even carbonate portions underneath periostracum at organic-rich tip portions of the shells lost some amounts Sr, Mg and Ca. It is hypothesized here that the intercrystalline organics exchanged ions with the doted ambient solution and thereby lost their protective capability. Sr was dissolved from the carbonate lattice, but was then absorbed to crystal surfaces and therefore did not become enriched the ambient water.

Some of the elements that were added to the starting solutions were absorbed by the shells, namely Mn, As, Ba and Pb (Figs. 5.1e-h). Most likely, these elements were absorbed by organics rather than shell carbonate itself as indicated by Figure 5.2. Larger, older shells seem to absorb more of these elements than young shells (Fig. 5.2). This is probably attributed to the greater amount of intercrystalline organics in older shells (e.g., Goodfriend & Weidman, 2001). Organics also include the periostracum. It is well known that some elements are absorbed by the periostracum. For example, Allen (1960) found that Mn may be enriched in the periostracum of shells which lived near the surface of the sediment. In addition, the Mn content far exceeded that of the bottom sediments. Sturesson (1976, 1978) also observed that ca 75% of Pb and Cd in *Mytilus edulis* shells were adsorbed onto the periostracum. This also applied to other elements such vanadium (Miramand et al., 1980).

The most startling finding was that the shell carbonate underneath the periostracum showed significant alterations in Ca, Mg and Sr after exposure to the doted solution. Despite the presence of the periostracum, Mg and Sr went out of the shell and Ca into the shell. The protective capability of the periostracum is there fore very limited. Even the valve that was not used in the experiment showed considerable variability from the outer shell surface toward the inside. Early diagenesis (even during life of the animal) may therefore strongly affect the chemical composition of bivalve shells, especially the outer shell surface of older, organic-rich shell portions. This should be kept in mind when shell carbonate is analyzed for trace element variations from the umbo toward the commissure. Samples should be taken at some distance from the outer shell surface.

## 6 Summary and conclusions

### 6.1 Main finding of the research

- (1) Trace elemental concentrations of bivalve shells are affected by early diagenesis by the leach or exchange of elemental ions, especially in shell tip part, even with the protection of periostracum.
- (2) The analytical methods also affect the results of trace elemental concentrations, especially for elements which are highly enriched in organic matrices, such as Mg.
- (3) Shell organic matrices play a dominating role on the concentration of trace elements on *A. islandica* shells. Most trace elements only occurred in insoluble organic matrices (IOM), and only Li, B, Na, Mg, Fe, Sb and Ba are found in the carbonate fraction. IOM of *A. islandica* shells is significantly enriched in Mg, and highly concentrated in B, Al, P, K, Fe, and Sb as well, while Li, and Na are more depleted in IOM, but enriched in shell carbonate. Ba is more or less even contented in IOM and shell carbonate. Because of slight difference in shell organic matrix in different shell layers, the concentrations of B, V, Cr, Mn, Co, Ga, Pb and U are slightly higher in the primary layer than in the secondary layer, while those of Li, Mg, Zn, Sr, Sn and Ba show the opposite. The reasons why trace elements are distributed differently on the shells might be the difference in shell organic matrices traits and content during life, the difference of physical and chemical properties of trace elements and inter-reaction between them function differently in different shell portions.
- (4) The vital /physiological controlling on trace elemental distributions on bivalve shells is identified at least in three aspects in the study. (a) Bivalves incorporate trace elements selectively. Comparing with Ca contents in ambient sea water, Mn, Fe, Co, Zn, Ga, Sn, Pb and U are enriched in shells, while the remaining elements such as Li, B, Na, Mg, Si, P, V, Rb, and Sr are depleted; (b) The concentrations of trace elements demonstrate four variable patterns with ontogenetic age. Moreover, six elemental (B, Na, Mg, Mn, Sr, and Ba) concentrations show a significant correlation with ontogenetic year. Exponential functions were found to fit these relationships well. B, Mg, Sr, and Ba are positively correlated with age, while Na and Mn are negatively correlated; (c) Regression analyses also show that the concentration of the same six elements (B, Na, Mg, Mn, Sr, and Ba) are highly correlated with shell grow rates (shell annual increment width) in logarithmic equations. The reasons of these three vital controlling might be ageing ion channels, changes in amino acid concentration and organic matrix effects.

## **6.2 Implications for the application of trace element-calcium ratios of bivalve shells as environmental and climatic proxies**

- (1) Because of early diagenesis, the ventral margin of the shells should be avoided to be investigated. Sr is more stable than Mg in the outer layer of umbonal and middle parts of the shells, and therefore might be a better proxy for environmental and climatic change.
- (2) Because of the high enrichment of Mg (and some other elements) in the insoluble organic matrix, wet chemical analyses are superior to in situ techniques (such as LA-ICP-MS) for the investigation of the Mg concentration and other elements with an affinity to organics.
- (3) Probably because of the different affinity to organics and shell carbonate, trace elements show different sensitivity to environment and climate, and therefore the proxies of trace element-calcium ratios should be chosen accordingly. Sr/Ca ratios are strongly related to the sun spot number, NAO, surface air temperature (SAT). Mg/Ca ratios are only strongly associated with SAT and solar irradiance. B/Ca is only correlated with SAT. Except for the SAT, Na/Ca showed distinct relation with precipitation, the sea level pressure and NAO. Mn/Ca is correlated with the number of hurricanes, SAT and sun spot number.
- (4) Although trace elemental distribution of *A. islandica* shells are confirmed to be affected many non-environmental and climatic controls, the correlation functions between trace elemental concentrations (B, Na, Mg, Mn, Sr, and Ba) and shell ontogenetic year and growth rates could be applied in the standardization to subtract the noise and to derive the environmental and climatic signals.

## **6.3 Prospective research in the near future**

There are two important problems that need to be addressed in the near future. One is new statistics should to be exploited to subtract the noise and reconstruct the environmental and climatic variations of the past. The other is studies should be conducted on the study of the process of biomineralization in bivalve shells.

## Zusammenfassung

Die aktuelle Studie beschäftigt sich mit Spurenelement-Zusammensetzungen innerhalb der Schalen von *Arctica islandica*. Wesentliche Erkenntnisse lassen sich wie folgt zusammenfassen:

### (1) Verteilung von Spurenelementen in verschiedenen Schalenlagen

Die meisten Elemente finden sich innerhalb der primären und sekundären Schalenlage in ähnlichen Konzentrationen. Die Konzentrationen von B, V, Cr, Mn, Co, Ga, Pb und U sind in der primären Lage angereichert, während die von Li, Mg, Zn, Sr, Sn und Ba reduziert sind. Im Vergleich zum umgebenden Meerwasser kommen die meisten Elemente, außer Na, Mg und Rb, im Schalenkarbonat in höherer Konzentration vor. Hingegen zeigen Metall/Calcium Verhältnisse unterschiedliche Resultate. Nur Cr/Ca, Ni/Ca, Cu/Ca und Ba/Ca befinden sich im gleichen Verhältnis, in der Schale und im Meerwasser. Relativ zum Meerwasser sind Werte für Mn/Ca, Fe/Ca, Co/Ca, Zn/Ca, Ga/Ca, Sn/Ca, Pb/Ca und U/Ca in der Schale erhöht, während Li/Ca, B/Ca, Na/Ca, Mg/Ca, Si/Ca, P/Ca, V/Ca, Rb/Ca und Sr/Ca in Schalen vermindert auftreten. Aus diesem Grunde scheinen Schalen von *A. islandica* Elemente aus dem umgebenden Meerwasser selektiv einzubinden (Vitaleffekt).

### (2) Organik-gebundene Spurenelemente

Aufgrund unterschiedlicher Bindung an die organische Matrix, können verschiedene Analysemethoden (*in situ* gegenüber nasschemischer Analyse) Ergebnisse über Elementzusammensetzungen beeinflussen. Obwohl das durchschnittliche, relative Auftreten an unlöslicher Matrix (IOM Proteine) in Schalen von *A. islandica* kaum 0.5 Gewichts-% übersteigt, können Analysen über die chemische Zusammensetzung der Schale wesentlich höhere Werte liefern, wenn mit LA-ICP-MS gemessen wird. Die IOM der *A. islandica*-Schale ist besonders angereichert an Mg, jedoch verarmt an Sr und Ca im Vergleich zum Anteil des inorganischen Karbonats (wie auch löslicher Organik, d.h. Zucker und Vorläufer von Zucker). Ebenso zeigten andere Elemente Bindung an die organische Matrix. Besonders B, Mg, Al, P, K, Fe und Sb sind höher in der IOM konzentriert als im Kristallgitter, wohingegen Li, B, Na, Mg, Fe, Sr und Ba nur in der Karbonatkomponente auftreten. Ba ist in beiden Schalenkomponenten gleichsam vertreten.

### (3) Frühdiagenese verändert die Signatur von Spurenelementverteilungen in Muschelschalen.

Experimente haben klar gezeigt, dass es ionische Austauschprozesse zwischen Schale und umgebendem Wasser gibt. Ca, Mg und B wurde aus der Schale ins Wasser überführt,

unabhängig des anfänglichen pH-Werts, der ionischen Zusammensetzung der Ausgangslösung und der Anwesenheit des Periostrakum. Der Verlust von Ca aus den Schalen an das umgebende Wasser fiel jedoch erheblich höher aus, wenn das Wasser angesetzt war an Mg, Sr, Pb, As, Mn and Ba. Innerhalb solcher Experimente wurden Mn, As, Ba und Pb von den Schalen absorbiert. Schalen mit Periostrakum gaben Si an das Wasser ab. Abschnitte nahe des ventralen Schalenrandes scheinen am größten von der Frühdiagenese beeinflusst zu werden.

Der erstaunlichste Fund war, dass das Schalenkarbonat unterhalb des Periostrakums bedeutende Veränderungen in Ca, Mg und Sr nach Immersion in der angesetzten Lösung zeigte. Trotz der Anwesenheit des Periostrakums wurde Mg und Sr aus der Schale exportiert, während Ca in die Schale importiert wurde. Die Schutzfähigkeit des Periostrakums ist deshalb nur begrenzt. Selbst die Schalen, die nicht im Experiment verwandt wurden, zeigten deutliche Unterschiede von der äußeren zur inneren Schalenoberfläche. Frühdiagenese (selbst während der Lebenszeit des Tieres) kann deshalb deutlich die chemische Zusammensetzung der Schale beeinflussen, insbesondere in der äußeren Schalenoberfläche älterer, organikreicher Schalenabschnitte. Dies sollte besondere Beachtung bei der Analyse von Spurenelementvariationen des Schalenkarbonats vom Wirbel bis zur Kommissur finden. Die Probennahme sollte im Abstand zur äußeren Schalenoberfläche erfolgen.

#### (4) Vitaleffekte

15 der 21 gemessenen Elemente zeigen keine Beziehung zum ontogenetischen Alter oder Biomineralisationsrate. Indes scheinen sie nicht durch Vitaleffekte beeinflusst. Sr, Mg, B, Ba, Na und Mn zeigten eine deutliche (nicht-lineare) Beziehung zum ontogenetischen Alter und der jährlichen Inkrementweite. Dies impliziert, dass gewisse Vitaleffekte einen starken Einfluss auf Konzentrationen dieser Elemente in den Schalen von *A. islandica* nehmen. Mögliche Gründe für die beobachteten Trends können beinhalten: (1) Alterung der Ionenkanäle oder Enzyme innerhalb der Mantelepithel, die kontrollieren welche Elemente zum Ort der Kalzifikation befördert werden und welche untersättigt in der extrapallialen Flüssigkeit verbleiben. (2) Wechselnde Aminosäuren-Konzentration und Zusammensetzungen innerhalb der Ontogenese.

#### (5) Umweltrekonstruktionen basierend auf Chemie des Schalenmaterials

Die aktuelle Studie zeigt, dass es möglich ist, physiologische Effekte von Elementkonzentrationen zu entfernen und Metall/Calcium Verhältnisse als Umwelt-Proxies zu benutzen. Um diese Hypothese im Weiteren zu evaluieren, wurden die Regressionskurven zwischen Elementkonzentrationen und dem ontogenetischen Alter einer mathematischen Trendentfernung unterzogen. Dies gilt insbesondere für Zeitreihen von Sr/Ca, Mg/Ca, B/Ca, Ba/Ca, Na/Ca und Mn/Ca. Standardisierte Indizes von Sr/Ca-Verhältnissen standen in deutlicher Korrelation mit der Anzahl von Sonnenflecken, der Nordatlantischen Oszillation (NAO), der Europäischen Oberflächen-Lufttemperatur im Herbst (SAT) und den Arktischen Meerestemperatur-Anomalien (TA). Zeitintervalle mit hoher Sonnenfleckenanzahl schienen positiv verknüpft mit den Sr/Ca-Verhältnissen. Mg/Ca Verhältnisse standen in deutlicher Verbindung mit TA, SAT und der Sonneneinstrahlung. Die SAT-Variationen zeigten eine größere Beziehung mit standardisierten Indizes von B/Ca als zu anderen Parameter. Außer dem SAT-Index zeigten standardisierte Indizes von Na/Ca keine besondere Beziehung zur Temperatur. Europäischer Niederschlag und der Index des arktischen Meeresspiegel-Luftdrucks zeigten eine gute Verbindung zu den Na/Ca-Werten in den Schalen. Das gleiche gilt für NAO. Standardisierte Indizes für Mn/Ca korrelierten mit der Anzahl von Hurrikans im Nord-Atlantik, wie auch SAT und der Anzahl von Sonnenflecken.

Die aktuelle Studie liefert wichtige Grundlagen und zeigt, dass es im Allgemeinen möglich ist Schlussfolgerungen über Umweltvariablen aus chemischen Eigenschaften in Schalen von *A. islandica* zu ziehen. Weitere Studien sind notwendig, um die Erkenntnisse dieser Studie noch weiter zu vertiefen. Insbesondere die Verknüpfung von direkten Umwelteinflüssen mit der Schalenchemie benötigt eine Quantifizierung, um mathematische Beziehungen aufstellen zu können, die eine präzise Ableitung von Umweltsignalen aus den Schalen von *A. islandica* erlauben.

Weitere Studien verlangen ebenso die Untersuchung der Rolle von strukturellen Änderungen im Kristallgefüge entlang des Schalenwachstums dieser Art. Zum Beispiel bedingen kleine Kristallgrößen eine größere Oberfläche an die gewisse Elemente adhäsiv gebunden werden können: Je kleiner der Kristall, desto größer die Verfügbarkeit an organischer Matrix, die einzelne Kristalle umgibt, wie auch die Zahl an Elementen, die sich an die Organik binden lassen.

Es sollte noch hervorgehoben werden, dass einige Elemente klare Zusammenhänge miteinander aufweisen. Zwei Elementpaare, d.h. Mg und Sr, wie auch Ga und Ba, zeigten sich als stark korreliert ( $R^2 = 0.61$  bis  $0.8$ ). Bis dato gibt es keine Dokumentation über eine Beziehung zwischen Ga und Ba. Weitere Untersuchungen werden benötigt, um die Hintergründe dieser Beziehung zu verstehen.



## Appendix

- File 1- Effect of organic matrix on trace elements.xls  
Sheet 'portion trace elemental content'- data for Fig. 3.4  
Sheet 'layer difference'- data for Fig. 3.5
- File 2- Continue laser results variations summary-Mainz.xls  
Data for Fig. 3.7, Figs. 4.1-10, Figs. 12-15.
- File 3- Water-shell reation experiment results.xls  
Data for Fig. 5.1-3.  
(in attached CD)

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## Publications

- (1) Schöne, B.R., Zhang, Z., Jacob, D., Gillikin, D.P., Tütken, T., Garbe-Schönberg, D., McConnaughey, T. & Soldati, A., 2009. Effect of organic matrices on the determination of the trace element chemistry (Mg, Sr, Mg/Ca, Sr/Ca) of aragonitic bivalve shells (*Arctica islandica*) – Comparison of ICP-OES and LA-ICP-MS data. *Geochemical Journal* 43 (in press).
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