Heavy metal uptake of near-shore benthic foraminifera during multi-metal culturing experiments

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Abstract. Heavy metal pollution originating from anthropogenic sources, e.g., mining, industry and extensive land use, is increasing in many parts of the world and influences coastal marine environments even after the source has ceased pollution. The elevated input of heavy metals into the marine system potentially affects the biota because of their toxicity, persistence and bioaccumulation. An emerging tool for environmental applications is the heavy metal incorporation into foraminiferal calcite tests, which facilitates monitoring of anthropogenic footprints on recent and past environmental systems. The aim of this study was to investigate whether the incorporation of heavy metals in foraminifera is a direct function of their concentration in seawater. Culturing experiments with a mixture of dissolved chromium (Cr), manganese (Mn), nickel (Ni), copper (Cu), zinc (Zn), silver (Ag), cadmium (Cd), tin (Sn), mercury (Hg) and lead (Pb) in artificial seawater were carried out over a wide concentration range to assess the uptake of heavy metals by the near-shore foraminiferal species *Ammonia aomoriensis*, *Ammonia batava* and *Elphidium excavatum*. Seawater analyses revealed increasingconcentrations for most metals between culturing phases and high metal concentrations in the beginning of the culturing phases due to the punctual metal addition. Furthermore, a loss of metals during the culturing process was discovered, by an offset between the added and the actual concentrations of the metals in seawater. Laser ablation ICP-MS analysis of the newly formed calcite revealed species-specific differences in the incorporation of heavy metals. The foraminiferal calcite of all three species exhibited Pb and Ag concentrations strongly correlated with concentrations in the seawater culturing medium (partition coefficients and standard deviation for Ag: *Ammonia aomoriensis*=0.50 ±0.02, *Ammonia batava*=0.17 ±0.01, *Elphidium excavatum*=0.47 ±0.04; for Pb: *Ammonia aomoriensis*=0.39 ±0.01, *Ammonia batava*=0.52 ±0.01, *Elphidium excavatum*=0.91 ±0.01). *Ammonia aomoriensis* further showed a correlation with Mn and Cu, *A. batava* with Mn and Hg and *E. excavatum* with Cr and Ni, and partially also with Hg. However, Zn, Sn and Cd showed no clear trend for the species studied, which in case of Sn was maybe caused by the lack of variation of the seawater Sn concentration . The calibrations and the calculated partition coefficients render *A. aomoriensis*, *A. batava* and *E. excavatum* as natural archives that enable the determination of variations of some heavy metal concentrations in seawater in polluted and pristine environments.

1 Introduction

Particular heavy metals e.g., zinc (Zn), iron (Fe), molybdenum (Mo), cobalt (Co) and copper (Cu) serve as micronutrients (e.g., Hänsch and Mendel, 2009) for eukaryotic life and play an important role for metabolism, growth, reproduction and enzymatic activity of organisms (e.g., Martín-González et al., 2005; Gallego et al., 2007). Other metals like mercury (Hg), on the other hand, are not known to have any positive effect on the body and are therefore believed to have a higher toxic potential (Jan et al., 2015). All these metals occur naturally in the environment as geogenic traces in soils, water, rocks and, consequently, in plants and animals. However, at higher

concentrations, most heavy metals become toxic and have hazardous effects on marine biota (Stankovic et al., 2014). Heavy metals are defined herein as elements with a density >7 g/cm^3 (Venugopal and Luckey, 1975) and an atomic number beyond calcium (Bjerrum, 1936; Thornton, 1995). Furthermore, they are highly persistent in the marine environment and are not easily excreted by organisms after the uptake of these metals into their system and cells (Flora et al., 2012; Kennish, 2019). Coastal environments act as natural catchments for anthropogenic pollutants because these areas are directly affected by industry, agriculture and urban runoff (e.g., Alloway, 2013; Julian, 2015; Tansel and Rafiuddin, 2016).

In marginal seas and coastal areas, benthic foraminifera are common, and the chemical composition of their calcite test can be used as proxies for changing environmental parameters like water temperature (Mg/Ca; e.g., Nürnberg et al., 1995; 1996), salinity (Na/Ca; e.g., Wit et al., 2013, Bertlich et al., 2018) and redox conditions (Mn/Ca; Groeneveld and Filipsson, 2013b; Koho et al., 2015; 2017; Kotthoff et al., 2017; Petersen et al., 2018; Guo et al., 2019). Foraminifera take up heavy metals and incorporate them into their calcium carbonate shells during calcification (e.g., Boyle, 1981; Rosenthal et al., 1997; Dissard et al., 2009; 2010a; 2010b; Munsel et al., 2010; Nardelli et al., 2016; Frontalini et al., 2018a; 2018b; Titelboim et al., 2018; Smith et al., 2020). Moreover, foraminifera have a short life cycle (< 1 year; e.g., Haake, 1967; Boltovskoy and Lena, 1969; Wefer, 1976; Murray, 1992) and thus, react immediately to changing environmental conditions and contamination levels of the surrounding environment. Therefore, foraminifera archive environmental signals and fossil records from sediments can be used to determine parameters of interest throughout space and time.

Species of the foraminiferal genera *Elphidium* and *Ammonia* are among the most abundant foraminiferal taxa in intertidal and shelf environments worldwide. They are found from subtidal water depths to the outer continental shelves (Murray, 1991). Furthermore, their calcite tests are often well preserved in the fossil record (Poignant et al., 2000; McGann, 2008; Xiang et al., 2008) and therefore provide the opportunity to assess past environmental conditions. The combination of all these properties make foraminifera, and especially *Elphidium* and *Ammonia* species, suitable indicators of anthropogenic pollution (e.g., Sen Gupta et al., 1996; Platon et al., 2005). As such, this group of organisms are excellent candidates for monitoring the spatial and temporal distribution of heavy metals in seawater to evaluate, for example, the effectiveness of contemporary measures of reducing emissions caused by anthropogenic inputs.

The majority of culturing studies on heavy metal incorporation into benthic foraminifera were designed to assess the influence and uptake of one particular metal, e.g., manganese (Mn) (Barras et al., 2018), copper (Cu) (De Nooijer et al., 2007), chromium (Cr) (Remmelzwaal et al., 2019), lead (Pb) (Frontalini et al., 2015), zinc (Zn) (e.g., Smith et al., 2020), mercury (Hg) (Frontalini et al., 2018a) or cadmium (Cd) (Linshy et al., 2013). This approach is adequate to detail the effects on shell chemistry, growth or physiology. Only two studies reported culturing experiments with elevated levels of Cu, Mn and Ni (Munsel et al., 2010) and elevated levels of Mn, Ni and Cd (Sagar et al., 2021b) in the same culturing medium. However, in reality there is rarely only one metal polluting environments but instead a combination of several pollutant metals is usually found (e.g., Mutwakil et al., 1997; Cang et al., 2004; Vlahogianni et al., 2007; Huang et al., 2011; Wokhe, 2015; Saha et al., 2017). How foraminifera incorporate and react to heavy metals when they are co-exposed to more than one metal at a time is less constrained to date. A mixture of different metals will lead to interactions, which may result in a more severe damage of tissue than exposure to each of them individually (Tchounwou et al., 2012). For example, a co-exposure to arsenic and cadmium causes more damage of human kidneys than only one of these elements (Nordberg et al., 2005).

79 Furthermore, a chronic low-dose exposure to multiple elements can cause similar synergistic effects (e.g., Wang
80 et al., 2008). It is therefore reasonable to assume that other organisms are likewise harmed more when exposed to
81 several potentially toxic elements simultaneously.

82 Here we present results from culturing studies with *Ammonia aomoriensis*, *Elphidium excavatum* and *Ammonia*
83 *batava* assessing the relationship between heavy metal concentrations in seawater and foraminiferal tests. The
84 partitioning factor between the concentration of an element in the ambient seawater and the calcium carbonate of
85 the foraminifers is constrained by determining both the dissolved metal concentrations in water and the metal
86 contents of individual chambers of the foraminiferal shell that have been precipitated in the culturing medium. In
87 particular, foraminifera were grown while exposed to a combination of ten different heavy metals, i.e., cadmium
88 (Cd), copper (Cu), chromium (Cr), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), silver (Ag), tin (Sn)
89 and zinc (Zn) over a range of concentrations that prevail in polluted near-shore environments today. These metals
90 are the most common representatives of marine heavy metal pollution (Alve, 1995; Martinez-Colon et al., 2009).
91 Once the carbonate/seawater metal partitioning coefficients are known, investigations of the chemistry of benthic
92 foraminiferal shells offer a reliable method to monitor short-term changes of the concentration of heavy metals in
93 seawater.

94

95 **2 Material and Methods**
96 **2.1 Field sampling**
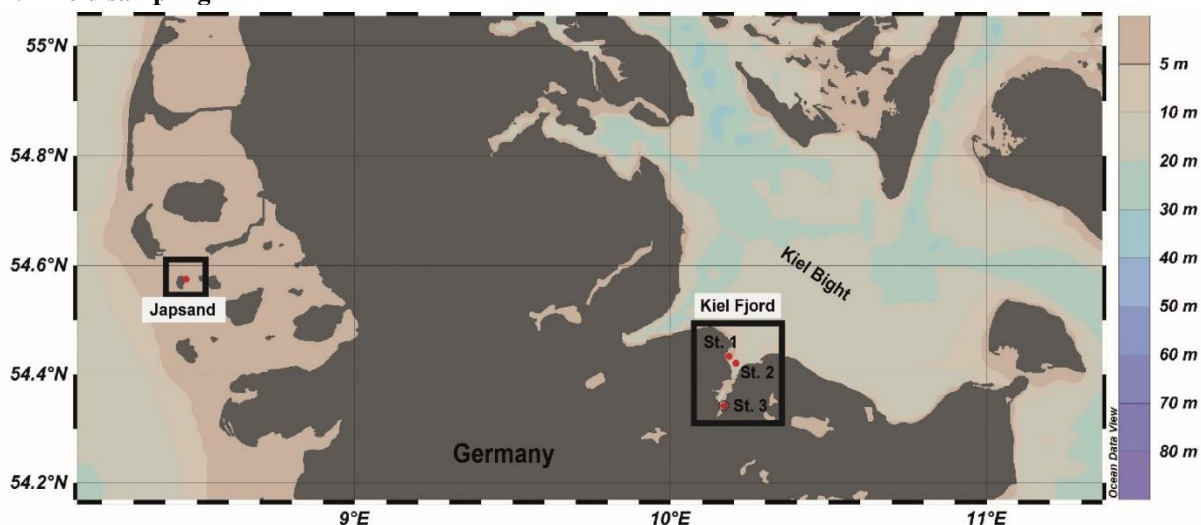
97
98 **Figure 1**: Location of the sampling stations in the North Sea (Japsand area) and in the Baltic Sea (Kiel Fjord, St.1
99 Strander Bucht, St. 2 Laboe, St. 3 Mönkeberg). The map was drawn with Ocean Data View (Schlitzer, 2016) on
100 the basis of bathymetric data. Water depths in m are indicated by the colour scale.

101

102 **2.1.1 North Sea, Japsand**
103 Living specimens of *A. batava* were collected at the barrier sand Japsand near Hallig Hooge in the German Wadden
104 Sea in July 2019 at two stations (St. 1: 54°34.480′N, 8°27.919′E; St. 2: 54°34.491′N, 8°27.895′E) (Fig. 1). The
105 sediment was a glacial till or Eemian clay at Station 1 and fine to medium sand at Station 2. Temperature and
106 salinity of seep waters were measured with a WTW 3210 conductivity meter in excavated holes in the vicinity.

3

107   The temperature at Station 1 was 21.1 ° C and at Station 2 21.6 ° C, respectively. Salinity was 34 PSU at station 1

108   and 33.6 PSU at station 2. The samples were recovered during low tide by scrapping off the uppermost centimetre

109   of the surface sediment with a spoon made out of stainless steel. Natural seawater (NSW) with a salinity of 30.3

110   PSU was collected near the sites for further processing of the samples. Once back on the nearby island Hallig

111   Hooge, the sediment was washed with NSW through stacked sieves with a mesh size of 2000 and 63 µm. The

112   2000 µm sieve was used to remove larger organisms and excess organic material (macroalgae, gastropods,

113   lugworms etc.) that could have induced anoxic conditions in the sediment during transport and storage. The residue

114   was stored in Mucasol soap-washed and acid-cleaned Emsa CLIP and CLOSE® boxes, sparged with air and some

115   algae food was provided. Back in the laboratory at GEOMAR, the residue was stored at 8 °C in a fridge until

116   culturing. These stock cultures were fed twice a week with green-coloured *Nannochloropsis* concentrate

117   (BlueBioTech) and water was partly exchanged with NSW from the sampling site once a week.

118

2.1.2 Baltic Sea, Kiel Bight

120   Living specimens of *A. aomoriensis* and *E. excavatum* were collected from different stations in Kiel Fjord, western

121   Baltic Sea (St.1, Strander Bucht, 54°26.001´N, 10°11.1078´E; St. 2, Laboe, 54°25.254´N, 10°12.346´E; St. 3,

122   Mönkeberg, 54°20.752´N, 10°10.150´E; water depth: 12.5 m, 12.3 m and 14.3 m, respectively) in September and

123   October 2019 with F.B. Polarfuchs and F.S. Alkor, respectively (Fig. 1). A Rumohr corer (inner diameter 55 mm)

124   was used on F.B. Polarfuchs and 9 cores were taken (2 at St. 1 and 7 at St. 3). The sediment from the cores was

125   collected in Mucasol treated and acid-cleaned plastic containers with NSW from the site.

126   On F.S. Alkor, a Reineck box corer was used (200 x 250 mm) and 3 replicates at each station were taken (St. 1-

127   3). The first 1 to 2 cm of the sediment surface of the box core were scrapped off with a spoon made out of stainless

128   steel and the material was stored in a Mucasol treated and acid-cleaned plastic box with NSW from the location.

129   Back in the laboratory at GEOMAR, the samples were treated the same way as Japsand samples from the North

130   Sea. Artificial seawater (ASW, Tropic Marin) with a salinity of 30 PSU was used for washing and storage of the

131   surface samples from Kiel Fjord. The use of artificial seawater ensured that no harmful microorganism could

132   invade the cultures.

133

2.2 Culturing setup
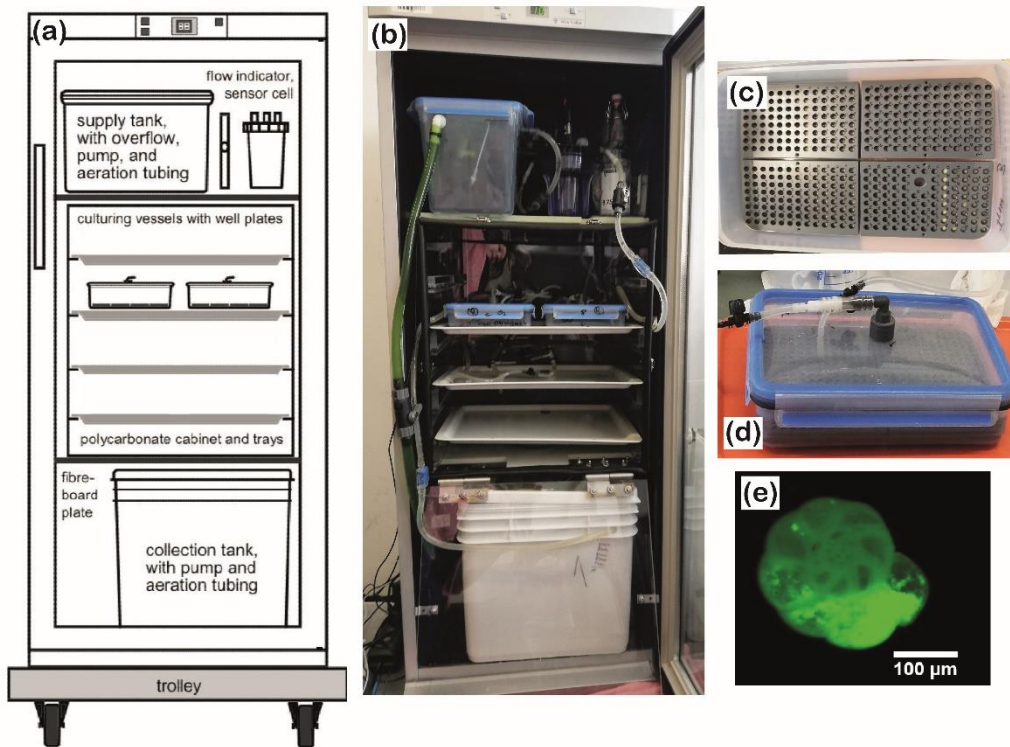
Figure 2: Culturing setup. a: conceptual draft and b: assembly of the system. Tubing and hoses were omitted from the draft for clarity. c: a well plate with mounted specimens and sand, d: closed culturing vessel with well plates and conduits. e: with calcein stained foraminifer under a fluorescence microscope. Please note that the last 2 ½ chambers are labelled and fluorescing brightly. The specimen shown in the picture was dead, cleaned and dried, which ensured that the test itself and not the cytoplasm showed the fluorescence.

2.2.1 Picking of the samples

The three foraminiferal species that were used in this study have been described in detail in the literature (e.g., Lutze, 1965; Nikulina et al., 2008; Schweizer et al., 2011; Francescangeli et al., 2021; Schmidt and Schönfeld, 2021). For extracting the foraminiferal specimens from the sediment, about 1 cm^3 of the 63 to 2000 µm size fraction was transferred to a petri dish. All living specimens were picked with a paintbrush from this subsample and collected in a small petri dish with ASW. All plastic utensils were treated with Mucasol water and rinsed with 5% HNO$_3$ prior to use. The paintbrush was cleaned with ethanol to protect the culture from harmful microorganisms. Only specimens with a glossy, transparent and undamaged test were chosen. After picking, a drop of concentrated food (pure culture of *Nannochloropsis*, green coloured algae) was added and the foraminifera were left untouched for a night.

Specimens that met one or more of the following criteria were considered as living and used for further procedures:

- The cytoplasm of the specimens was present in more than two chambers that were connected and including the innermost chambers,

- Specimens showed a structural infill of cytoplasm with a bright green colour, indicating they took up the food over night,

5

157   •   they developed a film or strings of pseudopodia firmly sticking to sediment particles or food,

158   •   they had covered themselves or gathered a cyst of sediment or food particles.

159 Specimens were identified and sorted by speciesand stained with calcein (10 mg l^{-1}, Bernhard et al., 2004)
160 (bis[N,N-bis(carboxymethyl)aminomethyl]-fluorescein) (Sigma-Aldrich) directly before each culturing phase to
161 ensure that freshly labelled foraminifera were inserted into the culturing system (Fig. 2e). Staining lasted for 14
162 days. Petri dishes were stored at 8 °C in a fridge, partial water exchanges and feeding of the foraminifera was
163 performed twice a week. After the staining, the foraminifera were transferred to a petri dish with ASW and left for
164 1 to 2 days to remove excess calcein from seawater vacuoles in their cytoplasm prior to the introduction into the
165 culturing system.

166

2.2.2 Culturing system

167
168 We used two closed-circulation incubation systems for foraminifera (Fig. 2a, b) provided by the Institute of
169 Microbiology, Kiel University (Woehle et al., 2018, their Fig. S4). The systems were further developed based on
170 earlier closed-circulation systems for culturing foraminifera (Hintz et al. 2004; Haynert et al., 2011). They were
171 slightly modified for the requirements of this study, but the basic operational principle is described by Woehle et
172 al., 2018. In detail, the systems consisted of three levels with different functions. They were built into a Bauknecht
173 WLE 885 fridges for temperature control. Each system accommodated two culturing vessels, which were arranged
174 pairwise on a tray in a polycarbonate cabinet (Fig. 2a, b). The water was pumped from the collection tank at the
175 lowest level to the top level into the supply tank. From the supply tank, the water was directed to the culturing
176 vessels and the flow was regulated ensuring that the same amount of water was provided to every culturing vessel.
177 After passing the culturing vessels, the water was redirected to the collection tank. The systems were filled with
178 15 L of ASW with a salinity of 30.5 PSU. The water was aerated in the supply and the collection tank with filtered
179 (0.2 µm) air from outside the system. Monitoring of temperature and salinity were performed with a WTW 3210
180 conductivity meter. Uncertainty of the conductivity measurements was ± 0.5% and ± 0.1 °C for temperature
181 according to the manufacturer's test certificate. pH was monitored using a pH electrode (GHL) for aquarium
182 purposes with uncertainties of ± 0.06. All parts that were introduced into the system were sterilised before use
183 either by autoclaving, UV-lamp exposure, or by applying DanKlorix®.

184

2.2.3 Preparation for incubation

185
186 For the incubation of the foraminifera, well plates with cavities made from PVC were used (Fig. 2c). All well
187 plates had been used in previous experiments for culturing foraminifera in seawater, which ensured that potentially
188 toxic substances or additives were already released from the material (Woehle et al., 2018). Before the foraminifera
189 were placed in the cavities, each cavity was filled with sterile quartz sand up to 1.5 mm height. The cavities were
190 subsequently filled with artificial seawater and the specimens were inserted randomly. Prepared well plates were
191 left untouched for one night, to make sure that the foraminifera were able to spread their pseudopodial network
192 before incubation. This ensures that they were stably anchored in the cavities and did not float when the culturing
193 vessels were filled and mounted (Haynert et al., 2011). Four well plates were assembled in each airtight Emsa
194 CLIP and CLOSE® box (Fig. 2d).Each culturing vessel had a lid with an inflow and an outflow conduit, for which
195 cleaned food grade Tygon® tubing was used. To guarantee that the foraminiferal specimens were not flushed away

by the incoming water, the inflow conduit reached almost the bottom of the culturing vessel and was placed between two well plates. Once all well plates were arranged in the culturing vessel, the lid was equipped with an additional, elastic sealing and closed. Before the culturing vessels were placed in the culturing systems, each chamber was slowly filled with ASW. Thereafter, the culturing vessels were placed on the shelve in the culturing system, and were connected to the supply hoses.

2.2.4 Culturing experiment

The culturing experiment had four different phases. The first, phase 0 was dedicated as control phase and no heavy metals were added. This phase allowed both systems to equilibrate in terms of physicochemical and biological processes and made it possible to determine the background values in terms of seawater constituents. This phase lasted 21 days. Afterwards, one system was used as the control system, where no heavy metals were added. In the other system, three phases with elevated heavy metal concentrations were performed. The phases lasted 21 days each. Tropic Marin Pro-Reef salt was mixed with deionized water for adjusting the salinity. This artificial salt contains all elements and nutrients in sufficient amounts required by marine organisms. A stock solution containing all metals of interest was mixed and this solution is called the multi metal stock solution hereafter. It was added to the supply tank of the system (see Fig. 2a) (phase 1 = 1 ml, phase 2 = 10 ml, phase 3 = 150 ml) at the beginning of each phase to reach the target concentration (Table 1). Additionally, a smaller aliquot of the same multi metal stock solution (Phase 1 = 0.1 ml, Phase 2 = 1 ml, Phase 3 = 10 ml) was introduced twice a week during the three weeks of a phase. This was to counteract the loss of metals during the culturing phase through e.g., uptake of metals by foraminifera or algae or by adsorption to surfaces of the culturing system. The target concentration of the elements at each phase were chosen after earlier culturing experiments with foraminifers (Mn, Cu, Ni: Munsel et al., 2010; Pb: Frontalini et al., 2015 & 2018b; Zn: Nardelli et al., 2016; Cd: Linshy et al., 2013; Cu: De Nooijer et al., 2007; Le Cadre and Debenary et al., 2006; Cr: Remmelzwaal et al., 2019, Hg: Frontalini et al., 2018a) and to resemble conditions observed in threatened environments. Examples for such environments are the San Francisco Bay, California (Thomas et al., 2002), the Black Sea, Turkey (Baltas et al., 2017) or the Gulf of Chabahar, Oman Sea (Bazzi, 2014). Furthermore, the Adriatic Sea (Ag; Barriada et al., 2007), Jakarta Bay (Williams et al., 2000; Putri et al., 2012), and polluted U.S. and European rivers (Byrd and Andreae, 1982; Kannan et al., 1998; Thomas et al., 2002) were considered. Table A4 summarizes the heavy metal concentration in seawater in different areas around the world to compare to the experimental values. Additionally, the maximum metal concentration as recommended by the EPA (Environmental Protection Agency, USA) is the lower boundary of the concentration range from this study (Prothro, 1993). This was taken into account to ensure that the foraminifera were not limited in their growth and able to maintain normal physiological functions. A lower concentration than the EPA value is also covered by our study during the control phase or in the control system. The heavy metal concentrations in the culturing media obtained during each phase were monitored by frequent water sampling.

Table 1: Heavy metal concentration in the multi metal stock solution, target concentration of these metals in each phase and used salt compounds. All salts used were provided in pro analysi quality and were purchased from Carl Roth ($CrCl_3 \cdot 6\ H_2O$; $SnCl_2 \cdot 2\ H_2O$ and $PbCl_2$), Walter CMP ($CdCl_2$) and Sigma Aldrich ($MnCl_2 \cdot 4\ H_2O$, $NiCl_2 \cdot 6\ H_2O$, $CuCl_2 \cdot 2\ H_2O$, $ZnCl_2$, $AgNO_3$ and $HgCl_2$).

	Target conc. in $\mu g\ l^{-1}$

	Salt compound	Conc. in mg l^{-1} Multi metal stock solution	Phase 1	Phase 2	Phase 3
Chromium (Cr)	CrCl$_3$ · 6 H$_2$O	25	0.5	5	50
Manganese (Mn)	MnCl$_2$ · 4 H$_2$O	40	40	400	4000
Nickel (Ni)	NiCl$_2$ · 6 H$_2$O	5	0.1	1	10
Copper (Cu)	CuCl$_2$ · 2 H$_2$O	2	0.05	0.5	5
Zinc (Zn)	ZnCl$_2$	50	0.8	8	80
Cadmium (Cd)	CdCl$_2$	4	0.08	0.8	8
Silver (Ag)	AgNO$_3$	3.5	0.1	1	10
Tin (Sn)	SnCl$_2$ · 2 H$_2$O	10	0.1	1	10
Mercury (Hg)	HgCl$_2$	0.04	0.01	0.1	1
Lead (Pb)	PbCl$_2$	10	0.1	1	10

Over the entire culturing period, both systems were exposed to a natural day and night cycle and the flow rate was adjusted to 1.02 ml min^{-1} (one drop per second) within the culturing vessels. The foraminifera were fed with *Nannochloropsis* concentrate twice a week (~ 2000 µg). After 21 days (meaning after each culturing phase) one culturing vessel per system was exchanged. Vessels and specimens were left in the culturing system for the complete culturing phase (21 days) and no exchange took place during a culturing phase.

Temperature and salinity were kept stable at 15.0 ±0.1 °C and 30.2 ±0.3 PSU (heavy metals) and at 14.9 ±0.2 °C and 30.4 ±0.4 PSU (control) over the complete culturing period. As the system was mostly closed, evaporation had a minor effect. Demineralized water was added when necessary to keep the salinity stable. The exchanges of culturing vessels between phases inferred a partial water exchange of approximately 10 % (= 1.5 l) every three weeks, which ensured a repetitive renewal of water with adequate quality.

2.3 Water samples

2.3.1 Collection of water samples

Water samples for determining the heavy metal concentrations were taken frequently from the supply tanks (see Fig. 2a) of both systems using acid cleaned syringes (Norm-Ject® disposable syringe, 20 ml, sterile) and sample bottles (LLG narrow neck bottles, 50 ml, LDPE = Low Density Polyethylene; Hg: GL 45 Laboratory bottle 250 ml with blue cap and ring, boro 3.3). From the beginning of phase 1, sampling was performed once a week. Water samples to be analysed for mercury concentrations had to be treated differently due to analytical constraints as detailed below. The water was filtered through a 0.2 µm PES filter (CHROMAFIL Xtra disposable filters, membrane material: polyether sulfone pore) for heavy metal samples and through a 0.2 µm quartz filter for Hg samples (HPLC syringe filters, 30 mm glass fibre syringe filters/ nylon). Filters were rinsed with the sample water before taking the sample. Every water sample was immediately acidified with concentrated ultrapure HCl to a pH of approximately 2 to avoid changes in the heavy metal concentrations due to adsorption to the sample bottle walls or the formation of precipitates.

2.3.2 Preparation of water samples before analysis

For Mn, Zn, Ni, Pb, Cu, and Cd concentration analyses, the water samples were pre-concentrated offline using a SeaFAST system (ESI, USA). Twelve mL of each sample were used to fill a sample loop and preconcentrated by a factor of 25 using the SeaFAST column into 1.5M HNO_3. All samples were spiked with indium as an internal standard for monitoring and the pre-concentration procedure. Both MilliQ water and bottle blanks of acidified MilliQ water (pH ~ 2) stored in the same bottles until the samples were passed through the pre-concentration system. Additionally, procedural blanks which were filtered as the samples were also pre-concentrated and measured. A variety of international (Open Ocean Seawater NASS-6, River Water SLRS-6, Estuarine Seawater SLEW-3, all distributed by NRC-CNRC Canada) and in-house (South Atlantic surface water, South Atlantic Gyre water) reference materials were pre-concentrated like the samples. All samples were subsequently analysed by ICP-MS (inductively coupled plasma mass spectrometry).

Other metals (Cr, Ag and Sn) were diluted 1/25 and directly introduced into the ICP-MS as they are not retained on the Nobias resin used by the SeaFAST system. The dilution was performed with indium-spiked nitric acid (2%) and to match the matrix of these samples, blanks and standards with added NaCl were prepared.

All heavy metals except mercury were measured using an Agilent 7500ce quadrupole ICP-MS. Raw intensities were calibrated with mixed standards, which were made from single element solutions covering a wide concentration range. Additionally, a dilution series (dilution factors: 1, 1/10, 1/100 and 1/1000) of SLRS-6 of river water reference material (NRC Canada; Yeghicheyan et al., 2019) was measured for quality control. Mean values and relative standard deviations (RSD) derived from the reference materials are summarised in the appendix (Table A2).

Prior to the measurements of Hg concentrations, all samples were treated with BrCl solution at least 24 hours before the analysis to guarantee the oxidation and release of mercury species that were possibly present in a different oxidation states or phases. The BrCl was removed again by adding hydroxylamine hydrochloride at least one hour prior to analysis before the Hg was reduced to the volatile Hg^0 species with acidic $SnCl_2$ (20 % w v^{-1}) during the measuring process. All preparations of the water samples took place in a Clean Lab within a metal clean atmosphere and all vials were acid cleaned prior to use. Mercury concentrations were determined using a Total Mercury Manual System (Brooks Rand Model III). The reduced volatile Hg^0 was nitrogen-purged onto a gold-coated trap and released again by heating before it was measured via cold vapour atomic fluorescence (CVAFS) under a continuous argon carrier stream. Quality control of the Hg measurements was carried out by measuring mixed standards, made from single element solutions and confirmed with replicate measurements throughout each analysis. The measurement uncertainty was smaller than 4.5 % RSD for all analyses.

The calcium concentration of culture seawater was analysed using a VARIAN 720-ES ICP-OES (inductively coupled plasma optical emission spectrometer). Yttrium was added as an internal spike and samples were diluted 1/10. IAPSO seawater standard (ORIL) was measured after every 15 samples for further quality control which revealed a measurement uncertainty < 0.35 (RSD %) for the elements analysed (mean Ca concentration IAPSO this study = 419.6 ± 0.15 mg l^{-1}; reference Ca concentration IAPSO Batch 161 = 423 mg l^{-1}).

2.4 Foraminiferal samples

After every culturing phase, the culturing vessels were taken out of the culturing system and foraminiferal specimens were collected from their cavities within one day. The individuals were cleaned with tap water and ethanol before they were mounted in cell slides to mechanically remove salt scale and organic coatings with a paintbrush. Dead specimens could be identified because they lost the colour of their cytoplasm and furthermore, they did not gather food and particles anymore and thus were lacking a detritus cyst by their aperture.

In order to check the growth of foraminifera during the culture experiment, the total number of chambers were counted before and after the experiment for every specimen (Table 2). This was performed to double check the growth in cases where calcein staining may have failed. As the foraminifera were stained with calcein before the experiment, it was possible to cross-check the growth with a fluorescent microscope (Zeiss Axio Imager 2) if new chambers without fluorescence were added, and hence whether the specimen had grown or not (Fig. 2e). Only individuals clearly showing new chambers were analysed by Laser ablation ICP-MS.

Prior to the laser ablation analyses, the foraminifera were transferred into individual acid-leached, 500 µl micro-centrifuge tubes and thoroughly cleaned, applying a procedure adapted from Martin and Lea (2002). The specimens were rinsed three times with MilliQ water and introduced into the ultrasonic bath for a few seconds at the lowest power setting after each rinse. Afterwards, clay and adhering particles were removed by twice rinsing the sample with ethanol, which was followed by three MilliQ rinses again with minimal ultrasonic treatment. Oxidative cleaning was applied using 250 µl of a 0.1M NaOH and 0.3 % H_2O_2 mixture added to each sample and the vials were kept for 20 min in a 90 °C water bath. Afterwards, the samples were rinsed with MilliQ three times to remove the remaining chemicals. The reductive step of the cleaning procedure was not applied. This step is necessary to remove metal oxides, which of course could also influence the heavy metal concentration within the foraminiferal shell carbonate but these are usually considered to be added during early deposition (e.g., Boyle, 1983) and therefore unlikely to occur during culture experiments. For Laser Ablation Inductively Coupled Plasma Mass Spectroscopy (LA-ICP-MS) measurements, all cleaned specimens were fixed on a double-sided adhesive tape (PLANO).

Micro-analytical analyses with LA-ICP-MS were performed at the Institute of Geosciences, Kiel University, using a 193nm ArF excimer GeoLasPro HD system (Coherent) with a large volume ablation cell (Zurich-type LDHCLAC, Fricker et al., 2011) and helium as the carrier gas with 14 mL min^{-1} H_2 added prior to the ablation cell. For the foraminiferal samples, the pulse rate was adjusted to 4 to 5 Hz with a fluence between 2 and 3.5 J cm^{-2}. The spot size was set to 44 or 60 µm depending on the size of the foraminiferal chamber. All chambers of a foraminifer that were built up in the culturing medium were analysed, starting from the earliest, inner chamber adjacent to the calcein-stained chamber. The laser was manually stopped once it broke through the foraminiferal shell. The ablated material was analysed by a tandem ICP-MS/MS instrument (8900, Agilent Scientific Instruments) in no gas mode. The NIST SRM 612 glass (Jochum et al., 2011) was used for calibration and monitoring of instrument drift while NIST SRM 614 was measured for quality control. The glass was chosen because all elements of interest (except Hg) were reported in the literature, which was not the case for established carbonate reference materials. Glasses were ablated with a pulse rate of 10 pulses per second, an energy density of 10 J cm^{-2} and a crater size of 60 µm. Dueñas-Bohórquez et al. (2009) demonstrated that different energy densities between the foraminiferal calcite and the glass standard does not affect the analyses. Carbonate matrix reference materials coral JCp-1, giant clam JCt-1, limestone ECRM752-1 and synthetic spiked carbonate MACS-

3 (Inoue et al., 2004; Jochum et al., 2019) in the form of nano-particle pellets (Garbe-Schönberg and Müller, 2014) were analysed for quality control. Carbonate reference material were ablated with a pulse rate of 5 pulses per second, an energy density of 5 J cm^{-2} and a crater size of 60 µm. MACS-3 was used for calibrating the mercury content in the samples as Hg is not present in the NIST SRM glasses. All results for the reference materials are given in the appendix (Table A3). Trace element-to-calcium ratios were quantified using the following isotopes: ^{26}Mg, ^{27}Al, ^{52}Cr, ^{55}Mn, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{68}Zn, ^{107}Ag, ^{111}Cd, ^{114}Cd, ^{118}Sn, ^{201}Hg, ^{202}Hg and ^{208}Pb normalised to ^{43}Ca. If more than one isotope was measured for an element, the average concentration of these was used after data processing. Analytical uncertainty (in % RSD) was better than 5 % for all TE/Ca ratios. The lowest RSD % based on the NIST SRM 612 glass was 2.1 % for Mn/Ca and the highest 5.0 % for Ag/Ca. Uncertainties of all used standards and reference materials are summarized in Table A3. Each acquisition interval lasted for 90 seconds, started and ended with measuring 20 s of gas blank, used as the background baseline to subtract from sample intensities during the data reduction process. Furthermore, the background monitoring ensured that the system was flushed properly after a sample. In cases when foraminiferal test walls were very fragile causing the test to break very quickly and, hence, the length of the sample data acquisition interval was less than 15 seconds, these profiles were excluded from further consideration.

Transient logs of raw intensities given in counts per seconds for all isotopes measured were processed with the software Iolite (Version 4, Paton et al., 2011) producing averages of every time-resolved laser profile. The determination of element/Ca ratios was performed after the method of Rosenthal et al. (1999). High values of ^{25}Mg, ^{27}Al or ^{55}Mn at the beginning of an ablation profile were related to contamination on the surface of the foraminiferal shell or remains of organic matter (e.g., Eggins et al., 2003) and these parts of the profiles were excluded from further data processing. The detection limit was defined by 3.3*SD of the gas blank in counts per seconds for every element in the raw data. Only values above this limit were used for further analyses and no data below the LOQ (limit of quantification = 10*SD) were interpreted. After processing the data with Iolite, an outlier detection of the TE/Ca ratios of the samples was performed. If trace metal values from a spot deviated more than ±2SD from the average of the samples from the corresponding culturing phase, values were defined as outliers and discarded. The number of rejected points is indicated in the supplementary material (Table S1).

All statistical tests of the TE/Ca values in the foraminiferal shell and the water were carried out using the statistical program PAST (Hammer, 2001). As the concentration of heavy metals in seawater varied during individual phases in the metal system (Table A1 and Fig. B1 in the appendix), the mean concentration was calculated by applying an individual curve fit for every phase. The curve was either linear, exponential or a power function depending on the trend the particular metal showed. If the type of trend was not clear, the curve type with the highest p and R^2 values were chosen. Based on these curves, water values were calculated for every day and the weighted average from all days was used for further calculations. This ensured that high concentrations in the beginning of each phase did not influence the mean value disproportionately. The partition coefficients of the different trace metal-to-calcium ratios were calculated using the trace element (TE) and calcium ratios in calcite and seawater. The following equation was used:

$$D_{TE} = (TE/Ca)_{calcite}/(TE/Ca)_{seawater}.$$

When the correlation between the metal concentration in seawater and the metal concentration in the foraminiferal test was positive and significant (R$^2 > 0.4$, p < 0.05), the D$_{TE}$´s are derived from the mean values of all phases and

376　represent the slope of the calculated regression line. In cases where a significant positive correlation between

377　phases could not be identified, the D_{TE} values were calculated from the means of each phase separately and the

378　ranges given. The regression line was forced through the origin, which is a common practice and is applied in

379　many other studies (e.g., Lea and Spero, 1994; Munsel et al., 2010; Remmelzwaal et al., 2019; Sagar et al., 2021a).

380　The reason for this approach is that foraminifers are expected not to incorporate any metals into their shell if the

381　metals concentration is zero in the seawater. In cases where there was clearly a non-zero intercept (Mn of *A. batava*

382　with phase 3 and Hg of *E. excavatum* without phase 3), obvious if the course of the regression line changed

383　significantly or the R^2 value decreased, then the trend line was not forced through the origin.

384　**3 Results**

385　**3.1 Survival Rates/ Growth rates / Reproductions**

386　**Table 2**: Number of inserted and recovered foraminifera from the different systems (C = control system, M =

387　metal system) and phases (0–3). Numbers of living individuals after the experiment and individuals that formed

388　chambers during their individual culturing phase are given in %. Note that the percentage of living foraminifera is

389　based on the number of foraminifera that could be recovered alive and not on the number of inserted individuals.

390　The number of laser spots is indicated as well.

	C0	C1	C2	C3	M0	M1	M2	M3	Total
No. of inserted individuals									
Ammonia aomoriensis	50	24	20	20	19	70	70	72	345
Ammonia batava	22	20	20	20	16	43	72	72	285
Elphidium excavatum	45	24	20	20	19	70	69	70	337
Total	117	68	60	60	54	183	211	214	967
No. of recovered individuals									
Ammonia aomoriensis	43	20	10	19	11	57	58	56	274
Ammonia batava	11	15	16	14	7	29	65	56	213
Elphidium excavatum	36	20	20	14	7	62	58	53	270
Total	90	55	46	47	25	148	181	165	757
Living individuals (end of experiment) in %									
Ammonia aomoriensis	86	100	80	100	90.9	100	81	98.2	92.0
Ammonia batava	81.8	100	100	92.9	100	100	100	100	96.8
Elphidium excavatum	91.7	100	95	92.9	100	88.7	91.4	94.3	94.3
Total	86.5	100	91.7	95.3	97.0	96.2	90.8	97.5	94.4
Ind. that formed chambers (end of the experiment) in %									
Ammonia aomoriensis	62.8	84.2	100	93.8	81.8	100	92.3	90	88.1
Ammonia batava	45.5	85.7	100	100	71.4	100	100	100	87.8
Elphidium excavatum	69.4	65	56.3	38.5	57.1	67.7	75	62.3	61.4
Total	59.2	78.3	85.4	77.4	70.1	89.2	89.1	84.1	79.1
No. of laser spots									
Ammonia aomoriensis	22	18	17	20	9	39	40	36	201

Ammonia batava	14	20	19	19	6	17	52	57	204
Elphidium excavatum	14	13	13	12	1	36	24	31	144
Total	50	51	49	51	16	92	116	124	549

On average 74.5 % of the specimens inserted into the experiment could be recovered after their individual culturing phase of 21 days and 94.4 % of these recovered specimens survived. Approximately 79.1 % of the surviving specimens also formed at least one new chamber. Fewer specimens of *E. excavatum* formed new chambers (61.4 %) than *A. batava* (87.8%) or *A. aomoriensis* (88.1 %) (Table 2). On average, *E. excavatum* formed only one or rarely two new chambers, whereas both *Ammonia* species formed usually more than four new chambers. Reproduction happened very sporadically occurring in between 2 and 6 specimens per phase, on average 5 %, for the two *Ammonia* species but not for *E. excavatum*. No malformed chambers were observed in specimens that were recovered from the heavy-metal contaminated system.
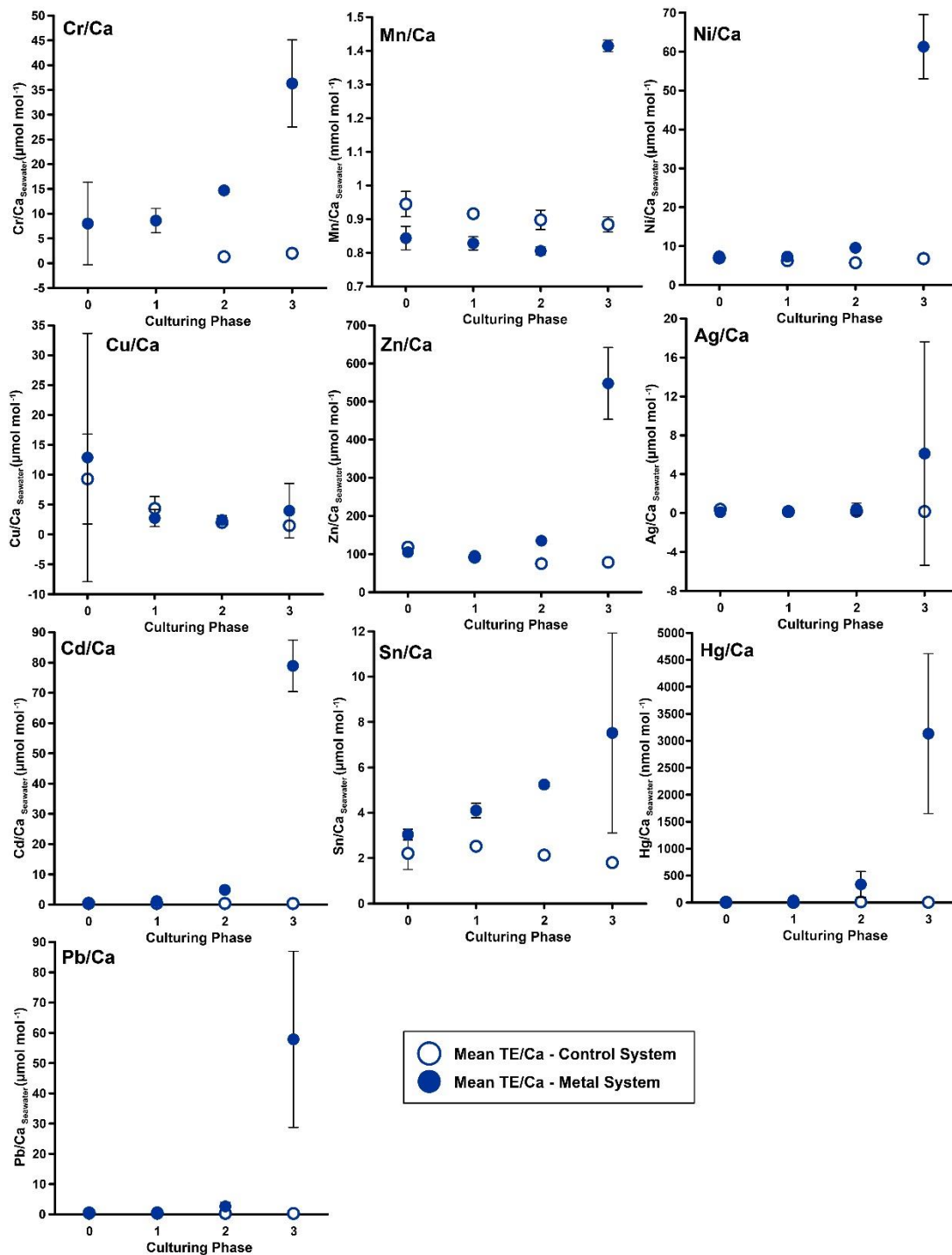
3.2 Culturing media

Table 3: Weighted mean TE/Ca values in the culturing medium of the control and the metal system ± the standard error of the mean (standard deviation $\sigma/\sqrt{n}$). Furthermore, the factors between the target concentrations (Table 1) and the measured concentrations as well as the factors between individual phases are given. Values given without a standard error originate from only one measurement. Averaged TE/Ca values of a phase were calculated based on single values measured on samples from different days during the culturing phase. These single values can be found in the Appendix (Table A1). BDL = below detection limit.

	Cr/Ca	Mn/Ca	Ni/Ca	Cu/Ca	Zn/Ca	Ag/Ca	Cd/Ca	Sn/Ca	Hg/Ca	Pb/Ca
Control System	μmol mol^{-1}	mmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	nmol mol^{-1}	μmol mol^{-1}
Phase 0	BDL	0.94 ± 0.02	7.0 ± 0.1	9.3 ± 4.3	118.3 ± 4.5	0.43 ± 0.214	0.41 ± 0.001	2.2 ± 0.4	5.8 ± 0.6	0.44 ± 0.06
Phase 1	BDL	0.92 ± 0.00	6.3 ± 0.1	4.4 ± 1.4	91.6 ± 1.1	0.19 ± 0.013	0.41 ± 0.002	2.5 ± 0.1	4.5 ± 1.0	0.39 ± 0.02
Phase 2	1.3 ± 0.3	0.90 ± 0.02	5.7 ± 0.1	2.1 ± 0.2	74.8 ± 2.0	0.19 ± 0.003	0.38 ± 0.006	2.1 ± 0.1	13.2 ± 5.8	0.31 ± 0.02
Phase 3	2.0 ± 0.4	0.89 ± 0.01	6.8 ± 0.3	1.5 ± 0.1	78.3 ± 0.8	0.16 ± 0.009	0.37 ± 0.006	1.8 ± 0.1	5.8 ± 1.8	0.28 ± 0.01
Metal System	μmol mol^{-1}	mmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	nmol mol^{-1}	μmol mol^{-1}
Phase 0	8.0 ± 1.8	0.84 ± 0.01	7.4 ± 0.1	12.9 ± 4.5	104.8 ± 1.4	0.09 ± 0.02	0.43 ± 0.002	3.0 ± 0.1	5.28	0.50 ± 0.04
Phase 1	8.6 ± 0.5	0.83 ± 0.004	7.3 ± 0.1	2.8 ± 0.3	95.2 ± 0.3	0.10 ± 0.02	1.12 ± 0.01	4.1 ± 0.1	39.7 ± 2.7	0.69 ± 0.03
Phase 2	14.7 ± 0.1	0.81 ± 0.003	9.6 ± 0.1	2.4 ± 0.2	134.8 ± 0.5	0.40 ± 0.14	4.86 ± 0.03	5.2 ± 0.03	337.6 ± 52.1	2.63 ± 0.3
Phase 3	36.3 ± 1.9	1.41 ± 0.004	61.3 ± 1.8	4.0 ± 1.0	547.5 ± 20.5	6.1 ± 2.5	78.92 ± 1.9	7.5 ± 1.0	3132.4 ± 323.7	57.84 ± 6.4
Factor between target conc. and measured conc.										
Phase 1	17.2	20.8	73.0	56.0	119.0	1.0	14.0	41.0	4.0	6.9
Phase 2	2.9	2.0	9.6	4.8	16.9	0.4	6.1	5.2	3.4	2.6
Phase 3	0.7	0.4	6.1	0.8	6.8	0.6	9.9	0.8	3.1	5.8
Factor between Phases										
Phase 0-1	1.1	1.0	1.0	0.2	0.9	1.1	2.6	1.4	7.5	1.4
Phase 1-2	1.7	1.0	1.3	0.9	1.4	4.0	4.3	1.3	8.5	3.8
Phase 2-3	2.5	1.7	6.4	1.7	4.1	15.3	16.2	1.4	9.3	22.0

Figure 3: Weighted mean TE/Ca values in the culturing medium in µmol mol^{-1}. Error bars display the standard error of the mean (standard deviation σ/√n). Open symbols represent the control system, where no extra metals were added during the complete culturing period (phase 0 to 3) and closed symbols represent the metal system. In this system, phase 0 is the control phase without any extra added metals and for phase 1 to 3, the heavy metal concentration in the culturing medium was elevated. Note that the standard error is comparably high in phase 3 because the heavy metal concentration in this phase varied more strongly, which is shown in the appendix (Table A1, Fig. B1). Therefore, this error is derived from the real values in the seawater and not from analytical uncertainties. Note that the Cr/Ca values from the control system in phase 0 and 1 are not given as these values were below the detection limit.

420 In phases 1 and 0 the concentration in both systems were nearly equal for most elements. Only Cr and Sn had
421 slightly elevated concentrations in the metal system. Furthermore, Cu concentration was higher in the metal system
422 in phase 0 and phase 3 (Fig. 3). In phase 2, all metals but Mn and Cu showed higher concentrations in the metal
423 system than in the control system. Mn concentrations were higher in the control system during phase 0 to phase 2.
424 In phase 3, the concentration of all heavy metals were elevated in the metal system compared to the control system.
425 The variation of the metal concentration was highest in phase 3, in both systems, for all elements but Cu, which
426 showed highest variation in phase 0 (Fig. 3). The control system generally displayed a smaller degree of variation
427 than the metal system.

428 Even though, the aim was to maintain the target concentrations shown in Table 1 during the 21 days of each
429 culturing period by the bi-weekly addition of an aliquot of the multi metal stock solution, the target concentration
430 of the metals was not obtained for most metals in phase 1 and 2, the only exception was Ag in phase 1 (Table 3).
431 The difference factors between the target and measured concentration was highest (> 50) for Ni, Cu and Zn in
432 phase 1 and decreased in phase 2 and 3. In phase 3, metals Cr, Mn, Cu, Ag and Sn reached concentrations closer
433 (factor 0.4-0.8) to the target concentration and Ni, Zn, Cd, Hg and Pb concentrations were higher (factor 3.1-9.9)
434 than expected. Furthermore, the change in metal concentration was small for the transition from phase 0 to 1 (factor
435 <1.4) for all elements but Cd (factor 2.6) and Hg (factor 7.5).

436

3.3.1 Incorporation of heavy metals into the foraminiferal shell

438 **Table 4**: Mean heavy metal–to–calcium values of *A. aomoriensis*, *A. batava* and *E. excavatum* in the control and
439 the metal system. Errors are standard errors of the mean (standard deviation $\sigma/\sqrt{n}$). Values marked with an asterisk
440 were derived from only one laser spot and thus are not considered for further discussion. Furthermore, the
441 calculated D_{TE} values, the slope of the linear regression line (OLS-Ordinary Least Squares) of all means, Pearson's
442 correlation coefficient (R^2) and its significance (p) are given for the calculation with all phases and when removing
443 phase 3 from the calculations. Cases where the regression lines were forced through the origin are indicated.. In
444 cases when a regression did not show significant correlation, the D_{TE} range calculated separately from the
445 individual phases is given. In cases when the regression was significant, the D_{TE} values represent the slope of the
446 regression line. Ph = Phase, SD = Standard deviation. Values in Table S1 are the basis of all calculations.

	Phase	Cr/Ca	Mn/Ca	Ni/Ca	Cu/Ca	Zn/Ca
Control System		$\mu mol\ mol^{-1}$	$mmol\ mol^{-1}$	$\mu mol\ mol^{-1}$	$\mu mol\ mol^{-1}$	$\mu mol\ mol^{-1}$
A. aomoriensis	0	18.6 ± 2.5	0.11 ± 0.02	1.3 ± 0.2	5.6 ± 0.9	53.2 ± 8.8
	1	12.6 ± 0.6	0.53 ± 0.12	5.9 ± 0.8	8.6 ± 1.0	34.2 ± 4.7
	2	13.6 ± 0.5	0.27 ± 0.07	2.1 ± 0.2	3.6 ± 0.2	18.6 ± 1.9
	3	10.2 ± 0.6	0.43 ± 0.08	4.3 ± 0.7	8.1 ± 2.0	29.5 ± 6.1
A. batava	0	11.6 ± 0.7	0.04 ± 0.01	1.4 ± 0.2	7.2 ± 1.1	23.9 ± 4.5
	1	10.9 ± 0.5	0.03 ± 0.00	2.6 ± 0.3	5.9 ± 0.6	17.8 ± 1.3
	2	9.0 ± 0.3	0.03 ± 0.00	0.9 ± 0.1	5.0 ± 1.0	12.9 ± 1.4
	3	9.1 ± 0.4	0.03 ± 0.01	1.9 ± 0.2	6.5 ± 1.3	14.9 ± 2.2
E. excavatum	0	22.9 ± 2.9	0.43 ± 0.13	9.4 ± 2.5	22.3 ± 7.9	28.1 ± 4.5
	1	88.9 ± 34.1	2.29 ± 0.56	7.8 ± 1.9	20.3 ± 8.0	48.9 ± 12.1
	2	16.2 ± 1.7	1.55 ± 0.26	5.9 ± 1.0	6.7 ± 1.4	21.9 ± 2.9
	3	26.7 ± 3.3	1.88 ± 0.55	4.4 ± 0.6	4.7 ± 0.7	16.8 ± 2.0
Metal System						
A. aomoriensis	0	16.0 ± 0.5	0.08 ± 0.02	5.5 ± 0.9	15.2 ± 2.6	29.8 ± 5.1
	1	14.0 ± 0.7	0.39 ± 0.08	3.1 ± 0.3	6.7 ± 0.7	30.0 ± 4.0

	Phase					
	2	11.1 ± 0.3	0.20 ± 0.05	5.3 ± 0.5	5.8 ± 0.5	28.3 ± 2.3
	3	14.1 ± 1.0	0.71 ± 0.12	3.8 ± 0.3	6.3 ± 1.5	42.2 ± 6.1
	0	16.5 ± 0.7	0.07 ± 0.01	1.1 ± 0.1	7.7 ± 1.6	68.0 ± 9.6
A. batava	1	15.2 ± 1.2	0.04 ± 0.01	1.8 ± 0.3	2.5 ± 0.6	20.7 ± 2.7
	2	9.7 ± 0.2	0.02 ± 0.00	1.8 ± 0.1	8.3 ± 1.8	12.9 ± 1.2
	3	12.2 ± 0.3	0.17 ± 0.04	2.9 ± 0.2	8.3 ± 1.2	49.8 ± 3.5
	0	17.30*	0.29*	4.30*	12.20*	26.70*
E. excavatum	1	32.9 ± 3.4	0.70 ± 0.12	8.2 ± 1.1	12.8 ± 1.8	18.5 ± 0.9
	2	41.8 ± 5.2	0.77 ± 0.15	8.6 ± 1.1	11.5 ± 1.5	29.8 ± 3.6
	3	54.1 ± 8.2	0.88 ± 0.15	17.0 ± 2.2	22.6 ± 3.6	43.1 ± 3.3

Calculations with Phase 3

A. aomoriensis

Slope of regression line ±SD		0.38 ±0.30		1.18 ±0.25	
Correlation coefficient (R^2)		0.83		0.80	
Significance (p)		0.05		0.05	
D_{TE} ±SD	0.4-10.3	0.38 ±0.30	0.06-0.94	1.18 ±0.25	0.08-0.45
Forced through origin	Single points	Yes	Single points	Yes	Single points

A. batava

Slope of regression line ±SD		0.23 ±0.04			
Correlation coefficient (R^2)		0.84			
Significance (p)		0.001			
D_{TE} ±SD	0.4-6.8	0.23 ±0.04	0.05-0.41	0.60-4.35	0.09-0.65
Forced through origin	Single points	No	Single points	Single points	Single points

E. excavatum

Slope of regression line ±SD	2.1 ±0.28		0.19 ±0.04		
Correlation coefficient (R^2)	0.82		0.79		
Significance (p)	0.01		0.003		
D_{TE} ±SD	2.1 ±0.28	0.34-2.50	0.19 ±0.04	0.95-5.67	0.08-0.53
Forced through origin	Yes	Single points	No	Single points	Single points

Calculations without Phase 3

A. aomoriensis

Slope of regression line ±SD					
Correlation coefficient (R^2)					
Significance (p)					
D_{TE} ±SD	0.74-10.3	0.09-0.53	0.19-0.94	0.61-5.42	0.21-0.45
Forced through origin	Single points	Single points	Single points	Single points	Single points

A. batava

Slope of regression line ±SD					
Correlation coefficient (R^2)					
Significance (p)					
D_{TE} ±SD	0.65-6.8	0.02-0.08	0.15-0.41	0.60-4.35	0.10-0.65
Forced through origin	Single points	Single points	Single points	Single points	Single points

E. excavatum

Slope of regression line ±SD					
Correlation coefficient (R^2)					
Significance (p)					
D_{TE} ±SD	2.5-13.4	0.34-2.50	0.64-1.35	0.95-4.73	0.22-0.53
Forced through origin	Single points	Single points	Single points	Single points	Single points

447

448 **Table 4** continued.

	Phase	Ag/Ca	Cd/Ca	Sn/Ca	Hg/Ca	Pb/Ca
Control System		µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	nmol mol^{-1}	µmol mol^{-1}
A. aomoriensis	0	0.27 ± 0.08	7.6 ± 1.0	0.33 ± 0.07	1.54 ± 0.46	1.23 ± 0.22
	1	0.28 ± 0.05	3.8 ± 0.3	1.60 ± 0.30	3.11 ± 0.68	1.14 ± 0.16
	2	0.16 ± 0.04	3.6 ± 0.2	0.21 ± 0.03	1.13 ± 0.31	0.81 ± 0.10
	3	0.31 ± 0.11	2.9 ± 0.2	0.19 ± 0.03	8.02 ± 1.72	1.45 ± 0.42
A. batava	0	0.09 ± 0.03	4.7 ± 0.5	0.27 ± 0.05	1.3 ± 0.4	0.67 ± 0.10
	1	0.07 ± 0.01	2.5 ± 0.2	0.65 ± 0.09	1.2 ± 0.3	0.29 ± 0.03

	Phase					
	2	0.05 ± 0.00	2.7 ± 0.1	0.08 ± 0.02	1.5 ± 0.4	0.39 ± 0.03
	3	0.06 ± 0.01	1.9 ± 0.1	0.10 ± 0.02	4.4 ± 0.6	0.36 ± 0.05
E. excavatum	0	0.22 ± 0.09	3.6 ± 1.1	0.99 ± 0.40	15.0 ± 4.4	1.83 ± 0.59
	1	0.07 ± 0.01	20.1 ± 9.2	8.21 ± 2.63	83.0 ± 33.4	2.22 ± 0.54
	2	0.10 ± 0.03	1.2 ± 0.2	0.45 ± 0.08	16.9 ± 3.8	0.94 ± 0.10
	3	0.04 ± 0.01	2.3 ± 0.4	0.27 ± 0.03	35.8 ± 6.3	0.55 ± 0.11
Metal System						
A. aomoriensis	0	0.08 ± 0.03	4.9 ± 0.3	0.62 ± 0.09	2.6 ± 0.6	1.17 ± 0.24
	1	0.25 ± 0.04	4.0 ± 0.4	0.84 ± 0.10	1.8 ± 0.2	0.90 ± 0.13
	2	0.52 ± 0.08	5.5 ± 0.4	1.70 ± 0.17	9.1 ± 1.7	3.85 ± 0.45
	3	3.03 ± 0.39	5.4 ± 0.4	0.55 ± 0.10	10.3 ± 1.3	22.14 ± 2.37
A. batava	0	0.06 ± 0.03	6.2 ± 0.2	0.19 ± 0.04	1.0 ± 0.2	1.27 ± 0.08
	1	0.04 ± 0.01	3.1 ± 0.3	0.59 ± 0.12	0.2 ± 0.0	0.42 ± 0.07
	2	0.18 ± 0.04	3.1 ± 0.2	0.46 ± 0.06	4.5 ± 1.1	0.52 ± 0.05
	3	1.05 ± 0.17	6.5 ± 0.3	0.21 ± 0.02	7.7 ± 1.0	29.82 ± 3.70
E. excavatum	0	0.40*	5.60*	0.18*	6.80*	1.59*
	1	0.03 ± 0.01	3.0 ± 0.3	2.63 ± 0.32	85.7 ± 19.7	1.36 ± 0.15
	2	0.69 ± 0.18	3.9 ± 0.5	2.89 ± 0.47	120.4 ± 44.7	4.61 ± 0.86
	3	2.84 ± 0.64	4.7 ± 0.5	2.74 ± 0.42	94.9 ± 16.2	52.51 ± 6.17
Calculations with Phase 3						
A. aomoriensis						
Slope of regression line ±SD		0.50 ±0.02				0.39 ±0.01
Correlation coefficient (R^2)		0.97				0.97
Significance (p)		< 0.0001				< 0.0001
D_{TE} ±SD		0.50 ±0.02	0.07-18.49	0.07-0.63	0.003-1.39	0.39 ±0.01
Forced through origin		Yes	Single points	Single points	Single points	Yes
A. batava						
Slope of regression line ±SD		0.17 ±0.01			0.003 ±0.001	0.52 ±0.01
Correlation coefficient (R^2)		0.98			0.63	1
Significance (p)		< 0.0001			0.01	< 0.0001
D_{TE} ±SD		0.17 ±0.01	0.08-14.42	0.03-0.26	0.003 ±0.001	0.52 ±0.01
Forced through origin		Yes	Single points	Single points	Yes	Yes
E. excavatum						
Slope of regression line ±SD		0.47 ±0.04				0.91 ±0.01
Correlation coefficient (R^2)		0.96				1
Significance (p)		< 0.0001				< 0.0001
D_{TE} ±SD		0.47 ±0.04	0.06-49.45	0.06-3.25	0.03-18.51	0.91 ±0.01
Forced through origin		Yes	Single points	Single points	Single points	Yes
Calculations without Phase 3						
A. aomoriensis						
Slope of regression line ±SD						1.6 ±0.17
Correlation coefficient (R^2)						0.91
Significance (p)						< 0.001
D_{TE} ±SD		0.70-2.57	1.14-18.49	0.10-0.63	0.003-1.39	1.60 ±0.17
Forced through origin		Single points	Single points	Single points	Single points	Yes
A. batava						
Slope of regression line ±SD		0.35 ±0.09				
Correlation coefficient (R^2)		0.91				
Significance (p)		0.03				
D_{TE} ±SD		0.35 ±0.09	0.63-14.42	0.04-0.26	0.005-0.76	0.20-5.52
Forced through origin		Yes	Single points	Single points	Single points	Single points
E. excavatum						
Slope of regression line ±SD					0.26 ±0.11	2 ±0.28
Correlation coefficient (R^2)					0.53	0.90
Significance (p)					0.05	0.003
D_{TE} ±SD		0.23-4.25	0.80-49.45	0.06-3.25	0.26 ±0.11	2.0 ±0.28
Forced through origin		Single points	Single points	Single points	No	Yes

449

450   Measurable incorporation into the foraminiferal calcite was found for all the heavy metals analysed but the degree

451   of incorporation varied profoundly within and between species (Fig. 4 and Table 4). In both systems, the heavy

452   metal concentration in *E. excavatum* was higher than in the other species (*A. aomoriensis* and *A. batava*) for Cr,
453   Mn, Ni, Hg and Sn. This trend is also visible but less pronounced in the Cu values of the control system.

454   Cr, Ni, Cu, Zn, Cd, Pb and Ag values of *A. aomoriensis* displayed the highest standard error of the mean paired
455   with highest concentrations in the water in the metal system. Sn, Mn and Hg did not show any clear pattern. In the
456   control system, all heavy metal concentrations had higher standard errors of the mean when the concentration of
457   these metals in the culturing medium was higher. The trend was also shown in *A. batava* and *E. excavatum* for all
458   heavy metals of the control and the metal system. Note that even though no extra metals were added to the culturing
459   medium of the control system, differences in the heavy metal concentration occurred (Fig. 3 and Table 3).

460   Calculations were performed with and without phase 3 of the metal system (Fig. 4, Fig. B2 and Table 4) to address
461   a possible overload effect when it comes to higher metal concentrations in the seawater.

462   When phase 3 was included, a strong positive correlation ($R^2 > 0.9$, $p \leq 0.05$) between Ag and Pb concentrations
463   in the foraminiferal shell and the culturing medium was found for all three species. Furthermore, *A. batava* also
464   displayed a positive correlation for Hg ($R^2 = 0.63$, $p < 0.01$), *A. aomoriensis* for Cu ($R^2 = 0.80$, $p < 0.05$) and *E.*
465   *excavatum* for Cr ($R^2 = 0.82$, $p < 0.01$) and Ni ($R^2 = 0.79$, $p < 0.003$). Weaker but still significant positive
466   correlations were recorded for Mn ($R^2 > 0.84$, $p \leq 0.05$) for both *Ammonia* species. An indistinct correlation of the
467   concentration in the seawater and in the foraminiferal test was recognised for Zn in all three species, whereas Cd
468   and Sn showed no covariance (Fig. 4 and Table 4).

469   When phase 3 was excluded from the calculations, *A. aomoriensis* and *E. excavatum* showed a positive correlation
470   for Pb ($R^2 > 0.9$, $p \leq 0.003$), *A. batava* for Ag ($R^2 = 0.91$, $p = 0.03$) and in *E. excavatum* Hg correlated weaker
471   positively ($R^2 > 0.53$, $p \leq 0.05$). All other elements show no significant correlation (Fig. 4 and Table 4).

472

473 **3.3.2 Partition coefficient (D$_{TE}$)**

474

475
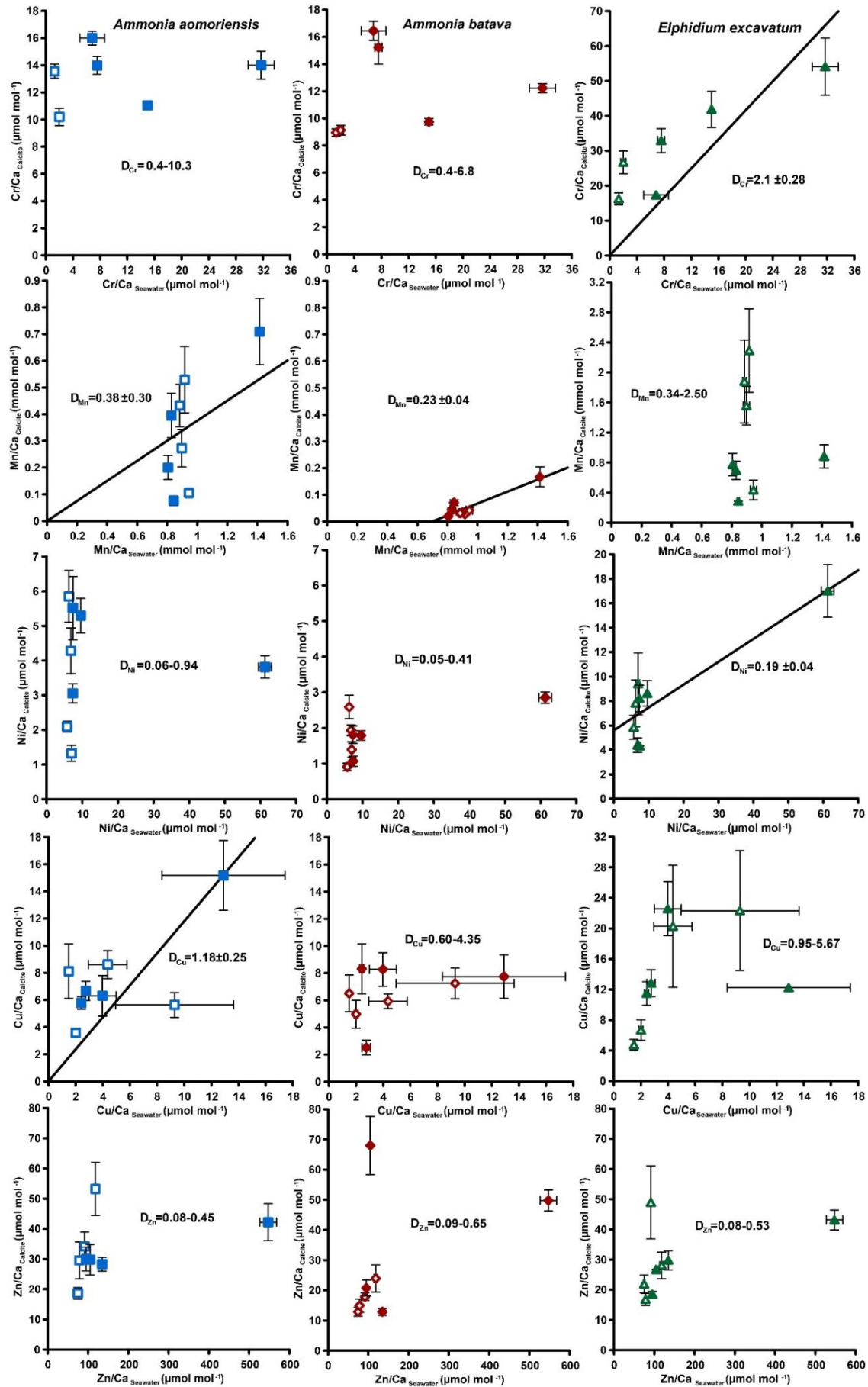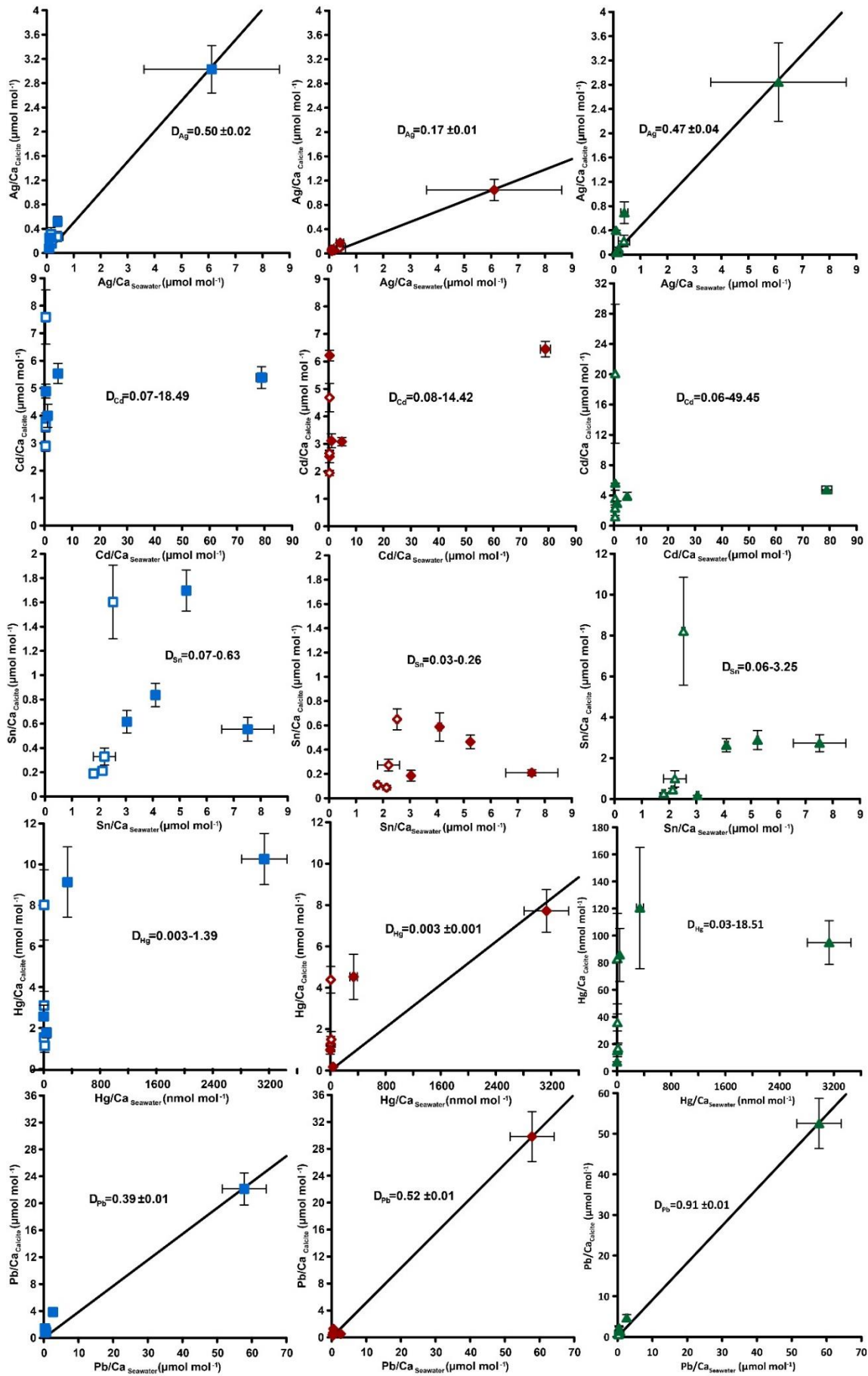
476

477 **Figure 4**: Mean TE/Ca values in the foraminiferal calcite versus the mean TE/Ca values in the corresponding

478 culturing medium based on phase 0 to 3. Each data point represents the mean value of all laser ablation ICP-MS

479 measurements on single foraminiferal chambers built up during the individual culturing phase plotted against the

480 mean metal concentrations in the seawater averaged over the culturing phase (Table 3). Because calculating p- and

481 R^2 values of the regression lines and the D_{TE}'s with the mean per phase resulted in comparable values to when

482 calculating with the overall dataset, we considered this approach adequate. Error bars symbolize the standard error

483 of the mean. The linear regression line (± standard deviation) is displayed when elements showed a significant

484 correlation between seawater and calcite. D_{TE}'s of *E. excavatum* were considered without values for Phase 0 of

485 the Metal System as only data from one newly formed chamber are available. All values can be found in Table 4.

486 An enlarged graph based on the calculations without phase 3 is provided in the appendix (Fig. B2).

487

488 The majority of D_{TE} were lower than 1 in *A. aomoriensis* (with phase 3 = 61 %, without phase 3 = 57%) and *A.*

489 *batava* (with phase 3 = 75%, without phase 3 = 73%), i.e., uptake but no enrichment took place. D_{TE} values derived

490 from *E. excavatum* on the other hand showed a smaller proportion < 1 (with phase 3 = 47%, without phase 3 =

491 42%). For most elements (Cr, Mn, Ni, Cu, Cd, Sn, Pb and Hg) D_{TE} derived from *E. excavatum* were higher than

492 D_{TE} from the two *Ammonia* species (Table 4, Fig. 4), which showed comparable D_{TE} values for most elements.

493 D_{Zn} built the exception because all values were within a similar range (D_{Zn} ~ 0.08-0.65) independent of the species.

494 For *A. aomoriensis* D_{Cu} was > 1 and D_{Cd} as well as D_{Pb} were also > 1 when phase 3 was excluded from the

495 calculations. *Elphidium excavatum* displayed D_{TE} values > 1 for Cr and Cu for the calculations with phase 3 and

496 also for Pb without phase 3. The highest variation between minimum and maximum D_{TE} for all species was found

497 for Cd and Hg.

498

499 **4. Discussion**

500 **4.1 Experimental Uncertainties**

501 Calcein was used for staining the foraminiferal test before they were placed into the culturing system. It can be

502 assumed, that a period of 1 or 2 days for removing excess calcein was sufficient because the youngest chambers

503 were not stained. Calcein binds to Ca and is incorporated into the mineralised calcium carbonate (Bernhard et al.,

504 2004). It is conceivable that the heavy metal incorporation could also be affected by calcein. However, no evidence

505 for such effects has been found so far in a variety of studies (e.g., Hintz et al., 2006; De Nooijer et al., 2007;

506 Dissard et al., 2009). Furthermore, calcein was only used prior to the experiment to mark the last chamber that

507 was grown outside the culturing system. Therefore, the incorporation of the metals measured in subsequent

508 chambers was not affected by the calcein application.

509 The element concentrations within the culturing medium of each culturing phase were comparably stable for most

510 elements in the control system. In the metal system, the variations were higher, which is due to the punctual input

21

of the multi metal stock solution for reaching the next phase concentration (Table A1, Fig. B1). This sudden addition of metals resulted in a high peak concentration in the beginning of the new phase, which equilibrated after a while. This trend was most pronounced in phase 3 as the added amount of the multi metal stock solution was highest for this phase, which was also why the standard error of this phase was comparably high. Furthermore, the variations of the metal concentrations were in a comparable range than those presented in other culturing studies (e.g., Marechal-Abram et al., 2004; De Nooijer et al., 2007; Munsel et al., 2010; Remmelzwaal et al., 2019). Generally, many other studies (e.g., Remmelzwaal et al., 2019; Sagar et al., 2021a; Titelboim et al., 2021) measured the heavy metal concentration in the seawater less frequently than done in this study. Therefore, the stability of metal concentrations during the culturing phases of those studies are often inferred. Furthermore, pollution events in nature are in most cases not persistent and stable but transient as was mirrored by the concentration changes in our experiments.

The measured metal concentrations in the culturing seawater were smaller than expected (Table 3). This in combination with the varying metal concentration within one phase suggested that several processes were affecting the concentration in such a complex culturing system. One possible mechanism was sorption of the metals onto surfaces (e.g., tubing, culturing vessels, plates, organic matter or the foraminiferal test itself), which could have lowered the metal concentration in the culturing medium. Therefore, sorption could have contributed to the overall budget of the metals. On the other hand, Cu appeared to have been released from components of the culturing system even though the system was cleaned before use and was operated with seawater for 14 days before the experiments begun. For instance, the concentration of Cu was high in phase 0, where no metals were added suggesting release from system parts. In phase 1, the Cu concentration decreased meaning the contamination derived from the system was removed by a process similar to that observed for the other metals after additions were made. Similar effects have been reported by De Nooijer et al. (2007) for Cu and Havach et al. (2001) for Cd. Other processes like the uptake of the metals by the foraminifera itself and the growth of algae could further have an influence on the metal concentration in the culturing medium. Germs of algae were introduced accidentally together with the living foraminifera and grew during the experiment. Such processes are difficult to predict and even more challenging to avoid but probably mirror real environments more realistically than sterile petri dish experiments (e.g., Havach et al., 2001; Hintz et al., 2004; Munsel et al., 2010).

Neither the survival rate nor the formation of new chambers was influenced by the elevated metal concentrations during the culturing period. These features were rather constant between the four different phases. Furthermore, no test morphology malformations were recognised. Elevated heavy metal concentrations are thought to induce a higher rate of malformations in benthic foraminifera (e.g., Sharifi et al., 1991; Yanko et al., 1998), whereas recent studies constrained them as a reaction to stressful environments, not necessarily created by high heavy metal concentrations (Frontalini and Coccioni, 2008; Polovodova and Schönfeld, 2008). The lack of malformations in our experiments suggested that the foraminifera were neither poisoned by elevated heavy metal concentrations nor stressed too much by strongly varying environmental parameters, maintaining a normal metabolism and growth. Reproduction was generally very rare, which may indicate that the conditions were not ideal. In field studies foraminiferal reproduction has been linked to short periods of elevated food supply (e.g., Lee et al., 1969; Gooday, 1988; Schönfeld and Numberger, 2007). The regular feeding of foraminifera in our experiment twice a week at constant rates therefore probably did not provide supply levels that trigger reproduction. Nevertheless, it can be assumed that a sufficient amount of food was provided because after the experiments, leftovers covering the

551 sediment surfaces in the cavities were evident. This would have likely been consumed by the foraminifera if they
552 would have needed more. Furthermore, the foraminifera calcified, which would not be the case if any
553 malnourishment occurred (e.g., Lee et al., 1991; Kurtarkar et al., 2019). Therefore, the nutritional status is unlikely
554 to have influenced the metal uptake by the foraminifera.

555 The calibrations between the heavy metal concentration in seawater and the foraminiferal shell rely on the TE/Ca
556 values from phase 3 because the difference in seawater concentration was highest compared to other phases.
557 Nevertheless, data points from other phases do play a role and forcing through the origin adds a further fixed point.
558 High variability for D_{TE} values like observed here for Cd or Cu is difficult to explain. Such variability suggests
559 there are factors affecting these metals we do not understand and therefore it is also important to show the data for
560 these elements. Furthermore, the experimental design, especially the mixture of metals, was chosen to best
561 simulate metal conditions in real environments, which could naturally enhance the variability of D_{TE}. This
562 knowledge is indispensable for the application of heavy metal concentrations in foraminifera as a proxy for the
563 heavy metal concentration in seawater.

564

565 **4.2 Incorporation of heavy metals in the foraminiferal test**
566 Many heavy metals have been demonstrated to be incorporated into the foraminiferal shell (e.g., Cr: Remmelzwaal
567 et al., 2019; Mn: Koho et al, 2015; 2017; Barras et al., 2018; Cu: De Nooijer et al., 2007; Ni: Munsel et al., 2010;
568 Hg: Frontalini et al., 2018a; Cd: Havach et al., 2001; Pb: Frontalini et al., 2018b; Titelboim et al., 2018; Sagar et
569 al., 2021a; 2021b; Zn: Marchitto et al., 2000; Van Dijk et al., 2017), and the incorporation of all of these metals
570 has been measured here. Additionally, to the best of our knowledge, Sn and Ag were investigated here for the first
571 time. The levels observed were well above control values indicating an elevated incorporation of Ag and Sn into
572 the foraminiferal test calcite with increasing metal concentrations in seawater.

573 Different factors can influence the incorporation of these metals into the foraminiferal test. First of all, the uptake
574 depends on metabolic pathways during the calcification process. Fundamental biomineralization processes of
575 foraminifera are the subject of an ongoing discussion and several (partly) competing models have been proposed
576 (e.g., Elderfield and Erez, 1996; Erez, 2003; De Nooijer et al., 2009b, 2014; Nehrke et al., 2013). One model
577 proposes that the foraminifera take up ions directly from the surrounding seawater by endocytosis or by building
578 seawater vacuoles, which are transported to the site of calcification (SOC) (Elderfield and Erez, 1996; Erez 2003;
579 De Nooijer et al., 2009b; 2009a; Khalifa et al., 2016). The SOC is located outside the foraminiferal cell and the
580 formation of new calcite takes place in this zone (see De Nooijer et al., 2014 for a summary and illustration). There
581 is evidence that this SOC is separated from the surrounding seawater (e.g., Spindler, 1978; Bé et al., 1979; De
582 Nooijer et al., 2009b; 2014; Glas et al., 2012; Nehrke et al., 2013). The other competing model suggests that the
583 uptake of ions and the transport to the SOC is performed directly from the seawater across the cell membrane by
584 active trans-membrane-transports (TMT) and/ or passive transport via gaps in the pseudopodial network of the
585 foraminifera (Nehrke et al., 2013; De Nooijer et al., 2014). The dependence of heavy metal concentrations in the
586 foraminiferal test on their seawater concentration relies on the prevailing mechanism. Biomineralization based on
587 endocytosis would infer that the metal concentration in the seawater is directly mirrored by their concentration in
588 the foraminiferal shell, which is not generally supported by the results of our study except for Ag and Pb. Several
589 metals showed partition coefficients > 1 or <1 when the D_{TE}´s were calculated separately for each culturing phase.

590   Only Pb and Cr in *E. excavatum* and Cu and Pb in *A. aomoriensis* consistently displayed mean D_{TE}'s > 1 paired

591   with a positive correlation of the concentration in seawater and in the foraminiferal shell, which could indicate a

592   non-selective uptake of these metals meaning uptake not only driven by the chemical properties of the ion such as

593   the size of the metal ion itself. If this would have been the case, D_{TE} values > than 1 would be expected especially

594   for metals ions that are smaller than Ca (Rimstidt et al., 1998). On the other hand, the D_{TE} values of many elements

595   (Ni, Zn, Cd, Hg, Pb) dramatically decreased with increasing concentration in the seawater in the highest metal

596   treatment in all species (Fig. 4). This kind of overload effect has also been noted by Nardelli et al. (2016) for Zn,

597   by Barras et al. (2018) for Mn, by Mewes et al., (2015) for Mg and by Munsel et al. (2010) for Ni. Nardelli et al.

598   (2016) suggested that some biological mechanism expulse or block these metals if the concentration is too high

599   and imminent intoxication is probable, which may be managed by controlling the ion uptake via TMT. Therefore,

600   it may well be possible that the highest concentration of the metals in our study was close to the tipping point of

601   the biological mechanism taking over and protecting the organism.

602   Besides biologically controlled factors, physicochemical properties also play an important role when it comes to

603   the uptake of ions. One chemical factor is the aqueous speciation and solubility of the metals. Metals with a free

604   ion form with a charge of 2+ are more similar to Ca^{2+}, which makes incorporation more likely (Railsback, 1999).

605   Nearly all metals in this study were added as dissolved chlorides and therefore had a charge of 2+. The only

606   exceptions were Ag, which was added as $AgNO_3$ with a charge of 1+ and Cr, which was added as $CrCl_3*6\,H_2O$.

607   The charge of the cation as such does not seem to make a major difference as Ag was incorporated into all three

608   species and Cr into *E. excavatum* with a significant positive correlation to concentrations in the culturing medium.

609   Furthermore, it is possible that the oxidative state of the elements changed due to their pH dependency, which will

610   be discussed for every element separately. Furthermore, other ions with a charge of 1+ are also known to be

611   incorporated in calcite. Examples are Li^+ (e.g., Delaney et al., 1985; Hall et al., 2004) and Na^+ (e.g., Wit et al.,

612   2013; Bertlich et al., 2018), which are believed to occupy interstitial positions in calcite where the calcite lattice

613   has defects (Ishikawa and Ichikuni, 1984; Okumura and Kitano, 1986). In addition, rare earth elements with a

614   charge of 3+ are also detected in the foraminiferal calcite (e.g., Haley et al., 2005; Roberts et al., 2012).

615   The aqueous speciation of many metals is strongly influenced by the pH (e.g., Förstner, 1993; Pagnanelli et al.,

616   2003; Spurgeon et al., 2006; Powell et al., 2015; Huang et al., 2017). As the pH during the experiment was stable

617   around 8.0 ± 0.1 (measured twice a week), speciation changes between phases due to varying pH values can be

618   excluded. However, it is possible that some metals were not available in a form that could be readily incorporated

619   in the calcite such as the free ion or carbonate species. Cr was not available in an optimal speciation to substitute

620   Ca as a pH of 8 would favour Cr^{3+} or Cr^{4+} as well as oxides and hydroxides (Elderfield, 1970; Geisler and Schmidt,

621   1991). Furthermore, the used Cr-salt may not have dissolved completely, even though the multi metal stock

622   solution was heated and stirred during the process. Both in combination may lead to the small variation in the

623   seawater concentrations between the different phases. Interferences that could possibly have influenced the Cr

624   measurements in the water samples are chlorine oxides or hydroxides (e.g., Tan and Horlick, 1986; McLaren et

625   al., 1987, Reed et al., 1994; Laborda et al., 1994). Measurements of reference materials revealed slightly elevated

626   Cr concentrations compared to those presented in the literature (Table A2), which indicates that interferences could

627   be responsible for some of the observed variability for Cr. Similar pH dependant processes could also have affected

628   Cu.Nevertheless, Cu and Cr were taken up by all species and therefore, this factor cannot be decisive when it

629   comes to incorporation of these metals into the foraminiferal shell.

630 If the incorporation of metals would be straightforward and would only depend on the speciation of the metal and

631 other physicochemical factors, the behaviour of the metals would mostly be influenced by the ionic radius in

632 combination with the charge of the metal ions as described for carbonate minerals by Rimstidt et al. (1998). The

633 endocytotic pathway of seawater into the foraminifer should produce a behaviour of ion incorporation comparable

634 to inorganic calcite precipitation. It was found that cations are incorporated into inorganic calcite by substitution

635 of Ca^{2+} (e.g., Reeder et al., 1999), especially when the effective ionic radius of these ions is comparable to the one

636 of calcium (= 1.0 Å).

637
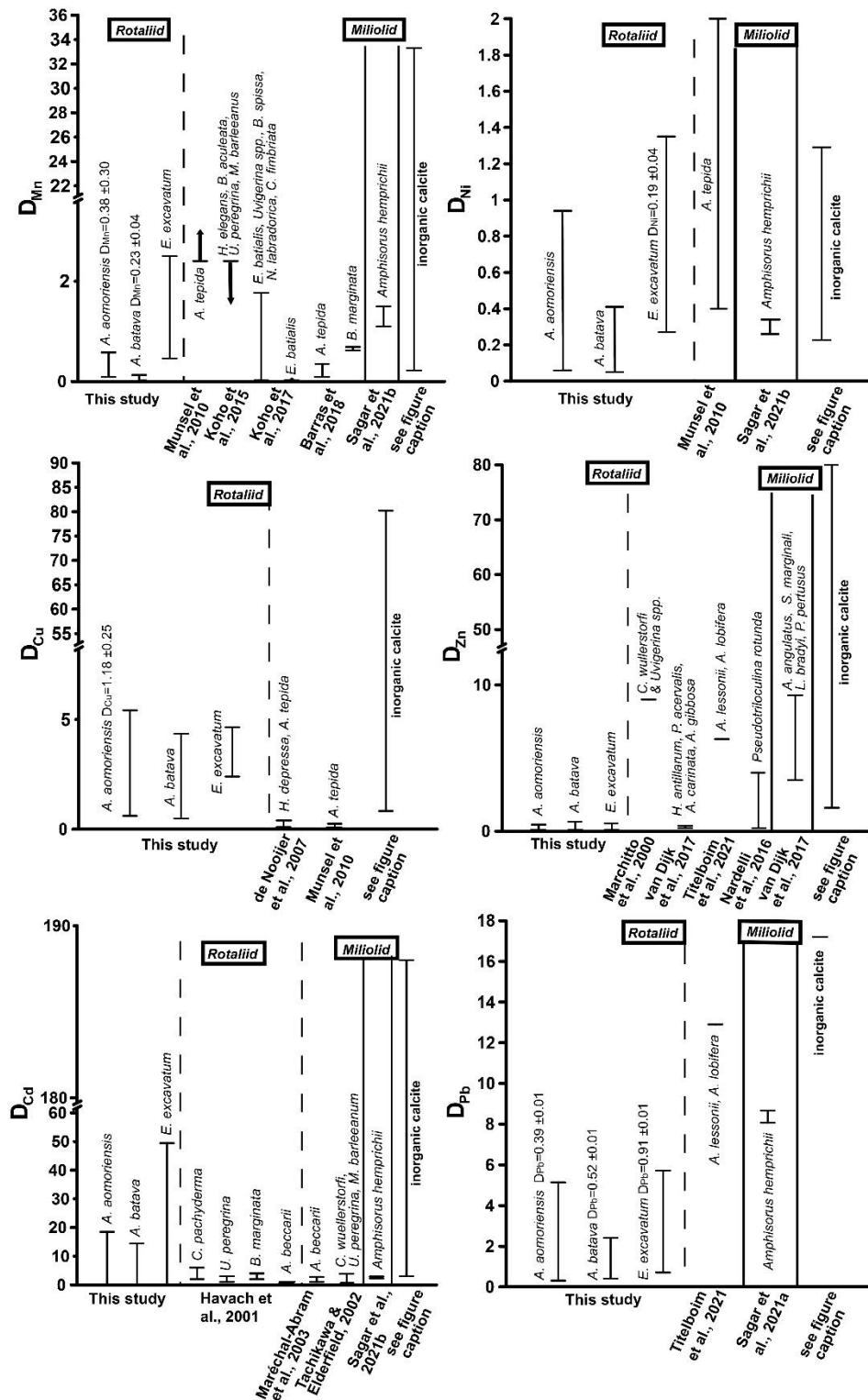
638   Figure 5: Comparison of D_{TE} values of this study with D_{TE} values from literature of different rotaliid and miliolid

639   foraminiferal species. The range of D_{TE} based on the different culturing phases is given and if a correlation between

640   the heavy metal concentration in seawater and the foraminiferal shell was detected, the mean D_{TE} value ±SD

641   (=slope of the regression line) is also indicated. Note that the x-axis was clipped for some elements. (Literature

642   for inorganic calcite D_{TE} values: Ni = Rimstidt et al., 1998; Alvarez et al., 2021; Mn = Lorens, 1981; Dromgoole

643   and Walter, 1990; Wang et al., 2021; Cu =Kitano et al., 1973; 1980; Wang et al, 2021; Zn = Kitano et al., 1973;

644   1980; Rimstidt et al., 1998; Wang et al., 2021; Cd = Rimstidt et al., 1998; Day and Henderson, 2013; Pb = Rimstidt

645   et al., 1998.)

646

647   Some metals like Mn, Zn and Cu are known to be fundamentally necessary as micro-nutrients to maintain

648   biological and physiological function of a cell (e.g., Mertz, 1981; Tchounwou et al., 2012; Martinez-Colon et al.,

649   2009; Maret, 2016). Therefore, these elements should preferentially be taken up into the foraminiferal cell, where

650   they are used for further processes. This in turn could lead to the consumption of these metals before they can be

651   incorporated into the foraminiferal tests. The artificial sea salt used in this study ensured that these elements were

652   present in a sufficient amount of micronutrients. All of these ions have a similar ionic radius (Cu = 0.73 Å, Mn =

653   0.67 Å, Zn = 0.74 Å) in six-fold coordination (Rimstidt et al., 1998), which would also suggest, that their behaviour

654   is comparable. The ionic radii are much smaller than that of Ca, but are rather similar to Mg (0.72 Å, Rimstidt et

655   al., 1998).

656   Mn showed a positive correlation between its concentration in seawater and the foraminiferal test in the two

657   *Ammonia* species when the calculations included phase 3. This indicates that this element serves as a well-behaved

658   proxy influenced mainly by its concentration in seawater. However, *E. excavatum* did not show this positive

659   correlation. D_{Mn} values of this study were comparable with rotaliid and miliolid species and partly with D_{Mn} values

660   from inorganic precipitation (Fig. 5). Species-specific partition coefficients of elements like Mg or Na are already

661   reported in the literature (e.g., Toyofuku et al., 2011; Barras et al., 2018; Wit et al., 2013) and could also explain

662   the different D_{TE} values of *E. excavatum* in this study (see below). Furthermore, it is known that the presence of

663   toxic metals such as Cd, Ni or Hg can inhibit the uptake of essential metals like Mn into the cell if these metals

664   are present in low concentrations (e.g., Sunda and Huntsman, 1998a, 1998b). It is possible that this mechanism is

665   more pronounced in *E. excavatum* than in the *Ammonia* species. Zn was clearly incorporated above control levels

666   into all three species, but it´s behaviour was influenced by more factors than the concentration of Zn in the culturing

667   medium(Fig. 4, Table 4). D_{Zn} values of this study are in good agreement with those calculated by Van Dijk et al.

668   (2017) for four hyaline species and Nardelli et al. (2016) for the miliolid *Pseudotriloculina rotunda* (Fig. 5) Other

669   studies reported higher values. It is again possible that the mixture of metals inhibited the uptake of essential metals

670   like Zn similar to Mn. Cu showed a simple well-behaved proxy behaviour with a significant positive correlation

671   in *A. aomoriensis* but not in the other two species. The D_{Cu} presented in the literature for rotaliid species are lower

672   than D_{Cu} from this study. Inorganic values were mostly higher (Fig. 5). These differences could arise from the

673   lower concentration of Cu in this study or from the mixture of metals. It is also reported, that the exposure to more

674   than one metal can cause an increased uptake of another metal into the cell (Archibald and Duong, 1984; Martinez-

675   Finley et al., 2012; Bruins et al., 2000; Shafiq et al., 1991). If more Cu is taken up into the cell after the usage of

676   Cu as micronutrient more Cu is left over and could possibly be deposited into the calcite. It is therefore conceivable

677   that one particular metal in our study was effecting a co-uptake of Cu, which lead to an elevated incorporation into

678   the calcite as compared to other studies.

679   The non-essential elements Hg, Cd and Pb are not used in physiological processes and are therefore believed to

680   have a higher toxic potential (Barbier et al., 2005; Raikwar et al, 2008; Ali and Khan, 2019). This could first of all

681   make the foraminifera prevent the uptake of these metals into their cell. But if the uptake of heavy metals into the

682   cell cannot be prevented, the foraminifera may remove the metals to their shell instead of keeping them in their

683   cell. This is a common mechanism for avoiding intoxication reported for various organisms (benthic foraminifera:

684   Bresler and Yanko, 1995; Yeast: Adle et al., 2007; Bacteria: Shaw and Dussan, 2015; Microalgae: Duque et al.,

685   2019). Furthermore, this would mean thatthe incorporation of these metals into the foraminiferal calcite increases.

686   The ionic radii of Pb^{2+} in calcite-coordination is 1.19 Å, which is remarkably higher than those of Hg^{2+} (1.02 Å)

687   and Cd^{2+} (0.95 Å), which are comparable to Ca.This similarity should also favour the incorporation of Cd and Hg

688   into calcite, which holds only partly true, as Cd showed no trends with complex behaviour, but Hg was linearly

689   incorporated in *A. batava* and in *E. excavatum* if the high concentrations of phase 3 are excluded. Pb emerged as

690   a well-behaved proxy under these experimental conditions with all three species incorporating Pb linearly (Fig. 4,

691   Table 4). When comparing D_{Pb} values in the literature, our D_{Pb} are slightly lower (Fig. 5). For Hg, no partition

692   coefficients were published so far. D_{Cd} values from different studies (Havach et al., 2001; Tachikawa and

693   Elderfield, 2002; Maréchal-Abram et al., 2004, Sagar at al., 2021b) have overall a smaller range of D_{Cd} values

694   than found here (Fig. 5). The greater variability in D_{Cd} of our study makes a comparison difficult.

695   The importance of other metals like Sn, Cr, Ag and Ni is not fully understood yet but some of them are believed

696   to have certain biological functions in the cells of animals or plants (Horovitz, 1988; Mertz, 1993; Lukaski, 1999;

697   Pilon-Smits et al., 2009; Hänsch & Mendel, 2009; Chen et al., 2009). For example, Ni is important for plants and

698   bacteria (Poonkothai and Vijayavathi, 2012; Maret, 2016). The ionic radii of these metals in calcite-coordination

699   is rather different (Sn = 1.18 Å; Ag = 1.15 Å; Cr = 0.62 Å; Ni = 0.69 Å) and deviate from the ionic radius of Ca^{2+},

700   too.

701   Ni was incorporated with a positive trend in *E. excavatum,* but with no clear trend in the *Ammonia* species (Fig. 4,

702   Table 4). D_{Ni} values from rotaliid and miliolid foraminifera and from inorganic calcite are in good agreement with

703   our results (Fig. 5).Ag exhibited a strong positive correlation between seawater and foraminiferal shell in all three

704   foraminiferal species. Partition coefficients for Ag (*A. aomoriensis* D_{Ag} = 0.50 ±0.02 , *A. batava* D_{Ag} = 0.17 ±0.01,

705   *E. excavatum* D_{Ag} = 0.47 ±0.04) cannot be compared to other studies as no literature data are available.

706   Cr and Sn, on the other hand, were not incorporated in a higher amount when the concentration of these metals in

707   the culturing medium was raised, except for Cr in *E. excavatum*, which showed a positive correlation. The D_{Cr}

708   values presented in Remmelzwaal et al. (2019) (D_{Cr} > 107), based on culturing experiments with the tropical,

709   symbiont bearing foraminifera *Amphistegina spp.,* are at least one order of magnitude higher than D_{Cr} values in

710   this study (*A. aomoriensis* D_{Cr} = 0.74-10.3, *A. batava* D_{Cr} = 0.4-6.8, *E. excavatum* D_{Cr} = 2.1 ±0.28). One possible

711   reason for dynamics of Cr are the comparable low concentrations in the culturing medium and furthermore, the

712   differences between the phases were also very low (Fig. 3, Fig. B1 and Table 3). It may be that the concentration

713   of Cr needs to be further elevated and the concentration range needs to be extended before the foraminifera are

714   able to incorporate Cr with significant differences between concentrations. For Sn, no comparative studies are

715   available so we may speculate that the same could apply for Sn. Nevertheless, we recognised a correlation between

the concentration of Cr in the culturing medium and in the foraminiferal calcite of *E. excavatum*, but not for both *Ammonia* species.

4.3 Interspecies variability

The three different species cultured in this study clearly incorporated the same metal in different ways, which is most visible in the overall higher TE/Ca values of *E. excavatum* compared to species from the genus *Ammonia* (Fig. 4 and 5, Table 4). Koho et al. (2017) suggested that these differences in the incorporation result from different microhabitats used by different foraminiferal species. This might be true in nature. In our experiments, however, the sediment in the cavities was only a few mm thick and no redox horizon was recognised when recovering the foraminifera after the experiment. Therefore, all foraminifera were living in the same microhabitat. Leftover food may have created a microhabitat but this effect would have been the same in all cavities and therefore cannot account for the differences between the species. In our experiment, dead *Nannochloropsis* were fed, which is certainly not the preferred food source for *E. excavatum* (Pillet et al., 2011). This could lead to a slower growth and *E. excavatum* built on average only 1 chamber during the individual culturing period of 21 days while *Ammonia* species built more than four chambers. Furthermore, *E. excavatum* did not reproduce, even though the culturing period is close to the generation time of this species (Haake, 1962). When growth is slower, it could be possible that a higher amount of a metal is incorporated into the shell, which would lead to higher TE/Ca values in this species. It is possible that a more preferred food source would have stimulated enhanced growth and influenced the incorporation of heavy metal into the shells of *E. excavatum*. For instance, the closely related species *E. clavatum* prefers bacillariophycean diatoms (Schönfeld and Numberger, 2007). It may also be possible that *E. excavatum* is simply a slower growing species than *Ammonia*, which seems not to be necessarily connected to a specific food source (e.g., Haynert et al., 2020). One could assume that a slower growth would provide more time to remove potentially toxic metals from the cell to the foraminiferal shell, which could explain why *E. excavatum* incorporated a higher metal concentration than *A. aomoriensis* and *A. batava*.

Another possibility for the higher metal concentration found in *E. excavatum* is the timing of chamber formation. As *E. excavatum* formed on average one new chamber, it is possible that this chamber was formed during the high peak in the metal concentration during the beginning of the culturing phases (Fig. B1, Table A1). This could in turn lead to a higher uptake of the metals and apparently higher D_{TE} values. Both *Ammonia* species on the other hand, formed more chambers, which makes it most likely that the first high concentrations did not particularly influence the overall D_{TE} value. Unfortunately, it is not possible to constrain exactly when the specimens formed their new chambers. It was checked whether the evolution of the metal concentration in seawater of phase 3 was reflected in the intra-test (chamber to chamber) data for the two *Ammonia* species. Particularly, the initial high concentration of certain heavy metals was found in the first chambers of very few individuals after the staining (i.e. the first chamber built in culture). This is most likely due to the individual timing of calcification. Furthermore, it could also be possible that the foraminifera did not calcify during the first high peak due to an initial intoxication. Therefore, a mean value over the whole culturing phase was considered as most representative.

Comparing *Ammonia* and *Elphidium* species showed that the D_{TE} of the *Ammonia* species of this study are partly comparable to literature data (Fig. 5).

754 D_{TE} values are known to be generally higher in tropical high-Mg calcite taxa like *Amphistegina* (e.g., Titelboim et

755 al., 2021) and also high-Mg miliolid taxa like *Amphisorus* (e.g., Sagar et al., 2021a) incorporate a higher amount

756 of metals compared to rotaliid low-Mg taxa like *Ammonia* or *Elphidium*. Comparing our data with high-Mg

757 species, it is visible that this trend can be partly confirmed (Fig. 5). For Mn, both *Ammonia* species of this study

758 show lower values than miliolid species but D_{Mn} of *E. excavatum* is comparable. D_{Ni} values of *A. hemprichii*

759 determined by Sagar et al. (2021b) display the same range as the values for low-Mg species here and furthermore

760 D_{Zn} values of the miliolid *P. rotunda* (Nardelli et al., 2017) overlap with our findings. On the other hand, D_{Zn}

761 values from miliolids in van Dijk et al., (2017) and high-Mg rotaliids from Titelboim et al. (2021) are much higher.

762 The same trend is observed for D_{Pb} (Titelboim et al., 2021; Sagar et al., 2021a). When comparing the Zn/Ca

763 concentration in the foraminiferal shell directly to values from Titelboim et al. (2018), who analysed the Cu, Zn

764 and Pb concentration in rotaliid and miliolid species from a field site, our values show similarities with both

765 groups. Zn/Ca in the foraminiferal calcite of our study was a maximal ~ 68 µmol/mol, which is slightly lower than

766 reported in Titelboim et al. (2018) for the low-Mg species *Pararotalia calcariformata* (195 µmol/mol), but much

767 lower than Zn/Ca reported for the high-Mg species *Lachlanella* (2540 µmol/mol). Differences between the low-

768 Mg species may be due to different concentrations in the seawater the foraminifera grew in. As the seawater metal

769 concentration is not given in Titelboim et al. (2018) this cannot be evaluated. It may also be possible hat high-Mg

770 species have more defects in their tests, which would result in more interstitial space, leading to more space for

771 ions other than Ca. Maximum Cu/Ca values of our study are ~ 23 µmol/mol in *E. excavatum*, which fits the findings

772 of Titelboim et al. (2018) for rotaliid species (*P. calcariformata* ~21 µmol/mol) and is lower than in high-Mg

773 species (*Lachlanella* ~ 186 µmol/mol). Pb/Ca of ~ 12 µmol/mol in *P. calcariformata* described by Titelboim et al.

774 (2018) are lower than found here (max. Pb/Ca in *E. excavatum* of this study ~ 53 µmol/mol), whereas our findings

775 are more comparable to *Lachlanella* (Pb/Ca ~ 125 µmol/mol).

776 **5 Conclusion**

777 Culturing experiments with different foraminiferal species (*A. aomoriensis*, *A. batava* and *E. excavatum*) that were

778 exposed to a mixture of ten different metals (Cr, Mn, Ni, Cu, Zn, Ag, Cd, Sn, Hg and Pb) at varying concentrations

779 (Table 3, Fig. 3, Fig. B1) were carried out and laser ablation ICP-MS analysis of the newly formed calcite revealed

780 the following:

781 1. All metals used in this study were incorporated into the foraminiferal calcite of all three species (Fig. 4,
782 Table 4).

783 2. Species-specific differences in the incorporation of heavy metals occurred.

784 3. The following metals showed a positive correlation between the metal concentration in seawater and the
785 foraminiferal calcite inferring that the uptake of these metals mainly depends on its concentration in
786 seawater:

787 a. *Ammonia aomoriensis*: $D_{Mn} = 0.38 \pm 0.3$, $D_{Cu} = 1.18 \pm 0.25$, $D_{Ag} = 0.50 \pm 0.02$, $D_{Pb} = 0.39 \pm 0.01$

788 b. *Ammonia batava*: $D_{Mn} = 0.23 \pm 0.04$, $D_{Ag} = 0.17 \pm 0.01$, $D_{Hg} = 0.003 \pm 0.001$; $D_{Pb} = 0.52 \pm 0.01$

789 c. *Elphidium excavatum*: $D_{Cr} = 2.1 \pm 0.28$, $D_{Ni} = 0.19 \pm 0.04$, $D_{Ag} = 0.47 \pm 0.04$, $D_{Pb} = 0.91 \pm 0.01$

790 4. Other metals like Zn, Sn and Cd showed no clear correlation between seawater and calcite, which may
791 be linked to the mixture of metals leading to synergetic effects.

5. D_{TE} values of Ni, Zn, Cd, Hg and Pb decreased with increasing heavy metal concentration in the seawater, which may be evidence for an early protective mechanism, prior to damage, reduced growth or death of the organism.

The results of this study facilitate the determination of variations in the heavy metal concentration in seawater for elements showing a correlation between TE/Ca ratios in calcite and seawater (*A. aomoriensis* = Mn, Cu, Ag, Pb; *A. batava* = Mn, Ag, Hg, Pb; *E. excavatum* = Cr, Ni, Ag, Pb). Such estimates can be based on foraminiferal samples from the fossil sediment record and recent surface sediments. This facilitates monitoring of anthropogenic footprints on the environment today and in the past. Foraminifera offer the opportunity of long- and short-term monitoring of the heavy metal concentration because they are storing environmental signals over a period of time and not only at one point in time.

Appendix

Appendix A: Additional Tables

Table A1: TE/Ca$_{Seawater}$ values from single weeks during the culturing period of the metal system. Measurements were carried out with ICP-MS. These values are the basis for the calculations of the mean TE/Ca values in Table 3 and for figure B1.

Metal System	Phase	Day	Sampling date	Cr/Ca	Mn/Ca	Ni/Ca	Cu/Ca	Zn/Ca	Ag/Ca	Cd/Ca	Sn/Ca	Hg/Ca	Pb/Ca
				μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	nmol mol^{-1}	μmol mol^{-1}
FR0 W2	0	10	10.2.20	12.80	818.54	7.60	27.75	100.19	0.16	0.44	3.20		0.63
FR0 W3	0	17	19.2.20	3.16	858.94	7.23	3.74	107.69	0.05	0.43	2.94	5.28	0.43
FR1 W1	1	2	27.2.20	13.59	862.52	7.08	6.25	97.45	0.37	1.00	4.98	43.07	1.03
FR1 W2	1	9	5.3.20	5.86	796.65	6.69	2.23	93.09	0.04	1.06	3.87	19.13	0.69
FR1 W3	1	13	9.3.20	7.03	819.38	6.86	2.14	95.50	0.06	1.08	4.23	27.17	0.62
FR1 W4	1	20	16.3.20	7.75	844.23	7.94	2.77	95.75	0.11	1.19	4.11	60.20	0.68
FR2 W1	2	2	19.3.20	13.68	825.59	10.02	4.15	129.09	1.88	5.20	5.37	933.50	5.70
FR2 W2	2	8	26.3.20	16.49	820.63	9.75	2.78	134.85	0.41	4.96	5.46	494.26	3.07
FR2 W3	2	15	2.4.20	13.31	811.64	9.44	2.23	132.12	0.31	4.89	5.10	287.70	2.50
FR2 W4	2	19	6.4.20	15.47	789.96	9.77	2.23	135.50	0.33	4.75	5.19	210.66	2.20
FR3 W1	3	2	9.4.20	52.74	1558.73	74.72	15.89	772.38	31.53	87.65	18.31	6123.75	125.25
FR3 W2	3	7	14.4.20	39.90	1281.58	46.73	3.67	455.31	7.95	61.37	11.84		70.27
FR3 W3	3	16	23.4.20	26.97	1469.59	66.07	3.55	579.52	4.13	84.82	5.87	2858.26	53.51
FR3 W4	3	20	27.4.20	25.59	1397.18	65.00	3.01	550.78	4.31	84.23	5.02	1640.01	45.72

Table A2: Average concentration, RSD (1σ in %), literature values, accuracy in comparison to literature values and number of measurements of the reference materials SLRS-6, SLEW-3, in-house reference materials (South Atlantic surface water and South Atlantic Gyre water) and NASS-6 measured with ICP-MS. Average concentration, RSD and accuracy values displayed here are averaged from single measuring days. Cr values are analysed after dilution of the samples and all other elements were analyses after preconcentration with a SeaFAST system. NRCC – National Research Council Canada. *Values originated from 1:10 dilution of SLRS-6.

30

Reference Materials	Cr	Mn	Ni	Cu	Zn	Cd	Pb
SLRS-6	nmol kg^{-1}	nmol kg^{-1}	nmol kg^{-1}	nmol kg^{-1}	nmol kg^{-1}	nmol kg^{-1}	nmol kg^{-1}
Average conc.	4732	52956	9811	338014*	31391*	62	786
RSD%	3.5	3.9	6.0	1.7*	7.2*	12.8	0.8
Yeghicheyan et al., 2019	4509	38616	10496	376378*	26920*	56	820
Accuracy	0.96	0.74	1.08	1.11*	0.86*	0.90	1.04
Number	4	11	11	13*	13*	7	7
SLEW-3							
Average conc.		40007	17508	22907	4442	343	
RSD%		4.3	3.5	4.2	9.1	4.8	
Leonhard et al., 2002		29326	20958	24409	3074	427	
Accuracy		0.74	1.21	1.07	0.78	1.28	
Number		12	12	12	12	12	
South Atlantic Gyre water							
Average conc.		1615	2189	2649	5614		
RSD%		6.2	3.7	5.3	13.2		
Number		10	10	10	10		
South Atlantic surface water							
Average conc.		1959	2417	2646	39718		
RSD%		6.8	2.8	5.8	2.2		
Number		6	6	6	6		
NASS-6							
Average conc.	6747	11162	3557	5206	5158	169	
RSD%	15.9	5.2	3.2	3.0	25.3	7.0	
NRCC	2293	9654	5129	3528	3931	165	
Accuracy	0.34	0.87	0.76	0.35	0.81	0.98	
Number	9	11	11	11	11	2	

816

Table A3: Average concentration, RSD (1 σ in %), literature values, accuracy in comparison to literature values and number of measurements of the reference materials, NIST SRM 614, JCt-1, JCp-1, MACS-3 and ECRM752-1 measured with LA-ICP-MS. Please note that for the ECRM752-1 no reported values for the elements of interest are available, which is also the case for some elements in other reference materials. It is important to note that the Hg/Ca values in the NIST glasses are not reliable as Hg is volatile and most likely volatilized during the glass formation. Average concentration, RSD and accuracy values displayed here are averaged from single measuring days.

Reference materials	Cr/Ca	Mn/Ca	Ni/Ca	Cu/Ca	Zn/Ca	Ag/Ca	Cd/Ca	Sn/Ca	Hg/Ca	Pb/Ca
NIST SRM 614	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	μmol mol^{-1}	nmol mol^{-1}	μmol mol^{-1}
Mean value	19.28	10.31	8.43	15.86	67.58	2.13	15.53	5.97	20.93	5.23
RSD%	10.57	4.47	4.66	3.03	2.44	4.92	5.69	2.98	20.69	1.98
Jochum et al., 2011	10.78	12.18	8.83	10.16	20.11	1.83	2.35	6.67		5.28
Accuracy	0.57	1.19	1.06	0.64	0.30	0.86	0.23	1.12		1.01
Number of spots	35	38	37	39	38	38	38	39	19	39

MACS-3	mmol mol^{-1}	mmol mol^{-1}	mmol mol^{-1}	mmol mol^{-1}	mmol mol^{-1}	mmol mol^{-1}	mmol mol^{-1}	mmol mol^{-1}	µmol mol^{-1}	mmol mol^{-1}
Mean value	0.21	0.97	0.093	0.17	0.13	0.065	0.041	0.042	5.11	0.026
RSD%	1.60	1.36	1.90	1.92	2.19	6.37	2.83	2.68	9.23	2.18
Jochum et al., 2019	0.23	0.99	0.10	0.19	0.20	0.054	0.051	0.049	5.41	0.031
Accuracy	1.13	1.02	1.09	1.11	1.50	0.84	1.24	1.15	1.07	1.16
Number of spots	45	45	44	46	46	42	46	46	44	46
JCt-1NP	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	nmol mol^{-1}	µmol mol^{-1}
Mean value	6.16	0.91	0.37	1.14	1.46	0.01	1.60	2.30	8.93	0.063
RSD%	14.25	15.59	9.56	7.44	10.37	6.57	11.75	5.06	23.95	5.86
Jochum et al., 2019	0.93	1.01	1.03	1.48						0.064
Accuracy	0.15	1.19	2.71	1.31						1.04
Number of spots	44	38	45	47	45	11	46	13	26	48
JCp-1NP	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	nmol mol^{-1}	µmol mol^{-1}
Mean value	9.61	2.11	0.50	0.84	1.81	0.02	0.98	0.06	8.25	0.13
RSD%	7.91	4.62	6.89	6.36	6.53	11.34	11.08	10.68	20.96	6.15
Jochum et al., 2019	1.27	2.16	1.05	1.29	3.53					0.15
Accuracy	0.15	1.06	2.10	1.25	1.96					1.19
Number of spots	37	41	41	40	41	21	36	30	21	47
ECRM752-1	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	µmol mol^{-1}	nmol mol^{-1}	µmol mol^{-1}
Mean value	14.75	144.44	3.87	2.34	8.40	0.01	1.54	0.04	19.14	0.86
RSD%	7.78	2.54	4.97	6.21	2.37	87.11	7.76	9.22	18.03	3.82
Number of spots	27	31	26	28	27	15	29	24	19	31

824

Table A4: Comparison of the heavy metal concentrations in seawater of different regions of the world to values used for the culturing experiments in this study. It is indicated whether the values of this study are comparable to environmental values or if values from this study are higher or lower. EPA = Environmental Protection Agency, USA, FI = Field Injection, SF-ICP-MS = Sector Field Inductively Coupled Plasma Mass Spectrometry, GF = Graphite Atomic, (F)AAS = (Flame) Graphite Atomic Absorption Spectrometry, APDC-MIBK = Ammonium Pyrrolidine Dithiocarbamat-Methyl Isobutyle Ketone, ASV = Anodic Stripping Voltammetry, AES = Atomic Emission Spectrometry, CVAFS = Cold Vapor Atomic Fluorescence Spectrometry, FPD = Flame Photometric Detector.

Element	Study area	Concentration in µg l^{-1}	Comparable?	Reference	Pretreatment + measurement technique
		0.06-4.61		**This study**	Dilution + ICP-MS
	EPA Recommended Values (acute)	1.9	yes	Prothro, 1993	
Ag	Restronguet Creek, U.K. + Adriatic Sea	0.0025-0.03	yes	Barriada et al., 2007	FI preconc.+ SF-ICP-MS
	Ibaraki coast + Watarase river	0.014-0.03	yes	Shijo et al., 1989	Solvent extraction, Microscale backextraction + GFAAS
Cd		0.14-30.61		**This study**	SeaFAST preconc. + ICP-MS

	Location	Value	Below EPA?	Reference	Method
	EPA Recommended Values (chronic)	7.9	yes	Prothro, 1993	-
	Suva, Fiji	150-250	no, low	Arikibe and Prasad, 2020	FAAS
	Black Sea in Rize, Turkey	1-3	yes	Baltas et al., 2017	ICP-MS
	Gulf of Chabahar, Oman Sea	0.15-0.19	yes	Bazzi, 2014	APDC-MIBK procedure + FAAS
	Gulf of Kutch, Arabian Sea	200-1580	no, low	Chakraborty et al., 2014	AAS
	East London + Port Elizabeth harbours, U.K.	200-72600	no, low	Fatoki and Mathabatha, 2001	APDC-MIBK procedure + AAS
	Yalujiang Estuary, China	0.83-1.33	yes	Li et al., 2017	ICP-MS
	Gulf San Jorge, Argentina	0.01-0.09	yes	Muse et al., 1999	APDC-MIBK procedure + AAS
	Alang–Sosiya ship scrapping yard, Gulf of Cambay, India	34-560	yes	Reddy et al., 2005	APDC-MIBK procedure + FAAS
	Kamal estuary, Jakarta	0.01-0.02	no, high	Putri et al., 2012	AAS
	Jakarta Bay	0.04-0.104	yes	Williams et al., 2000	ASV
	Kepez harbor of Canakkale, Turkey	19-73800	yes	Yılmaz and Sadikoglu, 2011	Sample mineralization + ICP-AES
Cr		0.1-14.0		**This study**	Dilution + ICP-MS
	EPA Recommended Values (chronic)	50	no, low	Prothro, 1993	-
	Gulf of Chabahar, Oman Sea	20.16-21.46	yes	Bazzi, 2014	APDC-MIBK procedure + FAAS
	Gulf of Kutch, Arabian Sea	260-3010	no, low	Chakraborty et al., 2014	AAS
	Yalujiang Estuary, China	0.113-0.14	yes	Li et al., 2017	ICP-MS
	Gulf San Jorge, Argentina	0.04-0.5	yes	Muse et al., 1999	APDC-MIBK procedure + AAS
	Jakarta Bay	0.511-5.25	yes	Williams et al., 2000	ASV
	Alang–Sosiya ship scrapping yard, Gulf of Cambay, India	35-765	no, low	Reddy et al., 2005	APDC-MIBK procedure + FAAS
Cu		0.6-6.2		**This study**	SeaFAST preconc. + ICP-MS
	EPA Recommended Values (chronic)	3.1	yes	Prothro, 1993	-
	Suva, Fiji	880-10290	no, low	Arikibe and Prasad, 2020	FAAS
	Black Sea in Rize, Turkey	30-242	no, low	Baltas et al., 2017	ICP-MS
	Gulf of Chabahar, Oman Sea	3.37-5.74	yes	Bazzi, 2014	APDC-MIBK procedure + FAAS
	Gulf of Kutch, Arabian Sea	1350-1850	no, low	Chakraborty et al., 2014	AAS
	East London + Port Elizabeth harbours, U.K.	500-42600	no, low	Fatoki and Mathabatha, 2001	APDC-MIBK procedure + AAS
	Yalujiang Estuary, China	1.8-4.7	yes	Li et al., 2017	ICP-MS
	Gulf San Jorge, Argentina	0.02-0.65	yes	Muse et al., 1999	APDC-MIBK procedure + AAS
	Jakarta Bay	0.405-4.04	yes	Williams et al., 2000	ASV
	Alang–Sosiya ship scrapping yard, Gulf of Cambay, India	32-3939	yes	Reddy et al., 2005	APDC-MIBK procedure + FAAS
Hg		0.00035-0.273		**This study**	amalgamation + CVAFS
	EPA Recommended Values (chronic)	0.94	yes	Prothro, 1993	-
	South Florida Estuaries	0.0034-0.0074	yes	Kannan et al., 1998	amalgamation + CVAFS

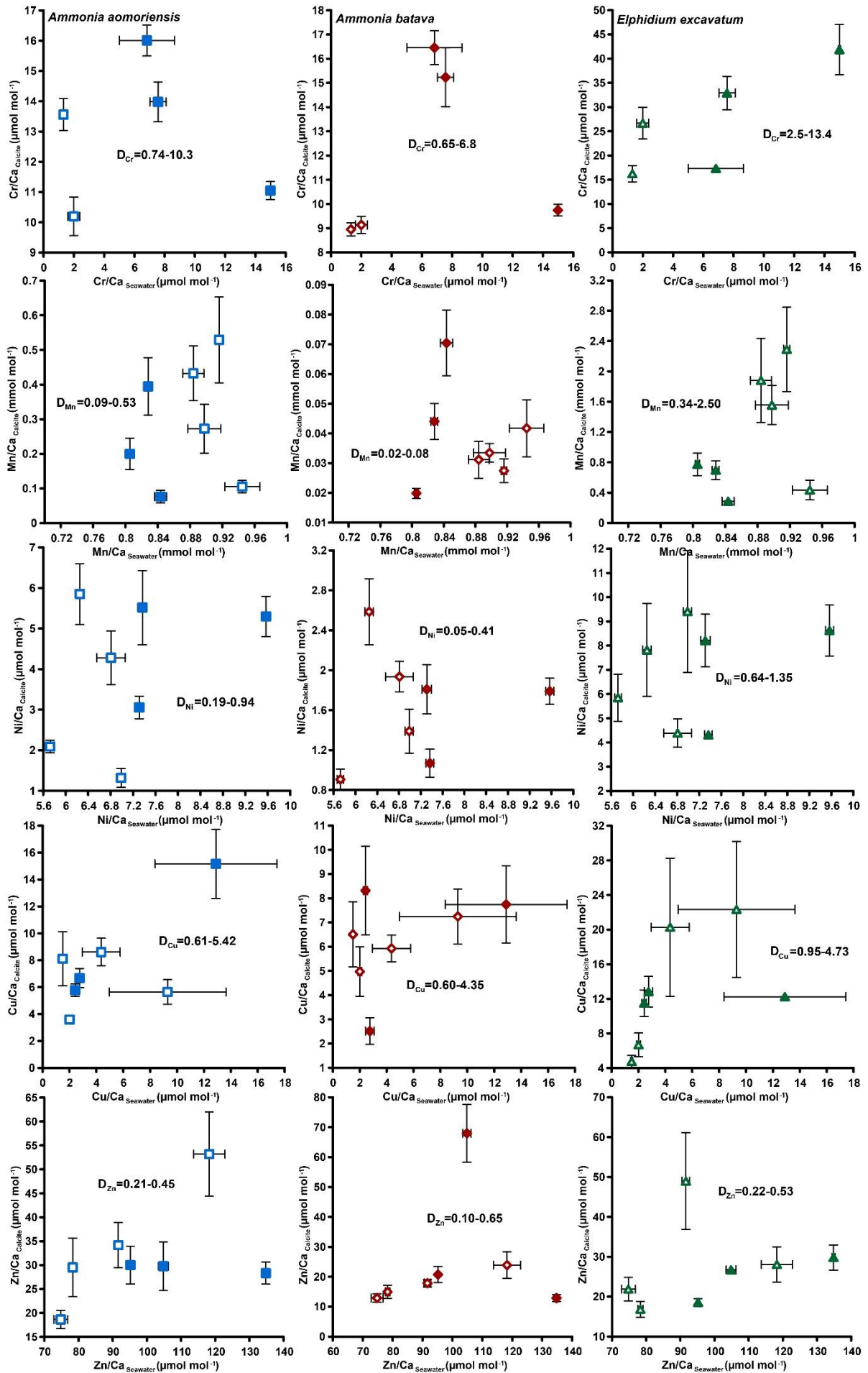|	Location	Value	Comparison	Reference	Method
	Guadalupe River and San Francisco Bay, California	0.0017-0.135	yes	Thomas et al., 2002	amalgamation + CVAFS
	Vembanad, India	0.0024-0.206	yes	Ramasamy et al., 2017	amalgamation + CVAFS
	Kamal estuary, Jakarta	0.1-0.2	yes	Putri et al., 2011	GFAAS
	Yalujiang Estuary, China	0.006-0.049	yes	Li et al., 2017	AFS
Mn		320-549		**This study**	SeaFAST preconc. + ICP-MS
	Black Sea in Rize, Turkey	3-14	yes	Baltas et al., 2017	ICP-MS
	Gulf of Chabahar, Oman Sea	15.43-24.76	no, high	Bazzi, 2014	APDC-MIBK procedure + FAAS
	Gulf of Kutch, Arabian Sea	13000-18000	no, low	Chakraborty et al., 2014	AAS
	East London + Port Elizabeth harbours, U.K.	300-23900	yes	Fatoki and Mathabatha, 2001	APDC-MIBK procedure + AAS
	Alang–Sosiya ship scrapping yard, Gulf of Cambay, India	31-4920	yes	Reddy et al., 2005	APDC-MIBK procedure + FAAS
Ni		2.3-24.3		**This study**	SeaFAST preconc. + ICP-MS
	EPA Recommended Values (chronic)	8.2	yes	Prothro, 1993	-
	Suva, Fiji	230-800	no, low	Arikibe and Prasad, 2020	FAAS
	Black Sea in Rize, Turkey	0.006-0.036	yes	Baltas et al., 2017	ICP-MS
	Gulf of Chabahar, Oman Sea	16.42-17.14	yes	Bazzi, 2014	APDC-MIBK procedure + FAAS
	Gulf of Kutch, Arabian Sea	190-330	no, low	Chakraborty et al., 2014	AAS
	Jakarta Bay	0.058-5.25	yes	Williams et al., 2000	ASV
	Alang–Sosiya ship scrapping yard, Gulf of Cambay, India	32-944	yes	Reddy et al., 2005	APDC-MIBK procedure + FAAS
Pb		0.11-28.35		**This study**	SeaFAST preconc. + ICP-MS
	EPA Recommended Values (chronic)	5.6	yes	Prothro, 1993	-
	Suva, Fiji	880-1770	no, low	Arikibe and Prasad, 2020	FAAS
	Black Sea in Rize, Turkey	6-130	yes	Baltas et al., 2017	ICP-MS
	Gulf of Chabahar, Oman Sea	4.24-4.25	yes	Bazzi, 2014	APDC-MIBK procedure + FAAS
	Gulf of Kutch, Arabian Sea	20-120	yes	Chakraborty et al., 2014	AAS
	East London + Port Elizabeth harbours, U.K.	600-16300	no, low	Fatoki and Mathabatha, 2001	APDC-MIBK procedure + AAS
	Yalujiang Estuary, China	0.4-1.8	yes	Li et al., 2017	ICP-MS
	Gulf San Jorge, Argentina	0.1-0.5	yes	Muse et al., 1999	APDC-MIBK procedure + AAS
	Alang–Sosiya ship scrapping yard, Gulf of Cambay, India	30-2036	yes	Reddy et al., 2005	APDC-MIBK procedure + FAAS
	Kamal estuary, Jakarta	1.3-4	yes	Putri et al., 2011	AAS
	Jakarta Bay	0.485-3.62	yes	Williams et al., 2000	ASV
	Kepez harbor of Canakkale, Turkey	49-9390	yes	Yılmaz and Sadikoglu, 2011	sample mineralization + ICP-AES
Sn		0.86-3.95		**This study**	Dilution + ICP-MS
	estuarine seawater, Galicia Coast, Spain	0.53-1.23	yes	Bermejo-Barrera et al., 1999	hydride generation + AAS
	U.S. and European rivers	0.0001-0.1	yes	Byrd and Andreae, 1982	hybride generation + FPD

		30.0-226.9		**This study**	SeaFAST preconc. + ICP-MS
Zn	EPA Recommended Values (chronic)	81	yes	Prothro, 1993	-
	Suva, Fiji	80-1450	yes	Arikibe and Prasad, 2020	FAAS
	Black Sea in Rize, Turkey	38-178	yes	Baltas et al., 2017	ICP-MS
	Gulf of Chabahar, Oman Sea	18.01-22.62	yes	Bazzi, 2014	APDC-MIBK procedure + FAAS
	Gulf of Kutch, Arabian Sea	11000-31000	no, low	Chakraborty et al., 2014	AAS
	East London + Port Elizabeth harbours, U.K.	500-27600	yes	Fatoki and Mathabatha, 2001	APDC-MIBK procedure + AAS
	Yalujiang Estuary, China	9.2-19.6	yes	Li et al., 2017	ICP-MS
	Gulf San Jorge, Argentina	0.01-0.55	no, high	Muse et al., 1999	APDC-MIBK procedure + AAS
	Jakarta Bay	2-30.1	yes	Williams et al., 2000	ASV
	Alang–Sosiya ship scrapping yard, Gulf of Cambay, India	33-5832	yes	Reddy et al., 2005	APDC-MIBK procedure + FAAS

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835 **Appendix B: Additional Figures**

836
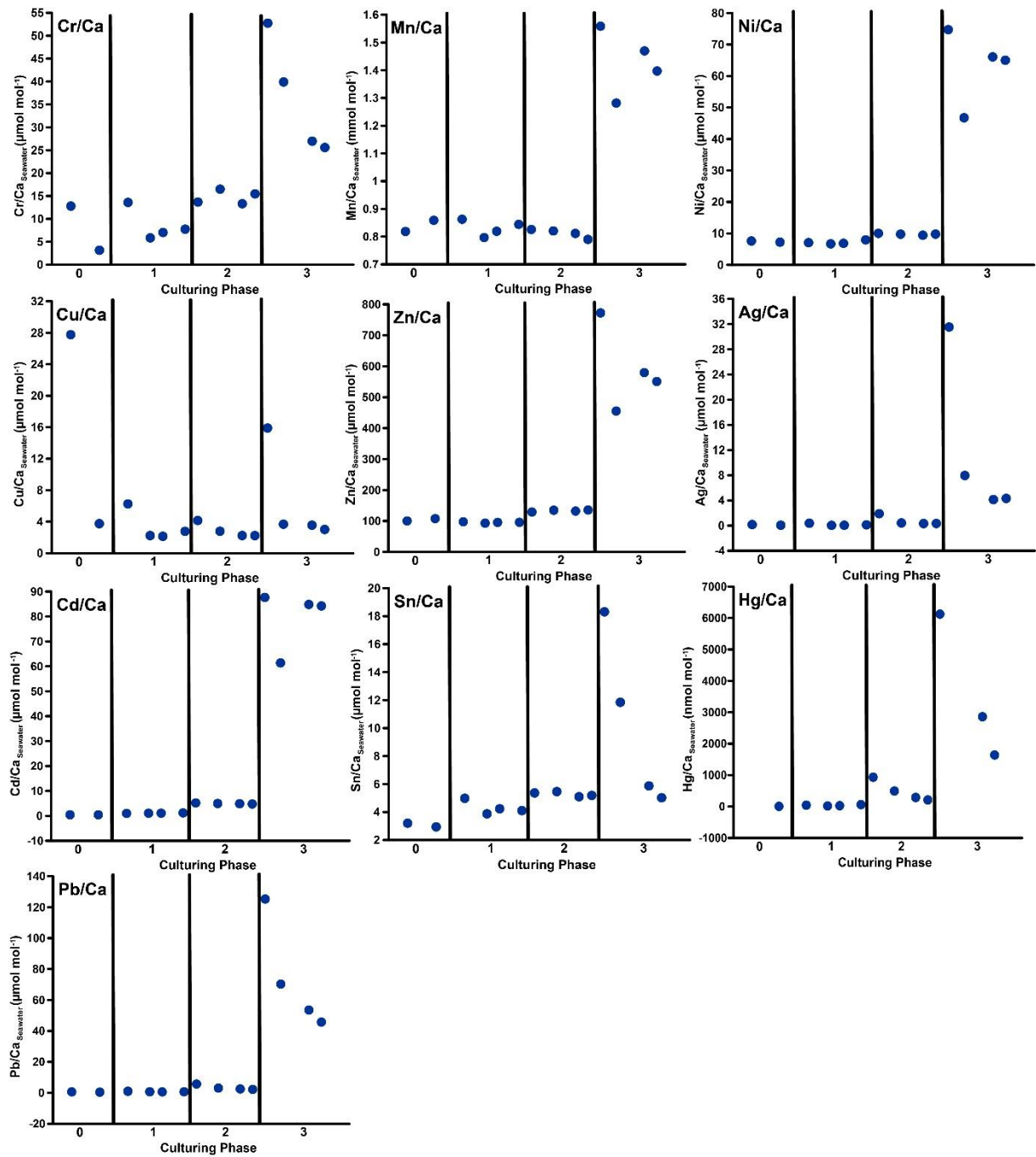
837

838 **Figure B1**: TE/Ca values in the culturing medium of the metal system in µmol mol^{-1} or nmol mol^{-1} divided by

839 individual culturing phases. In this system, phase 0 is the control phase without any extra added metals and for

840 phase 1 to 3, the heavy metal concentration in the culturing medium was elevated. The data the figure is based on

841 can be found in Table A1.

843

☐	Mean TE/Ca Control System - *A. aomoriensis*
■	Mean TE/Ca Metal System - *A. aomoriensis*
◇	Mean TE/Ca Control System - *A. batava*
◆	Mean TE/Ca Metal System - *A. batava*
△	Mean TE/Ca Control System - *E. excavatum*
▲	Mean TE/Ca Metal System - *E. excavatum*
──	Regression line
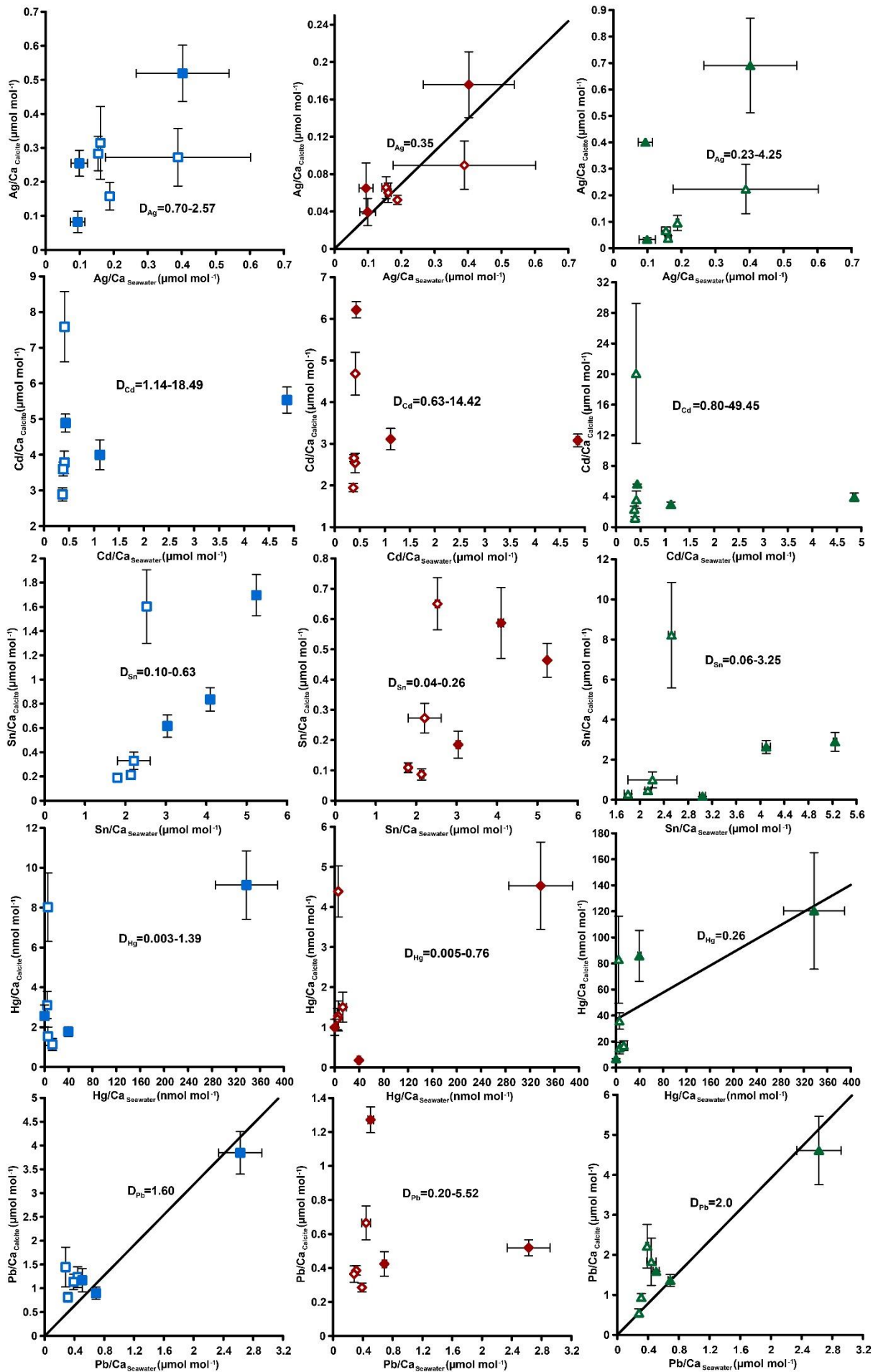
Figure B2: Mean TE/Ca values in the foraminiferal calcite versus the mean TE/Ca values in the corresponding culturing medium without phase 3. Each data point represents the mean value of all laser ablation ICP – MS measurements on single foraminiferal chambers built up during the individual culturing phase plotted against the mean metal concentrations in the seawater averaged over the culturing phase (Table 3). Error bars symbolize the standard error of the mean. The linear regression line is based on the calculations excluding phase 3 and is only displayed when elements showed a significant correlation between seawater and calcite. D_{TE}'s of *E. excavatum* where considered without values for Phase 0 as only data from one newly formed chamber are available. All values can be found in Table 4.

Supplements

Table S1–S3: TE/Ca$_{Calcite}$ values from *Ammonia aomoriensis* (Table S1)*, Ammonia batava* (Table S2) and *Elphidium excavatum* (Table S3). Values represent single laser ablation spots on foraminiferal chambers that were formed during the individual culturing period in the control and the metal system. Only values above the detection limits of the individual element are presented. Furthermore, outliers are also excluded. These values are the basis for the calculation of the mean TE/Ca values in Table 4 and Fig. 4. The sample ID indicates the species (AA = *A. aomoriensis*, AB = *A. batava*, E = *E. excavatum*), the culturing phase, the system (R = metal system, L = control system), the individual and the chamber that was ablated, starting from the innermost chamber going to the youngest one.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Author contribution

This study was initiated by JS and EH. SS collected the samples, cultured the foraminifera, processed the samples in the laboratory and acquired, analysed and interpreted the water and foraminiferal data. JS helped with the sampling logistics, design and implementation of the culturing experiments. EH advised and helped with the processing and analysis of the water samples and EH and DGS advised and helped with the measurements of the foraminiferal samples. SS wrote the manuscript with all the authors contributing to the discussion and data interpretation, and editing of the work.

Competing interests

The authors declare that they have no conflict of interest.

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