



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

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8 **Abstract.** Heavy metal pollution originating from anthropogenic sources, e.g., mining, industry and extensive land
9 use, is increasing in many parts of the world and influences coastal marine environments for a long time. The
10 elevated input of heavy metals into the marine system potentially affects the biota because of their toxicity,
11 persistence and bioaccumulation. An emerging tool for environmental applications is the heavy metal
12 incorporation into foraminiferal tests calcite, which facilitates monitoring of anthropogenic footprints on recent
13 and past environmental systems. The aim of this study is to investigate whether the incorporation of heavy metals
14 in foraminifera is a direct function of their concentration in seawater. Culturing experiments with a mixture of
15 dissolved chromium (Cr), manganese (Mn), nickel (Ni), copper (Cu), zinc (Zn), silver (Ag), cadmium (Cd), tin
16 (Sn), mercury (Hg) and lead (Pb) in artificial seawater were carried out over a wide concentration range to assess
17 the uptake of heavy metals by the near-shore foraminiferal species *Ammonia aomoriensis*, *Ammonia batava* and
18 *Elphidium excavatum*. Seawater analysis exhibited the increasing metal concentrations between culturing phases
19 and revealed high metal concentrations in the beginning of the culturing phases due to the punctual metal addition.
20 Furthermore, a loss of metals during the culturing process was discovered, which lead to a deviation between the
21 expected and the actual concentrations of the metals in seawater. Laser ablation ICP-MS analysis of the newly
22 formed calcite revealed species-specific differences in the incorporation of heavy metals. The foraminiferal calcite
23 of all three species reveals a strong positive correlation with Pb and Ag concentrations in the culturing medium.
24 *Ammonia aomoriensis* further showed a correlation with Mn and Cu, *A. batava* with Mn and Hg and *E. excavatum*
25 with Cr and Ni, and partially also with Hg. Zn, Sn and Cd showed no clear trend for the species studied, which
26 may be caused by the little variation of these metals in seawater. Our calibrations and the calculated partition
27 coefficients render *A. aomoriensis*, *A. batava* and *E. excavatum* as natural archives that enable the direct
28 quantification of metals in polluted and pristine environments. This in turn allows monitoring of the ecosystem
29 status of areas that are potentially under the threat of anthropogenic pollution in order to evaluate contemporary
30 emission reduction measures.

31

32 **1 Introduction**

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



39 concentrations, most heavy metals become toxic and have hazardous effects on marine biota (Stankovic et al.,
40 2014). Furthermore, they are highly persistent in the marine environment and can be hardly degraded by organisms
41 after the uptake of these metals into their system and cells (Flora et al., 2012; Kennish, 2019). Coastal environments
42 act as natural catchments for anthropogenic pollutants because these areas are directly affected by industry,
43 agriculture and urban runoff (e.g., Alloway, 2013; Julian, 2015; Tansel and Rafiuddin, 2016).

44 In marginal seas and coastal areas, benthic foraminifera are common, and they can be used as proxies for changing
45 environmental parameters like water temperature (Mg/Ca; e.g., Nürnberg et al., 1995; 1996), salinity (Na/Ca; e.g.,
46 Wit et al., 2013, Bertlich et al., 2018), oxygen content or redox conditions (Mn/Ca; Groeneveld and Filipsson,
47 2013b; Koho et al., 2015; 2017; Kotthoff et al., 2017; Petersen et al., 2018). Foraminifera take up heavy metals
48 and incorporate them into their calcium carbonate shells during calcification (e.g., Boyle, 1981; Rosenthal et al.,
49 1997; Dissard et al., 2010a; 2009; 2010b; Munsel et al., 2010; Nardelli et al., 2016; Frontalini et al., 2018a; 2018b;
50 Titelboim et al., 2018; Smith et al., 2020). Moreover, foraminifera have a short life cycle (< 1 year; e.g., Haake,
51 1967; Boltovskoy and Lena, 1969; Wefer, 1976; Murray, 1992) and thus, react immediately to changing
52 environmental conditions and contamination levels of the surrounding environment.

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

62 The majority of culturing studies on heavy metal incorporation into benthic foraminifera were designed to assess
63 the influence and uptake of one particular metal, e.g., copper (Cu) (De Nooijer et al., 2007), chromium (Cr)
64 (Rommelzwaal et al., 2019), lead (Pb) (Frontalini et al., 2015), zinc (Zn) (e.g., Smith et al., 2020), mercury (Hg)
65 (Frontalini et al., 2018a) or cadmium (Cd) (Linshy et al., 2013). This approach is adequate to detail the effects on
66 shell chemistry, growth or physiology. Only one study reported a culturing experiment with elevated levels of Cu,
67 Mn and Ni in the same culturing medium (Munsel et al., 2010). However, there is rarely only one but mostly a
68 combination of several pollutants that occur in nearly all environments affected by heavy metal pollution (e.g.,
69 Mutwakil et al., 1997; Cang et al., 2004; Vlahogianni et al., 2007; Huang et al., 2011; Wokhe, 2015; Saha et al.,
70 2017). How foraminifera incorporate and react to heavy metals when they are co-exposed to more than one metal
71 at a time is less constrained to date. A mixture of different metals will lead to interactions, which may result in a
72 more severe damage of tissue than exposure to each of them individually (Tchounwou et al., 2012). For example,
73 a co-exposure to arsenic and cadmium causes a more distinct damage of human kidneys than only one of these
74 elements (Nordberg et al., 2005). Furthermore, a chronic low-dose exposure to multiple elements can cause similar
75 synergistic effects (e.g., Wang et al., 2008). It is therefore reasonable to assume that other organisms are likewise
76 threatened more harmfully when exposed to several potentially toxic elements simultaneously.

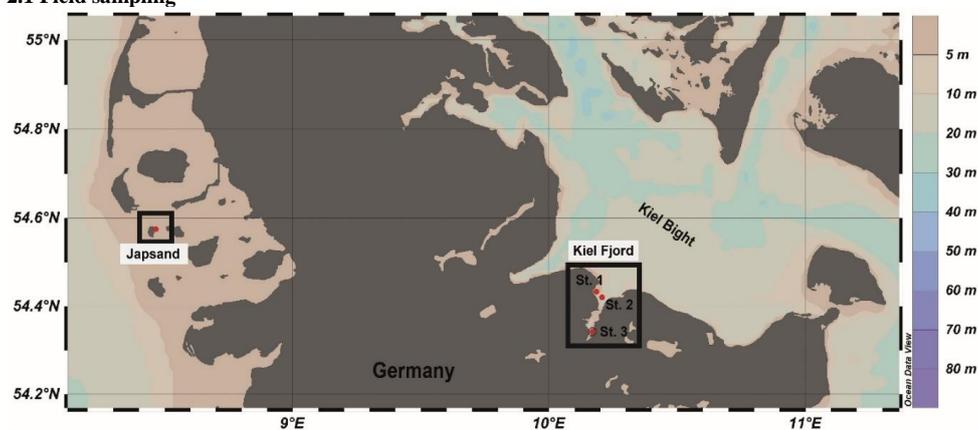


77 Here we present results from culturing studies with *Ammonia aomoriensis*, *Elphidium exvacatum* and *Ammonia*
78 *batava* addressing the relationship of heavy metal concentration in seawater and foraminiferal tests. The
79 partitioning factor between concentration of an element in the ambient seawater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

89

90 2 Material and Methods

91 2.1 Field sampling



92

93 **Figure 1:** Location of the sampling stations in the North Sea (Japsand area) and in the Baltic Sea (Kiel Fjord, St.1
94 Strander Bucht, St. 2 Laboe, St. 3 Mönkeberg). The map was drawn with Ocean Data View (Schlitzer, 2016) on
95 the basis of bathymetric data. Depth in m are indicated by the color code.

96

97 2.1.1 North Sea, Japsand

98 Living specimens of *A. batava* were collected at the barrier sand Japsand near Hallig Hooge in the German Wadden
99 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
100 sediment was a glacial till or Eemian clay at Station 1 and fine to medium sand at Station 2. The samples were
101 recovered during low tide by scrapping off the uppermost centimetre of the surface sediment with a spoon made
102 out of stainless steel. Natural seawater (NSW) with a salinity of 30.3 PSU was collected near the sites for further
103 processing of the samples. Once back on Hallig Hooge, the sediment was washed with NSW through stacked
104 sieves with a mesh size of 2000 and 63 μm . The 2000 μm sieve was used to remove larger organisms and excess
105 organic material (macroalgae, gastropods, lugworms etc.) that could induce anoxic conditions in the sediment



106 during transport and storage. The residue was stored in Emsa CLIP and CLOSE® boxes, sparged with air and
107 some algae food was provided. Back in the laboratory at GEOMAR, the residue was stored at 8 °C in a fridge until
108 culturing. These stock cultures were fed twice a week with *Nannochloropsis* concentrate (BlueBioTech) and water
109 was partly exchanged with NSW from the sampling site once a week.

110

111 **2.1.2 Baltic Sea, Kiel Bight**

112 Living specimens of *A. aomoriensis* and *E. excavatum* were collected from different stations in Kiel Fjord, western
113 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,
114 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
115 October 2019 with F.B. Polarfuchs and F.S. Alkor (Fig. 1). A Rumohr corer (inner diameter 55 mm) was used on
116 F.B. Polarfuchs and 9 cores were taken (2 at St. 1 and 7 at St. 3). One core at both stations was used for
117 foraminiferal assemblage analysis and the first 2 cm of the sediment from all other cores was collected in plastic
118 containers with NSW from the site.

119 The sediment surface was nearly horizontal and comprised a ~ 5 mm thick fluffy layer consisting of organic
120 detritus of a dark brownish color. Mussels, worm burrows and plant debris was found. The sediment underneath
121 the surface layer was a very fine mud. The redox boundary was shallower than 0.5 to 1 cm as indicated by the
122 color turning black underneath this depth, and the sediment smelling of H₂S.

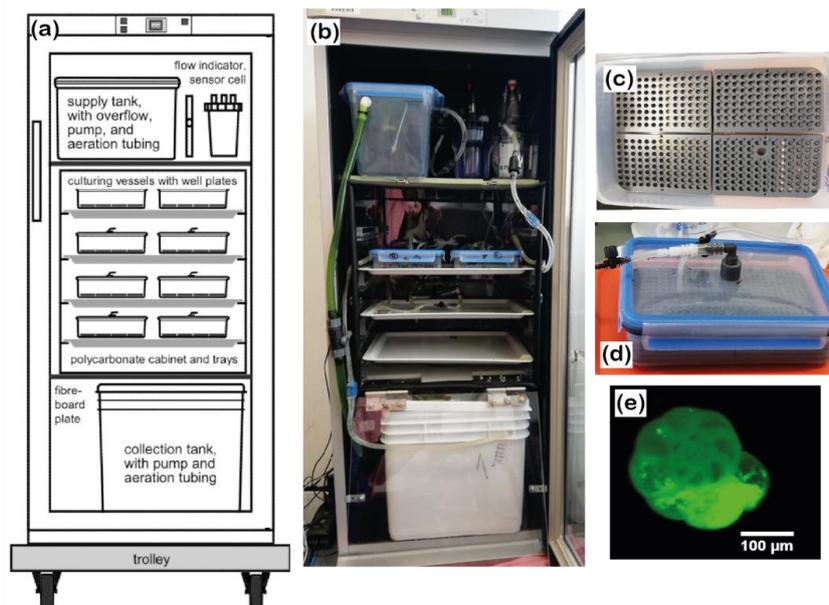
123 On F.S. Alkor, a Reineck box corer was used (200 x 250 cm) and 3 replicates at each station were taken (St. 1 –
124 3). The first 1 to 2 cm of the sediment surface of the box core were scrapped off with a spoon made out of stainless
125 steel and the material was stored in a plastic box with NSW from the location. Additional samples for foraminiferal
126 assemblage analysis were taken at each station.

127 Back in the laboratory at GEOMAR, the samples were treated the same as Japsand samples from the North Sea.
128 Artificial seawater (ASW, Tropic Marin) with a salinity of 30 PSU was used for washing and storage of the surface
129 samples from Kiel Fjord.

130



131 **2.2 Culturing setup**



132

133 **Figure 2:** Culturing setup. a: conceptual draft (left, modified after Dagan et al., 2016) and b: assembly of the
134 system (right). Tubing and hoses were omitted from the draft for clarity. c: a well plate with mounted specimens
135 and sand, d: closed culturing vessel with well plates and conduits. e: with calcein stained foraminifer under a
136 fluorescence microscope. The chambers formed in calcein are fluorescing brightly.

137

138 **2.2.1 Picking of the samples**

139 For extracting the foraminiferal specimens from the sediment, about 1 cm³ of the 63 to 2000 µm size fraction was
140 transferred to a petri dish. For maintaining optimal conditions for the foraminifera, the petri dish was filled with
141 artificial seawater (ASW) with a salinity of 30 PSU. All living specimens were picked with a paint brush from this
142 subsample and collected in a small petri dish of 55 mm diameter with ASW. The procedure was repeated until the
143 whole sample residue was screened. Only specimens with a glossy, transparent and undamaged test were chosen.
144 Furthermore, only individuals with the cytoplasm present in more than just a couple of chambers that were
145 connected and included the innermost chambers were chosen. After picking, a drop of concentrated food (pure
146 culture of *Nannochloropsis*, green colored algae) was added and the foraminifera were left untouched for a night.
147 Specimens that met one or more of the following criteria were considered as living and used for further procedures:

- 148 • Specimens showed a structural infill of cytoplasm with a bright green color, indicating they took up the
149 food over night,
- 150 • they developed a film or strings of pseudopodia firmly sticking to sediment particles or food,
- 151 • they had covered themselves or gathered a cyst of sediment or food particles.



152 Specimens were identified and sorted by species, rinsed, sediment particles were removed, and the color of the
153 cytoplasm was checked before inserting them into a petri dish with calcein (16 mg l^{-1}) (bis[N,N-
154 bis(carboxymethyl)aminomethyl]-fluorescein) (Sigma-Aldrich) for staining (Fig. 2e). Staining took place
155 immediately before the individual culturing phase 0 to 3 and lasted for 14 days. Petri dishes were stored at $8 \text{ }^{\circ}\text{C}$ in
156 a fridge, partial water exchanges and feeding of the foraminifera was performed twice a week. After the staining,
157 the foraminifera were transferred to a petri dish with ASW and left for 1 to 2 days to remove excess calcein from
158 seawater vacuoles in their cytoplasm prior to the introduction into the culturing system.

159

160 2.2.2 Culturing system

161 We used two closed-circulation incubation systems for foraminifera (Fig. 2a, b) provided by the Institute of
162 Microbiology, Kiel University (Dagan et al., 2016, their Fig. 5; Woehle et al., 2018, their Fig. S4). The systems
163 were a further development based on earlier closed-circulation systems for foraminiferal culturing (Hintz et al.
164 2004; Haynert et al., 2011). They were slightly modified for the requirements of this study, but the basic
165 operational principle is described by Dagan et al., 2016. In detail, the systems consisted of three levels with
166 different functions. They are built into Bauknecht WLE 885 fridges for temperature control. Each incubation
167 system accommodated two culturing vessels, which were arranged pairwise on a tray in a polycarbonate cabinet
168 (Fig. 2a, b). The water was pumped from the collection tank at the lowest level of the fridge to the top level into
169 the supply tank. From the supply tank, the water was directed to the culturing vessels and the flow was regulated
170 by 3-way wheels, which ensured that the same amount of water was provided to every culturing vessel. After
171 passing the culturing vessels, the water was redirected to the collection tank. The systems were filled with 15 L of
172 ASW with a salinity of 30.5 PSU. The water was aerated in the supply and the collection tank with air from outside
173 the system, which passed through a $0.2 \text{ }\mu\text{m}$ filter. Monitoring of temperature and salinity were performed with a
174 WTW 3210 conductivity meter. Uncertainty of the conductivity measurements was $\pm 0.5\%$ and $\pm 0.1 \text{ }^{\circ}\text{C}$ for
175 temperature according to the manufacturer's test certificate. All parts that were introduced into the system were
176 sterilized before use either by autoclaving, an UV-lamp exposure, or by applying DanKlorix®.

177

178 2.2.3 Preparation for incubation

179 For the incubation of the foraminifera, well plates made from PVC were used (Fig. 2c). Each well plate measured
180 $117 \times 82 \text{ mm}$ with 104 cavities of which each one had a diameter of 6 mm and a depth of 5 mm. All well plates
181 had been used in previous experiments for culturing foraminifera in seawater, which ensured that potentially toxic
182 substances or additives were already released from the PVC material (Dagan et al., 2016; Woehle et al., 2018) and
183 should therefore not harm the foraminifera. Before the foraminifera were placed in the cavities, each cavity was
184 filled with sterile quartz sand up to 1.5 mm. The cavities were subsequently filled with artificial seawater and the
185 specimens were inserted randomly. Prepared well plates were left untouched for one night, to make sure that the
186 foraminifera are able to spread their pseudopodial network before incubation. This ensures that they are stable
187 anchored in the cavities and do not float when the culturing vessels are filled and mounted (Haynert et al., 2011).
188 Four well plates respectively were assembled in an airtight Emsa CLIP and CLOSE® box of $226 \times 167 \times 59 \text{ mm}$
189 (Fig. 2d). Of these four well plates, only three were engrafted with living foraminifera, one species per plate. The
190 fourth plates were left barren and were used to stabilize the arrangement in the culturing vessels. Each culturing



191 vessel had a lid with an inflow and an outflow conduit, for which Tygon® tubing were used. The tubing had an
192 outside diameter of 6.3 mm, and the PVC material was considered as nontoxic because no phthalate-based
193 emollients were used. To guarantee that the foraminiferal specimens are not flushed away by the incoming water,
194 the inflow conduit reached almost the bottom of the culturing vessel and was placed between two well plates. The
195 outflow conduit located at the lid of the vessel was attached to a custom-made PVC fitting (Fig. 2d). Once all well
196 plates were arranged in the culturing vessel, the lid was equipped with an additional, elastic sealing and closed.
197 Before the culturing vessels were placed in the incubators, each chamber was slowly filled with ASW. Thereafter,
198 the culturing chambers were placed on the shelves in the incubator, and were connected to the supply hoses. The
199 water supply was turned on once the system was completely filled.

200

201 2.2.4 Culturing experiment

202 The culturing experiment had four different phases. The first, phase 0 was dedicated as control phase. No heavy
203 metals were added. This phase allowed both systems to equilibrate in terms of physicochemical and biological
204 processes and made it possible to determine the background values in terms of seawater constituents. This phase
205 lasted 21 days. Afterwards, one system was used as the control system, where no heavy metals were added. In the
206 other system, three phases with elevated heavy metal concentrations were performed. The phases lasted 21 days
207 each. Tropic Marin Pro-Reef salt was mixed with deionized water for adjusting the salinity. A stock solution
208 containing all metals of interest was mixed and before each phase, the heavy metal concentration was elevated by
209 1 to 1.5 order of magnitude by adding an appropriate amount of the stock solution into the supply tank of the
210 system (see Fig. 2a) (phase 1 = 1 ml, phase 2 = 10 ml, phase 3 = 150 ml). The elevation was performed all at once
211 and for keeping the metal concentration at the same level over the culturing phase, a smaller amount of the stock
212 solution was fed into the system bi-weekly. Target concentrations are given in Table 1. The target concentration
213 of the elements at each phase were chosen after earlier culturing experiments with foraminifers (Mn, Cu, Ni:
214 Munsel et al., 2010; Pb: Frontallini et al., 2015 & 2018a; Zn: Nardelli et al., 2016; Cd: Linshy et al., 2013; Cu: De
215 Nooijer et al., 2007; Le Cadre and Debenary et al., 2006; Cr: Remmelzwaal et al., 2019) to resemble conditions
216 observed in polluted areas like Jakarta Bay (e.g., Williams, 2000) or at European ports (e.g., Fatoki and
217 Mathabatha, 2001). Furthermore, recommended threshold values provided from the EPA (Environmental
218 Protection Agency, USA) were taken into account to ensure that the foraminifera are not limited in their growth
219 and are able to maintain normal physiological functions. However, the heavy metal concentrations in the culturing
220 media obtained during each phase were monitored by frequent water sampling.

221 **Table 1:** Heavy metal concentration in the stock solution, target concentration of these metals in each phase and
222 used salt compounds. All salts used were provided in p.a. quality.

	Salt compound	Conc. in mg l ⁻¹ Stock solution	Target conc. in µg l ⁻¹		
			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

223

224 Over the entire culturing period, both systems were exposed to a natural day and night cycle and the flow rate was
225 adjusted to 0.017 ml s⁻¹ (one drop per second) within the culturing vessels. The foraminifera were fed with
226 *Nannochloropsis* concentrate twice a week (~ 2000 µg). After 21 days (meaning after each culturing phase) one
227 culturing vessel per system was exchanged. Vessels and specimens were left in the culturing system for the
228 complete culturing phase (21 days) and no exchange took place during a culturing phase. One culturing vessel
229 containing all three species was left in the system from the beginning until the end of the experiment (from phase
230 0 to phase 3) for 84 days. Data of these specimens are not available due to time constraints caused by the outbreak
231 of the COVID-19 pandemic.

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

237

238 2.3 Water samples

239 2.3.1 Collection of water samples

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

250

251 2.3.2 Preparation of water samples before analysis

252 For Mn, Zn, Ni, Pb, Cu, and Cd concentration analyses, the water samples were pre-concentrated offline by using
253 a SeaFAST system (ESI, USA). Twelve ml of each sample were used to fill a 10 mL sample loop and concentrated
254 by a factor of 25 into 1.5M HNO₃. All samples were spiked with indium as an internal standard for monitoring
255 and correcting for instrumental drift. Both MilliQ water and bottle blanks of acidified MilliQ water (pH ~ 2) stored
256 in the same bottles until the samples were passed through the pre-concentration system. Additionally, procedural
257 blanks which were filtered as the samples were also pre-concentrated and measured. A variety of international



258 (Open Ocean Seawater NASS-6, River Water SLRS-6, Estuarine Seawater SLEW-3, all distributed by NRC-
259 CNRC Canada) and in-house (South Atlantic surface water, South Atlantic Gyre water) reference materials were
260 pre-concentrated like the samples. All samples were subsequently analysed by ICP-MS (inductively coupled
261 plasma mass spectrometry).

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

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

271 Prior to the measurements of Hg concentrations, all samples were treated with BrCl solution at least 24 hours
272 before the analysis to guarantee the oxidation and release of mercury species that are possibly present in a different
273 oxidation states or phases. The BrCl was removed again from the sample by adding hydroxylamine hydrochloride
274 at least one hour prior to analysis before the Hg was reduced to the volatile Hg⁰ species with acidic SnCl₂ (20 %
275 w v⁻¹) during the measuring process. All preparations of the water samples took place in a Clean Lab within a
276 trace metal clean atmosphere and all vials were acid cleaned prior to use. Mercury concentrations were determined
277 using a Total Mercury Manual System (Brooks Rand Model III). The reduced volatile Hg⁰ is nitrogen-purged onto
278 a gold-coated trap and released from again by heating before it is measured via cold vapour atomic fluorescence
279 (CVAFS) under a continuous argon carrier stream. Quality control of the Hg measurements was carried out by
280 measuring mixed standards, made from single element solutions and confirmed with replicate measurements
281 throughout each analysis. The measurement uncertainty was smaller than 4.5 % RSD for all analyses.

282 The calcium concentration of culture seawater was analysed using a VARIAN 720-ES ICP-OES (inductively
283 coupled plasma optical emission spectrometer). Yttrium was added as an internal spike and samples were diluted
284 1/10. IAPSO seawater standard (ORIL) was measured after every 15 samples for further quality control which
285 revealed a measurement uncertainty < 0.35 (RSD %) for the elements analysed (mean Ca concentration IAPSO
286 this study = 419.6 ± 0.15 mg l⁻¹; reference Ca concentration IAPSO Batch 161 = 423 mg l⁻¹).

287

288 **2.4 Foraminiferal samples**

289 After every culturing phase, the culturing vessels were taken out of the system and foraminiferal specimens where
290 collected from their cavities within one day. The individuals were cleaned with tap water and ethanol before they
291 were mounted in cell slides to mechanically remove salt scale and organic coatings with a paintbrush.

292 In order to check, whether the foraminifera had grown during the experiment, the total number of chambers was
293 counted before and after the experiment for every specimen (Table 2). As the foraminifera were stained with
294 calcein before the experiment, it was possible to cross-check the growth with a fluorescent microscope (Zeiss Axio



295 Imager 2) if new chambers without color were added, hence whether the particular specimen had grown or not
296 (Fig. 2e). Only individuals clearly showing new chambers were analysed by Laser ablation ICP – MS.

297 Prior to the laser ablation analyses, the foraminifera were transferred into individual acid-leached, 500 µl micro-
298 centrifuge tubes and thoroughly cleaned, applying a procedure adapted from Martin and Lea (2002). The
299 specimens were rinsed three times with MilliQ water and introduced into the ultrasonic bath for a few seconds at
300 the lowest power setting after each rinse. Afterwards, clay and adhering particles were removed by rinsing the
301 sample with Ethanol twice, which was followed by three MilliQ rinses again with minimal ultrasonic treatment.
302 Oxidative cleaning was applied using 250 µl of a 0.1M NaOH and 0.3 % H₂O₂ mixture added to each sample and
303 the vials were kept for 20 min in a 90 °C water bath. Afterwards, the samples were rinsed with MilliQ three times
304 to remove the remaining chemicals. The reductive step of the cleaning procedure by Martin and Lea (2002) was
305 not applied. This step is necessary to remove metal oxides, which of course could also influence the trace metal
306 concentration within the foraminiferal shell carbonate but these are usually considered to be added during early
307 deposition (e.g., Boyle, 1983) and therefore unlikely to occur during culture experiments. For Laser Ablation
308 Inductively Coupled Plasma Mass Spectroscopy (LA-ICP-MS) measurements, all cleaned specimens were fixed
309 on a double-sided adhesive tape (PLANO).

310 Micro-analytical analyses with LA-ICP-MS were performed at the Institute of Geosciences, Kiel University, using
311 a 193nm ArF excimer GeoLasPro HD system (Coherent) with a large volume ablation cell (Zurich-type
312 LDHCLAC, Fricker et al., 2011) and helium as the carrier gas with 14 mL min⁻¹ H₂ added prior to passing the
313 ablation cell. For the foraminiferal samples, the pulse rate was adjusted to 4 to 5 Hz with a fluence between 2 and
314 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
315 of a foraminifer that were built up in the culturing medium were analysed, starting from the earliest, inner chamber
316 adjacent to the calcein-stained chamber. The laser was manually stopped once it broke through the foraminiferal
317 shell. The ablated material was analysed by an ICP-MS/MS instrument (8900, Agilent Scientific Instruments) in
318 no gas mode. The NIST SRM 612 glass (Jochum et al., 2011) was used for calibration and monitoring of instrument
319 drift while NIST SRM 614 was measured for quality control. Glasses were ablated with a pulse rate of 10 pulses
320 per second, an energy density of 10 J cm⁻² and a crater size of 60 µm. Carbonate matrix reference materials coral
321 JcP-1, giant clam JcT-1, limestone ECRM752-1 and synthetic spiked carbonate MACS-3 (Inoue et al., 2004;
322 Jochum et al., 2019) in the form of nano-particle pellets were analysed (Garbe-Schönberg and Müller, 2014).
323 MACS-3 was used for calibrating the mercury content in the samples as Hg is not present in the NIST SRM
324 glasses. All results for the reference materials are displayed in the appendix (Table A3). Trace element-to-calcium
325 ratios were quantified using the following isotopes: ²⁶Mg, ²⁷Al, ⁵²Cr, ⁵⁵Mn, ⁶⁰Ni, ⁶³Cu, ⁶⁵Cu, ⁶⁸Zn, ¹⁰⁷Ag, ¹¹¹Cd,
326 ¹¹⁴Cd, ¹¹⁸Sn, ²⁰¹Hg, ²⁰²Hg and ²⁰⁸Pb normalised to ⁴³Ca. If more than one isotope was measured for an element, the
327 average concentration of these was used after data processing. Uncertainty (in % RSD) was better than 5 % for all
328 TE/Ca ratios. The lowest RSD % based on the NIST SRM 612 glass was 2.1 % for Mn/Ca and the highest 5.0 %
329 for Ag/Ca. Uncertainties of all used standards and reference materials are expressed are summarized in Table A3.
330 Each acquisition interval lasted for 90 seconds, started and ended with measuring 20 s of gas blank, used as the
331 background baseline to subtract from sample intensities during the data reduction process. Furthermore, the
332 background monitoring ensured that the system was flushed properly after a sample. In cases when foraminiferal
333 test walls were very fragile causing the test to break very quickly and, hence, the length of the sample data
334 acquisition interval was less than 15 seconds, these profiles were excluded from further consideration.



335 Transient logs of raw intensities given in counts per seconds for all isotopes measured were processed with the
336 software Iolite (Version 4, Paton et al., 2011) producing averages of every time-resolved laser profile. The
337 determination of element/Ca ratios were performed after the method of (Rosenthal et al., 1999). High values of
338 ^{25}Mg , ^{27}Al or ^{55}Mn at the beginning of an ablation profile were related to contamination on the surface of the
339 foraminiferal shell or remains of organic matter (e.g., Eggins et al., 2003) and these parts of the profiles were
340 excluded from further data processing. The detection limit was defined by $3.3 \times \text{SD}$ of the gas blank in counts per
341 seconds for every element in the raw data. Only values above this limit were used for further analyses. After
342 processing the data with Iolite, an outlier detection of the TE/Ca ratios of the samples was performed. If trace
343 metal values from a spot deviated more than $\pm 2\text{SD}$ from the average of the samples from the corresponding
344 culturing phase, values were defined as outliers and discarded. The number of rejected points is indicated in the
345 supplementary material (Table S1).

346 All statistical tests were carried out using the statistical program PAST (Hammer, 2001). As the concentration of
347 heavy metals in seawater was varying during individual phases in the metal system (Table A1 and Fig. B1 in the
348 appendix), the mean concentration was calculated by applying an individual curve fit for every phase. The curve
349 was either linear, exponential or a power function depending on the covariance trend the particular metal showed.
350 Based on these curves, water values were calculated for every day and the weighted average from all days was
351 used for further calculations. This ensured that high concentrations in the beginning of each phase did not influence
352 the mean value disproportionately. The partition coefficients of the different trace metal-to-calcium ratios were
353 calculated using the trace element (TE) and calcium ratios in calcite and seawater. The following equation was
354 used:

$$355 \quad D_{\text{TE}} = (\text{TE}/\text{Ca})_{\text{calcite}} / (\text{TE}/\text{Ca})_{\text{seawater}}.$$

356 When the correlation between the metal concentration in seawater and the metal concentration in the foraminiferal
357 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
358 represent the slope of the calculated regression line. In cases where a significant positive correlation between
359 phases could not be identified, the D_{TE} values were calculated from the means of each phase separately and the
360 ranges given.

361

362 **3 Results**

363 **3.1 Survival Rates/ Growth rates / Reproductions**

364 **Table 2:** Number of inserted and recovered foraminifera from the different systems (C = control system, M =
365 metal system) and phases (0–3). Numbers of living individuals after the experiment and individuals that formed
366 chambers during their individual culturing phase are given in %. Note that the percentage of living foraminifera is
367 based on the number of foraminifera that could be recovered alive and not on the number of inserted individuals.
368 The number of laser spots is indicated as well.

	C0	C1	C2	C3	M0	M1	M2	M3	Total
No. of inserted individuals									
<i>Ammonia aomoriensis</i>	50	24	20	20	19	70	70	72	345
<i>Ammonia batava</i>	22	20	20	20	16	43	72	72	285



<i>Elphidium excavatum</i>	45	24	20	20	19	70	69	70	337
Total	117	68	60	60	54	183	211	214	967
No. of recovered individuals									
<i>Ammonia aomoriensis</i>	43	20	10	19	11	57	58	56	274
<i>Ammonia batava</i>	11	15	16	14	7	29	65	56	213
<i>Elphidium excavatum</i>	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 %									
<i>Ammonia aomoriensis</i>	86	100	80	100	90.9	100	81	98.2	92.0
<i>Ammonia batava</i>	81.8	100	100	92.9	100	100	100	100	96.8
<i>Elphidium excavatum</i>	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 %									
<i>Ammonia aomoriensis</i>	62.8	84.2	100	93.8	81.8	100	92.3	90	88.1
<i>Ammonia batava</i>	45.5	85.7	100	100	71.4	100	100	100	87.8
<i>Elphidium excavatum</i>	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									
<i>Ammonia aomoriensis</i>	22	18	17	20	9	39	40	36	201
<i>Ammonia batava</i>	14	20	19	19	6	17	52	57	204
<i>Elphidium excavatum</i>	14	13	13	12	1	36	24	31	144
Total	50	51	49	51	16	92	116	124	549

369

370 On average 74.5 % of the specimens inserted into the experiment could be recovered after their individual culturing
 371 phase of 21 days and 94.4 % of these recovered specimens survived. Approximately 79.1 % of the surviving
 372 specimens also formed at least one new chamber. Fewer specimens of *E. excavatum* formed new chambers (61.4
 373 %) than *A. batava* (87.8%) or *A. aomoriensis* (88.1 %) (Table 2). On average, *E. excavatum* formed only one or
 374 rarely two new chambers, whereas both *Ammonia* species formed usually more than four new chambers.
 375 Reproduction happened very sporadically concerning between 2 and 6 specimens per phase, on average 5 %, for
 376 the two *Ammonia* species but not for *E. excavatum*. No malformed chambers were observed in specimens that
 377 were recovered from the trace-metal contaminated system.

378

379 3.2 Culturing media

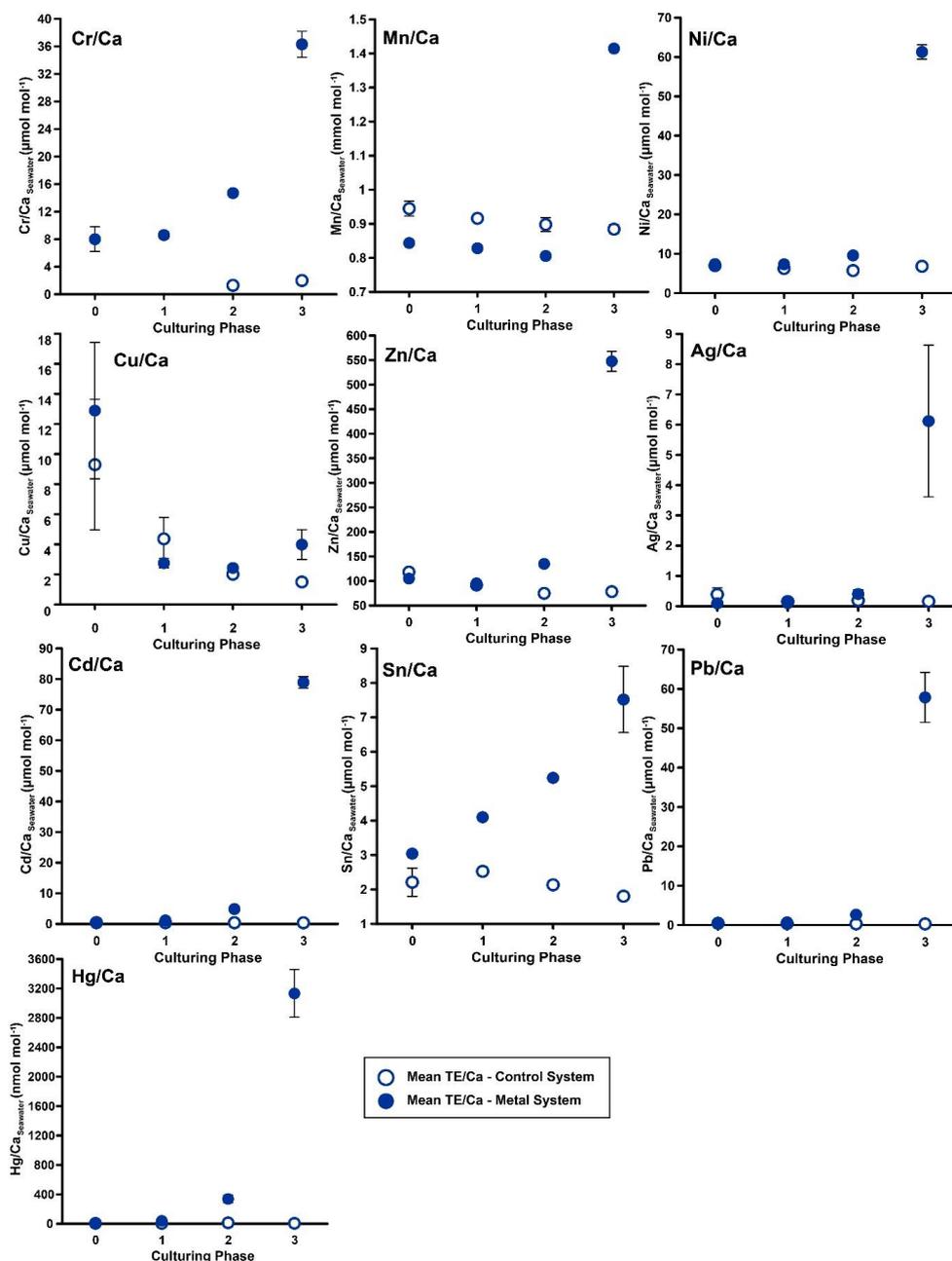
380 **Table 3:** Weighted mean TE/Ca values in the culturing medium of the control and the metal system \pm the standard
 381 error of the mean (standard deviation σ/\sqrt{n}). Furthermore, the factors between the target concentrations (Table 1)
 382 and the measured concentrations as well as the factors between individual phases are given. Values given without
 383 a standard error originate from only one measurement. All values the calculations are based on can be found in the
 384 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	$\mu\text{mol mol}^{-1}$	mmol mol^{-1}	$\mu\text{mol mol}^{-1}$	nmol mol^{-1}	$\mu\text{mol mol}^{-1}$					
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	$\mu\text{mol mol}^{-1}$	mmol mol^{-1}	$\mu\text{mol mol}^{-1}$	nmol mol^{-1}	$\mu\text{mol mol}^{-1}$					
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

385



386

387 **Figure 3:** Weighted mean TE/Ca values in the culturing medium in $\mu\text{mol mol}^{-1}$. Error bars display the standard
 388 error of the mean (standard deviation σ/\sqrt{n}). Open symbols represent the control system, where no extra metals
 389 were added during the complete culturing period (phase 0 to 3) and closed symbols represent the metal system.
 390 In this system, phase 0 is the control phase without any extra added metals and for phase 1 to 3, the heavy metal
 391 concentration in the culturing medium was elevated. Note that the standard error is comparably high in phase 3
 392 because the heavy metal concentration in this phase varied more strongly, which is shown in the appendix (Table



393 A1, Fig. B1). Therefore, this error is derived from the real values in the seawater and not from analytical
 394 uncertainties. Note that the Cr/Ca values from the control system in phase 0 and 1 are not given as these values
 395 were below the detection limit.

396

397 In phases 1 and 0 the concentration in both systems were nearly equal for most elements. Only Cr and Sn had
 398 slightly elevated concentrations in the metal system, whereas Cu and Mn concentration were higher in the control
 399 system in phase 0 (Fig. 3). This also holds true for Mn in phase 2, when all other metals showed higher
 400 concentrations in the metal system than in the control system. In phase 3, the concentration of all heavy metals
 401 were elevated in the metal system as compared to the control system. The variation of the metal concentration was
 402 in both systems highest in phase 3 for all elements but Cu, which showed the highest variation in phase 0 (Fig. 3).
 403 The control system generally displayed a smaller degree of variation than the metal system.

404 The target concentration of the metals was not accomplished for most metals in phase 1 and 2, the only exception
 405 is Ag in phase 1 (Table 3). The factors between the target and measured concentration was highest (> 50) for Ni,
 406 Cu and Zn in phase 1 and gets smaller in phase 2 and 3. Generally, all elements but Mn were concentrated higher
 407 in phases 1 and 2 than expected. In phase 3 Cr, Mn, Cu, Ag and Sn reached concentrations closer (factor 0.4 – 0.8)
 408 to the target concentration and Ni, Zn, Cd, Hg and Pb were concentrated higher (factor 3.1 – 9.9) than expected.
 409 Furthermore, the factor between individual phases (Table 3) was small for the transition from phase 0 to 1 (factor
 410 < 1.4) for all elements but Cd (factor 2.6) and Hg (factor 7.5). Same patterns can be seen between phase 1 and 2,
 411 while the difference between phase 2 and 3 was more distinct (factor > 4) for Ni, Zn, Ag, Cd, Pb and Hg. Mn, Cu
 412 and Sn showed little variation between phase 2 and 3 (factor < 1.7). Generally, the factor between each phase
 413 should have been approximately 10, which was not achieved in most cases. Exceptions were Ag, Cd and Pb, which
 414 had factors >15 between phase 2 and 3. Furthermore, Hg showed concentrations that were higher by a factor
 415 around 10 between all phases (phase 0-1 = 7.5, phase 1-2 = 8.5, phase 2-3 = 9.3).

416

417 3.3.1 Incorporation of trace metals into the foraminiferal shell

418 **Table 4:** Mean trace element-to-calcium values of *A. aomoriensis*, *A. batava* and *E. excavatum* in the control and
 419 the metal system. Errors are standard errors of the mean (standard deviation σ/\sqrt{n}). Values marked with an asterisk
 420 were derived from only one laser spot and thus are not considered for further discussion. Furthermore, the
 421 calculated D_{TE} values, the slope of the linear regression line (OLS – Ordinary Least Squares) of all means,
 422 Pearson's correlation coefficient R^2 and its significance (p) are given for the calculation with all phases and when
 423 removing phase 3 from the calculations. It's also indicated whether the regression line is forced through the origin
 424 or not. In cases when a regression did not show significant correlation, the D_{TE} range separately calculated from
 425 the individual phases is given. In cases when the regression was significant, the D_{TE} values represent the slope of
 426 the regression line. Ph = Phase. Values in Table S1 are the basis of all calculations.

	Phase	Cr/Ca	Mn/Ca	Ni/Ca	Cu/Ca	Zn/Ca
Control System		$\mu\text{mol mol}^{-1}$	mmol mol^{-1}	$\mu\text{mol mol}^{-1}$	$\mu\text{mol mol}^{-1}$	$\mu\text{mol mol}^{-1}$
	0	18.6 ± 2.5	0.11 ± 0.02	1.3 ± 0.2	5.6 ± 0.9	53.2 ± 8.8
<i>A. aomoriensis</i>	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



	0	11.6 ± 0.7	0.04 ± 0.01	1.4 ± 0.2	7.2 ± 1.1	23.9 ± 4.5
<i>A. batava</i>	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
	0	22.9 ± 2.9	0.43 ± 0.13	9.4 ± 2.5	22.3 ± 7.9	28.1 ± 4.5
<i>E. excavatum</i>	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						
	0	16.0 ± 0.5	0.08 ± 0.02	5.5 ± 0.9	15.2 ± 2.6	29.8 ± 5.1
<i>A. aomoriensis</i>	1	14.0 ± 0.7	0.39 ± 0.08	3.1 ± 0.3	6.7 ± 0.7	30.0 ± 4.0
	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
<i>A. batava</i>	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*
<i>E. excavatum</i>	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						
<i>A. aomoriensis</i>						
			0.38		1.18	
			0.83		0.80	
			0.05		0.05	
	D _{TE}	0.4-10.3	0.38	0.06-0.94	1.18	0.08-0.45
	Forced through origin	Single points	Yes	Single points	Yes	Single points
<i>A. batava</i>						
			0.23			
			0.84			
			0.001			
	D _{TE}	0.4-6.8	0.23	0.05-0.41	0.60-4.35	0.09-0.65
	Forced through origin	Single points	No	Single points	Single points	Single points
<i>E. excavatum</i>						
		2.1		0.19		
		0.82		0.79		
		0.01		0.003		
	D _{TE}	2.1	0.34-2.50	0.19	0.95-5.67	0.08-0.53
	Forced through origin	Yes	Single points	Yes	Single points	Single points
Calculations without Phase 3						
<i>A. aomoriensis</i>						
	D _{TE}	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				
<i>A. batava</i>						
	D _{TE}	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				
<i>E. excavatum</i>						
	D _{TE}	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				



428 **Table 4** continued.

	Phase	Ag/Ca	Cd/Ca	Sn/Ca	Hg/Ca	Pb/Ca
Control System						
<i>A. aomoriensis</i>	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
<i>A. batava</i>	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
	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
<i>E. excavatum</i>	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						
<i>A. aomoriensis</i>	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
<i>A. batava</i>	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
<i>E. excavatum</i>	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						
<i>A. aomoriensis</i>						
Slope of regression line		0.56				0.39
Correlation coefficient (R ²)		0.97				0.97
Significance (p)		< 0.0001				< 0.0001
D _{TE}		0.56	0.07-18.49	0.07-0.63	0.003-1.39	0.39
Forced through origin		Yes	Single points	Single points	Single points	Yes
<i>A. batava</i>						
Slope of regression line		0.17			0.003	0.52
Correlation coefficient (R ²)		0.98			0.63	1
Significance (p)		< 0.0001			0.01	< 0.0001
D _{TE}		0.17	0.08-14.42	0.03-0.26	0.003	0.52
Forced through origin		Yes	Single points	Single points	Yes	Yes
<i>E. excavatum</i>						
Slope of regression line		0.47				0.91
Correlation coefficient (R ²)		0.96				1
Significance (p)		< 0.0001				< 0.0001
D _{TE}		0.47	0.06-49.45	0.06-3.25	0.03-18.51	0.91
Forced through origin		Yes	Single points	Single points	Single points	Yes
Calculations without Phase 3						
<i>A. aomoriensis</i>						
Slope of regression line						1.6
Correlation coefficient (R ²)						0.91
Significance (p)						< 0.001
D _{TE}		0.70-2.57	1.14-18.49	0.10-0.63	0.003-1.39	1.60
Forced through origin		Single points	Single points	Single points	Single points	Yes
<i>A. batava</i>						
Slope of regression line		0.35				
Correlation coefficient (R ²)		0.91				
Significance (p)		0.03				
D _{TE}		0.35	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



<i>E. excavatum</i>					
Slope of regression line				0.26	2
Correlation coefficient (R^2)				0.53	0.90
Significance (p)				0.05	0.003
DTE	0.23-4.25	0.80-49.45	0.06-3.25	0.26	2.0
Forced through origin	Single points	Single points	Single points	No	Yes

429

430 Measurable incorporation into the foraminiferal calcite was found for all the trace metals analysed but the degree
 431 of incorporation varied profoundly within and between species (Fig. 4 and Table 4). In both systems, the trace
 432 metal concentration in *E. excavatum* was higher than in the other species (*A. aomoriensis* and *A. batava*) for Cr,
 433 Mn, Ni, Cu, Hg and Sn. This trend is also visible but less pronounced in the Cu values of the control system.

434 Cr, Ni, Cu, Zn, Cd, Pb and Ag values of *A. aomoriensis* displayed the highest standard error of the mean paired
 435 with highest concentrations in the water in the metal system. Sn, Mn and Hg did not show any clear pattern. In the
 436 control system, all trace metal concentrations had higher standard errors of the mean when the concentration of
 437 these metals in the culturing medium was higher. The trend is also shown in *A. batava* and *E. excavatum* for all
 438 trace metals of the control and the metal system.

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

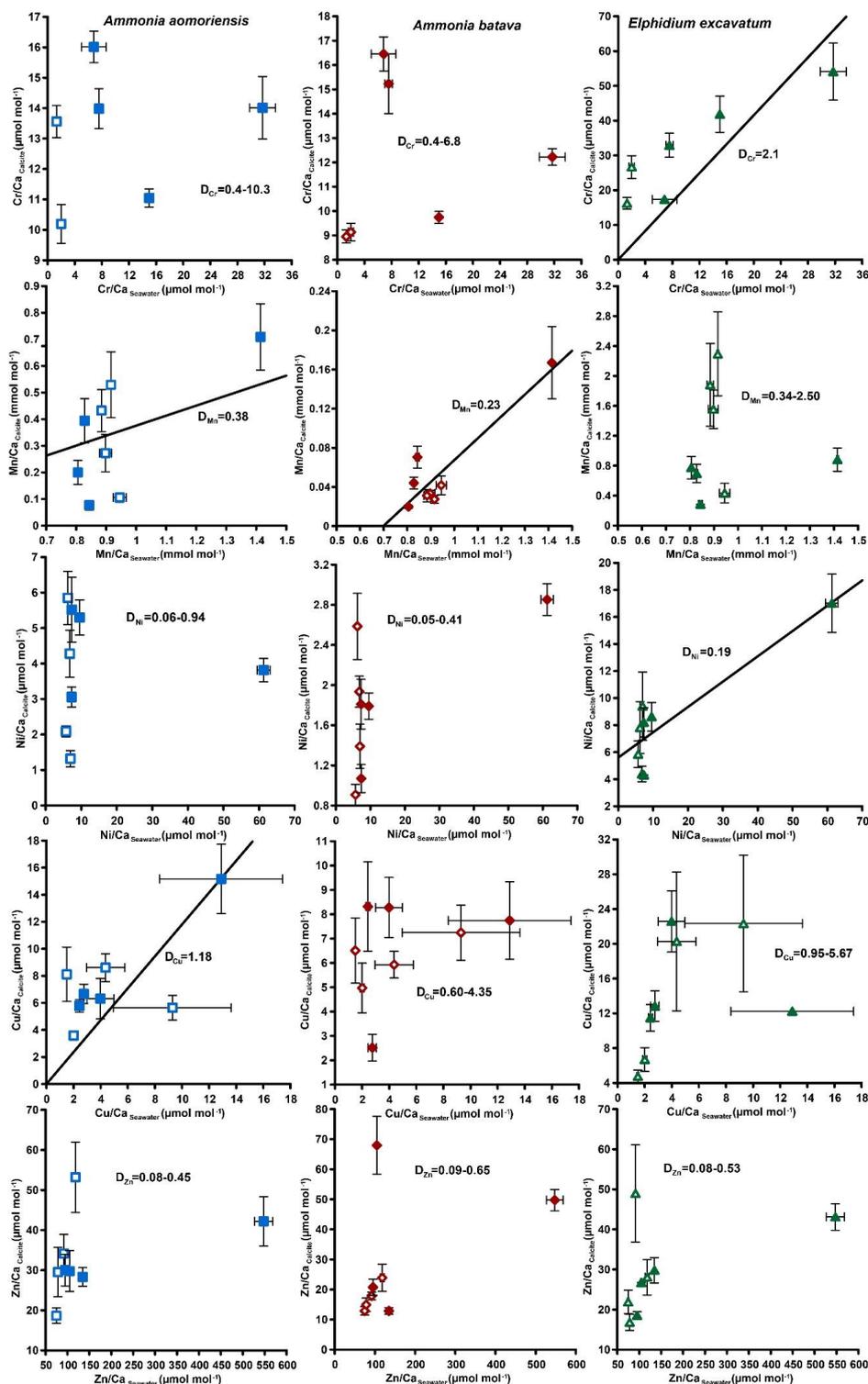
441 When phase 3 was included, a strong positive correlation ($R^2 > 0.9$, $p \leq 0.05$) of Ag and Pb concentrations in the
 442 foraminiferal shell and in the culturing medium was recognised in all three species. Furthermore, *A. batava* also
 443 revealed a positive correlation for Hg ($R^2 = 0.63$,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

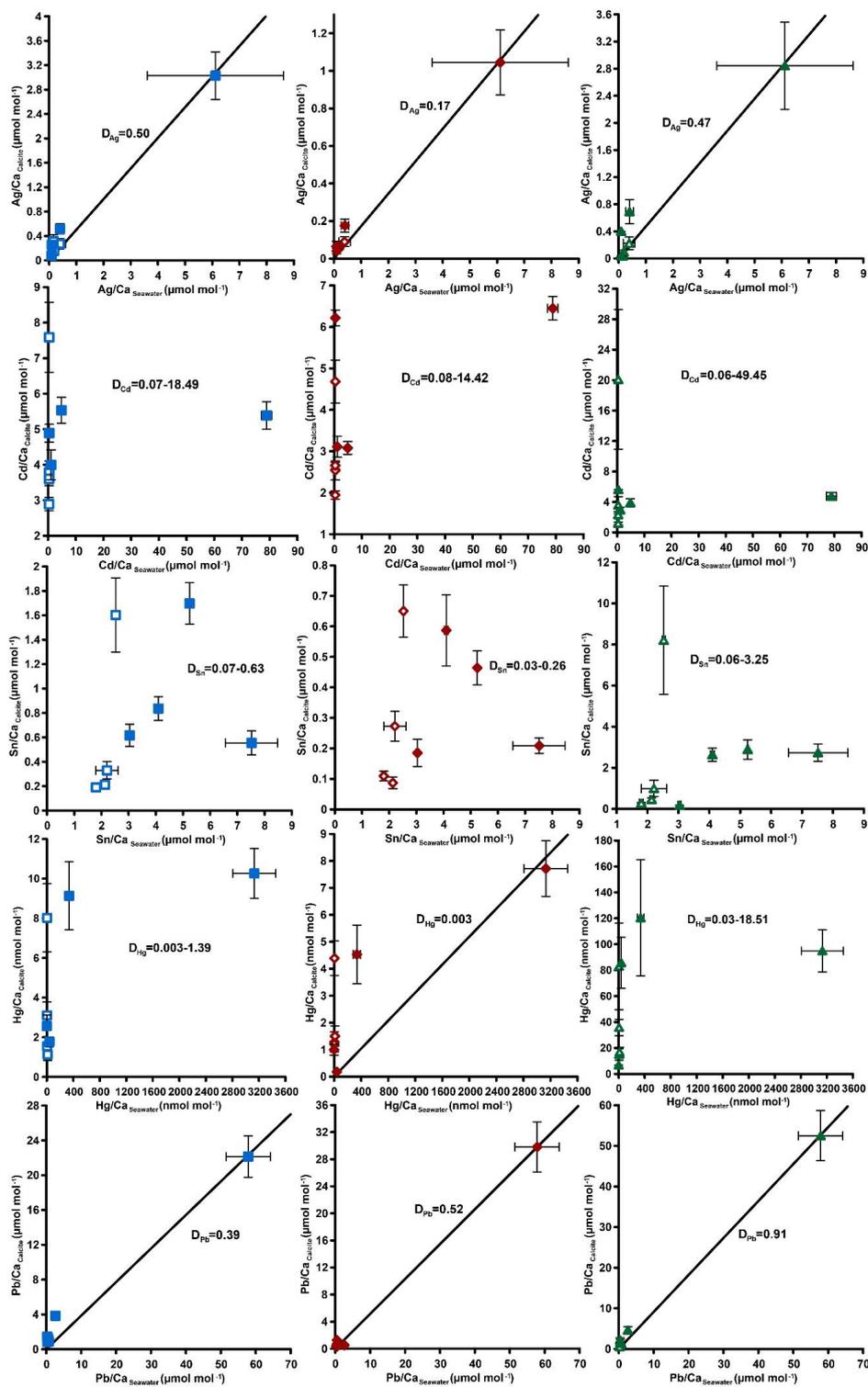
448 When phase 3 was excluded from the calculations, *A. aomoriensis* and *E. excavatum* showed a positive correlation
 449 for Pb ($R^2 > 0.9$, $p \leq 0.003$), *A. batava* for Ag ($R^2 = 0.91$, $p = 0.03$) and in *E. excavatum* Hg correlated weaker
 450 positively ($R^2 > 0.53$, $p \leq 0.05$). All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

451



452 3.3.2 Partition coefficient (D_{TE})







455



456 **Figure 4:** Mean TE/Ca values in the foraminiferal calcite versus the mean TE/Ca values in the corresponding
457 culturing medium based on phase 0 to 3. Each data point represents the mean value of all laser ablation ICP – MS
458 measurements on single foraminiferal chambers built up during the individual culturing phase plotted against the
459 mean metal concentrations in the seawater averaged over the culturing phase (Table 3). Error bars symbolize the
460 standard error of the mean. The linear regression line is displayed when elements showed a significant correlation
461 between seawater and calcite. D_{TE} 's of *E. excavatum* were considered without values for Phase 0 of the Metal
462 System as only data from one newly formed chamber are available. All values can be found in Table 4. An enlarged
463 graph based on the calculations without phase 3 is provided in the appendix (Fig. B2).

464

465 Partition coefficients for the different trace elements were deduced from molar foraminiferal test TE/Ca and the
466 values from the corresponding culturing medium. Note that the D_{TE} values represent the slope of the regression
467 line when there was a positive correlation detected and were furthermore separately calculated for every individual
468 phase. The range of the calculations based on every individual phase are given when no positive correlation could
469 be detected. Furthermore, calculations were performed with and without phase 3 (Fig. 4, Fig. B2 and Table 4).

470 Generally, the values varied between 0.003 and 49.45. The majority of D_{TE} were lower than 1 in *A. aomoriensis*
471 (with phase 3 = 61 %, without phase 3 = 57%) and *A. batava* (with phase 3 = 75%, without phase 3 = 73%), i.e.,
472 uptake but no enrichment took place. D_{TE} values derived from *E. excavatum* on the other hand showed a smaller
473 proportion < 1 (with phase 3 = 47%, without phase 3 = 42%). Note that D_{TE} of *E. excavatum* were considered
474 without values for Phase 0 as there were data from only one newly formed chamber available. For most elements
475 (Cr, Mn, Ni, Cu, Cd, Sn, Pb and Hg) D_{TE} derived from *E. excavatum* are higher than D_{TE} from the two *Ammonia*
476 species (Table 4, Fig. 4), which showed comparable D_{TE} values for most elements. One exception is Zn, where all
477 D_{Zn} values are within a similar range ($D_{Zn} \sim 0.08-0.65$) independent of the species. For *A. aomoriensis* D_{Cu} was >
478 1 and D_{Cd} as well as D_{Pb} were also > 1 when phase 3 was excluded from the calculations. *A. batava* showed D_{TE}
479 values > 1 for most Cr and Cd values (excluding phase 3 and 2) when considering calculations from the individual
480 phases. *E. excavatum* displayed D_{TE} values > 1 for Cr and Cu for the calculations with phase 3 and also for Pb
481 without phase 3. Furthermore, D_{Cd} and D_{Hg} of *E. excavatum* were also mostly > 1 when looking at calculations
482 based on individual phases (Table 4, Fig. 4). The highest variation between minimum and maximum D_{TE} for all
483 species was found for Cd and Hg. In addition, some elements showed partition coefficients > 1 in a couple of
484 phases and/ or in one of the two culturing systems (e.g., *E. excavatum*: Mn = Control System Phase 1 to 3; *A.*
485 *batava*: Cu = all but Control System Phase 0 and Metal System Phase 1; Pb = Control System and Metal System
486 Phase 1; *A. aomoriensis*: Cr = Control System Phase 2 and 3, Metal System Phase 1; Ag = Control System Phase
487 1 and 3, Metal System Phase 2 and 3, Hg: Control System Phase 3, see Table 4, Fig. 4).

488



489 **4. Discussion**

490 **4.1 Experimental Uncertainties**

491 The element concentrations within the culturing medium of each culturing phase were comparably stable for most
492 elements in the control system. In the metal system, the variations were higher, which is due to the punctual input
493 of the stock solution for reaching the next phase concentration (Table A1, Fig. B1). This sudden adding of metals
494 resulted in a high peak concentration in the beginning of the new phase, which equilibrated after a while. This
495 trend is most pronounced in phase 3 as the added amount of the stock solution was highest for this phase, which
496 is why the standard error of this phase is comparably high. Furthermore, the variations of the metal concentrations
497 were in a comparable range than those presented in other culturing studies (e.g., Marechal-Abram et al., 2004; De
498 Nooijer et al., 2007; Munsel et al., 2010; Rimmelzwaal et al., 2019).

499 When taking into account that the amount of the stock solution added to the culturing medium of the metal system
500 at the beginning of each culturing phase was elevated 1 to 1.5 order of magnitudes between phases, the measured
501 metal concentrations are smaller than expected for phases 0, 1 and 2. This in combination with the varying metal
502 concentration within one phase suggests that several processes are affecting the concentration in such a complex
503 culturing system. One possible mechanism is sorption of the metals onto surfaces (e.g., tubing, culturing vessels,
504 plates, organic matter or the foraminiferal test itself), which could have lowered the metal concentration in the
505 culturing medium. Therefore, sorption could have contributed to the overall budget of the metals. On the other
506 hand, Cu appears to have been released from components of the culturing system even though the system was
507 cleaned before and was operated with seawater for 14 days before the experiments begun. For instance, the
508 concentration of Cu was high in phase 0, where no metals were added suggesting release from system parts. In
509 phase 1, the Cu concentration decreased meaning the contamination derived from the system was removed by a
510 process similar to that observed for the other metals after additions were made. Similar effects have been reported
511 by De Nooijer et al. (2007) for Cu and Havach et al. (2001) for Cd. Other processes like the uptake of the metals
512 by the foraminifera itself and the growth of algae could further have an influence on the metal concentration in the
513 culturing medium. Such processes are difficult to predict and even more challenging to avoid but probably mirror
514 real environments better than sterile petri dish experiments do (e.g., Havach et al., 2001; Hintz et al., 2004; Munsel
515 et al., 2010).

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



529 Calcein was used for staining the foraminiferal test before they were placed into the culturing system. Calcein
530 binds to Ca and is incorporated into the mineralised calcium carbonate (Bernhard et al., 2004). It is conceivable
531 that the trace metal incorporation could also be affected by calcein. However, no evidence has been found by a
532 variety of studies (e.g., Hintz et al., 2006; De Nooijer et al., 2007; Dissard et al., 2009). Furthermore, calcein was
533 only used prior to the experiment to mark the last chamber that was grown outside the culturing system. Therefore,
534 the incorporation of the metals measured in subsequent chambers was not affected by the calcein application.

535

536 **4.2 Incorporation of heavy metals in the foraminiferal test**

537 Many heavy metals have been demonstrated to be incorporated into the foraminiferal shell (e.g., Cr: Rimmelzwaal
538 et al., 2019; Mn: Koho et al., 2015; 2017; Cu: De Nooijer et al., 2007; Ni: Munsel et al., 2010; Hg: Frontalini et
539 al., 2018; Cd: Havach et al., 2001; Pb: Titelboim et al., 2018; Sagar et al., 2021; Zn: Marchitto et al., 2000; Van
540 Dijk et al., 2017), and the incorporation of all of these metals was measured here. Additionally, to our knowledge,
541 Sn and Ag were investigated here for the first time. The levels observed were well above control values indicating
542 an elevated incorporation of Ag and Sn into the foraminiferal test calcite with increasing metal concentrations in
543 seawater.

544 Different factors can influence the incorporation of these metals into the foraminiferal test. First of all, the uptake
545 depends on metabolic pathways during the calcification process. Fundamental biomineralization processes of
546 foraminifera are the subject of an ongoing discussion and several (partly) competing models have been proposed
547 so far (e.g., Elderfield and Erez, 1996; Erez, 2003; De Nooijer et al., 2009b, 2014; Nehrke et al., 2013). One model
548 proposes that the foraminifera take up ions directly from the surrounding seawater by endocytosis or by building
549 seawater vacuoles, which are transported to the site of calcification (SOC) (Elderfield and Erez, 1996; Erez 2003;
550 De Nooijer et al., 2009b; 2009a; Khalifa et al., 2016). The SOC is located outside the foraminiferal cell and the
551 formation of new calcite takes place in this zone (see e.g., De Nooijer et al., 2014 for a summary and illustration).
552 There are evidence that this part is separated from the surrounding seawater (e.g., Spindler, 1978; Bé et al., 1979;
553 De Nooijer et al., 2009b; 2014; Glas et al., 2012; Nehrke et al., 2013). The other competing model suggests that
554 the uptake of ions and the transport to the SOC is performed directly from the seawater across the cell membrane
555 by active trans-membrane-transport (TMT) and/ or passive transport via gaps in the pseudopodial network of the
556 foraminifera (Nehrke et al., 2013; De Nooijer et al., 2014). The dependency of heavy metal concentrations in the
557 foraminiferal test on their seawater concentration relies on the prevailing mechanism. A biomineralization based
558 on endocytosis does not control the amount of ions that is introduced into the foraminiferal cell and is transported
559 to the SOC. Consequently, processes like Rayleigh fractionation are most important (e.g., Elderfield and Erez,
560 1996). This would also mean that the metal concentration in the seawater is directly mirrored by their concentration
561 in the foraminiferal shell, which cannot be supported by the results of our study. Indeed, several elements show
562 partition coefficients > 1 when the D_{TE} 's are calculated separately for each phase (see Results section in this
563 manuscript). Only Pb and Cr in *E. excavatum* and Cu and Pb in *A. aomoriensis* consistently display mean D_{TE} 's $>$
564 1 paired with a positive correlation of the concentration in seawater and in the foraminiferal shell, which could
565 indicate a non-selective uptake of these metals. On the other hand, the D_{TE} values of many elements (Ni, Zn, Cd,
566 Hg, Pb) dramatically decrease with increasing concentration in the seawater in the highest metal treatment in all
567 species. This kind of overload effect was also noted by Nardelli et al. (2016) for Zn or by Munsel et al. (2010) for
568 Ni. Nardelli et al. (2016) suggested, that biological mechanism expulse or block these metals if the concentration



569 is getting too high and an imminent intoxication is probable, which may be managed by controlling the ion uptake
570 via TMT. Therefore, it may well be possible that the highest concentration of the metals in our study was close to
571 the tipping point of the biological mechanism taking over and protecting the organism.

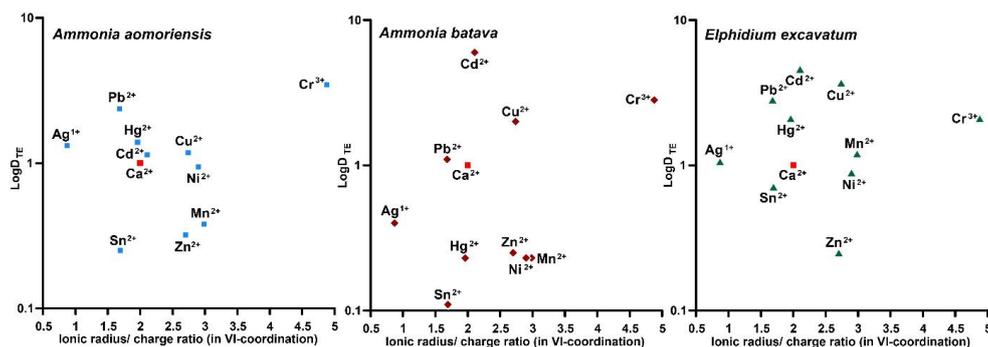
572 Besides biologically controlled factors, also physicochemical properties play an important role when it comes to
573 the uptake of ions. One chemical factor is the aqueous speciation and solubility of the metals. Metals with a free
574 ion form with a charge of 2+ are more similar to Ca^{2+} , which makes incorporation more likely (Railsback, 1999).
575 Nearly all metals in this study were added as dissolved chlorides and therefore had a charge of 2+. The only
576 exceptions were Ag, which was added as AgNO_3 with a charge of 1+ and Cr, which was added as $\text{CrCl}_3 \cdot 6 \text{H}_2\text{O}$.
577 The charge of the cation as such does not seem to make a major difference as Ag was incorporated into all three
578 species and Cr into *E. excavatum* with a significant positive correlation to concentrations in the culturing medium.
579 Furthermore, it is possible that the oxidative state of the elements is changing due to their pH dependency, which
580 will be discussed for every element separately. Furthermore, other ions with a charge of 1+ are also known to be
581 incorporated in calcite. Examples are Li^+ (e.g., Delaney et al., 1985; Hall et al., 2004) and Na^+ (e.g., Wit et al.,
582 2013; Bertlich et al., 2018), which are believed to occupy interstitial positions in calcite where the calcite lattice
583 has defects (Ishikawa and Ichikuni, 1984; Okumura and Kitano, 1986). In addition, rare earth elements with a
584 charge of 3+ are also detected in the foraminiferal calcite (e.g., Haley et al., 2005; Roberts et al., 2012).

585 The aqueous speciation of many metals is strongly influenced by the pH (e.g., Förstner, 1993; Pagnanelli et al.,
586 2003; Spurgeon et al., 2006; Powell et al., 2015; Huang et al., 2017). As the pH during the experiment was stable
587 around 8.0 ± 0.1 (measured twice a week), speciation changes between phases due to varying pH values can be
588 excluded. However, it is possible that some metals were not available in a form that could be readily incorporated
589 in the calcite such as the free ion or carbonate species. Cr is not available in an optimal speciation to substitute Ca
590 as a pH of 8 would favour Cr^{3+} or Cr^{4+} as well as oxides and hydroxides (Elderfield, 1970; Geisler and Schmidt,
591 1991). Furthermore, the used Cr-salt may not have dissolved completely, even though the stock solution was
592 heated and stirred during the process. Both in combination may lead to the small variation between the different
593 phases in the seawater. Interferences that could possibly influence the Cr measurements in the water samples are
594 chlorine oxides or hydroxides (e.g., Tan and Horlick, 1986; McLaren et al., 1987; Reed et al., 1994; Laborda
595 et al., 1994). As NaCl blanks were measured, these interferences are most likely monitored and can be excluded as
596 a biasing factor. Furthermore, measurements of reference materials revealed accurate Cr concentrations compared
597 to those presented in the literature (Table A2), which also corroborates the assumption that these interferences can
598 be neglected. Similar pH processes could also have effected Cu as a pH around 8, like in this experiments, favours
599 copper carbonates over free Cu^{2+} ions (e.g., Escudero et al., 2008; Millero et al., 2009), which means that the best
600 available speciation was not prevailing during the experiments. Nevertheless, Cu and Cr were taken up by all
601 species and therefore, this factor cannot be decisive when it comes to incorporation of these metals into the
602 foraminiferal shell.

603 If the incorporation of metals would be straightforward and would only depend on the speciation of the metal and
604 other physicochemical factors, the behaviour of the metals would mostly be influenced by the ionic radius in
605 combination with the charge of the metal ions as described for carbonate minerals by Rimstidt et al. (1998). The
606 endocytotic pathway of seawater components into the foraminifer provokes a behaviour of ion incorporation
607 comparable to inorganic calcite precipitation. It was found that cations are incorporated into inorganic calcite by



608 substitution of Ca^{2+} (e.g., Reeder et al., 1999), especially when the effective ionic radius of these ions is comparable
609 to the one of calcium ($= 1.0 \text{ \AA}$).



610
611 **Figure 5:** Partition coefficients ($\log D_{\text{TE}}$) in dependency of the ionic radius-to-charge ratio (values from Shannon
612 and Prewitt, 1969) in 6-fold coordination for the trace elements analysed in this study. Displayed mean D_{TE} 's are
613 derived from single values calculated for each culturing phase individually (Metal and Control System Phase 0 to
614 3) and not from the regression lines. Calcium is marked in red. Blue squares represent values from *A. aomoriensis*
615 (left), red diamonds represent values from *A. batava* (middle) and green triangles represent values from *E.*
616 *excavatum* (right).

617

618 Some metals like Mn, Zn and Cu are known to be fundamentally necessary as micro-nutrients to maintain
619 biological and physiological function of a cell (e.g., Mertz, 1981; Tchounwou et al., 2012; Martinez-Colon et al.,
620 2009; Maret, 2016). Therefore, these elements should preferentially be taken up into the foraminiferal cell, where
621 they were used for further processes. This in turn could lead to the consumption of these metals before they can
622 be incorporated into the foraminiferal tests. All of these ions have a similar ionic radius ($\text{Cu} = 0.73 \text{ \AA}$, $\text{Mn} = 0.67$
623 \AA , $\text{Zn} = 0.74 \text{ \AA}$) in six-fold coordination (Rimstidt et al., 1998), which would also suggest, that their behaviour is
624 comparable. The ionic radii are much smaller than that of Ca, but are rather similar to Mg (0.72 \AA , Rimstidt et al.,
625 1998) (Fig. 5).

626 Mn shows a positive correlation between its concentration in seawater and the foraminiferal test in the two
627 *Ammonia* species when the calculations include phase 3. This indicates that this element serves as a well-behaved
628 proxy influenced mainly by its concentration in seawater. However, *E. excavatum* do not show this positive
629 correlation. Furthermore, D_{Mn} values of this study calculated with phase 3 (*A. aomoriensis* $D_{\text{Mn}} = 0.38$, *A. batava*
630 $D_{\text{Mn}} = 0.23$, *E. excavatum* $D_{\text{Mn}} = 0.34\text{-}2.50$) are comparable in range of those presented in Koho et al. (2015, 2017:
631 $D_{\text{Mn}} = 0.34\text{-}2.50$) and Barras et al. (2018: $D_{\text{Mn}} = 0.09\text{-}0.35$), but lower than Munsel et al. (2010) reported ($D_{\text{Mn}} >$
632 2.4) except for *E. excavatum*. Species-specific differences in partition coefficients not only for Mn but also for
633 other elements like Mg or Na are common phenomena (e.g., Toyofuku et al., 2011; Barras et al., 2018; Wit et al.,
634 2013) and could also explain the offset of the D_{TE} values from *E. excavatum* in this study. Furthermore, it is known
635 that the presence of toxic metals such as Cd, Ni or Hg can inhibit the uptake of essential metals like Mn if these
636 metals are present in low concentrations (e.g., Sunda and Huntsman, 1998a, 1998b). It is possible that this
637 mechanism is more pronounced in *E. excavatum* than in the *Ammonia* species. Zn was clearly incorporated above
638 control level into all three species, but it's behaviour is influenced by more factors than the concentration of Zn in



639 the culturing medium. This can be inferred by the fact that there was no significant correlation recognised between
640 Zn concentration in calcite and seawater (Fig. 4, Table 4). D_{Zn} values of this study are in good agreement with
641 those calculated by Van Dijk et al. (2017) for four hyaline species ($D_{Zn} = 0.2-0.36$) and Nardelli et al. (2016) for
642 the miliolid *Pseudotriloculina rotunda* ($D_{Zn} = 0.2-4.0$), especially when phase 3 is excluded from the calculation
643 (this study, without phase 3: *A. aomoriensis* $D_{Zn} = 0.21-0.45$, *A. batava* $D_{Zn} = 0.10-0.65$, *E. excavatum* $D_{Zn} = 0.22-$
644 0.53). Other studies reported higher values between 3.5 and 9, though for rotaliid taxa like *Cibicidoides*
645 *wullerstorfi* and *Uvigerina spp.* (Marchitto et al., 2000; Van Dijk et al., 2017). It is again possible that the mixture
646 of metals is inhibiting the uptake of essential metals like Zn similar to Mn. Cu shows a simple well-behaved proxy
647 behaviour with a significant positive correlation in *A. aomoriensis* but not in the other two species. The D_{Cu}
648 presented by Munsel et al. (2010) ($D_{Cu} = 0.08-0.25$) and De Nooijer et al. (2007) ($D_{Cu} = 0.1-0.4$) is lower than D_{Cu}
649 from this study (*A. aomoriensis* $D_{Cu} = 1.18$, *A. batava* $D_{Cu} = 0.60-4.35$, *E. excavatum* $D_{Cu} = 0.95-5.67$). Only the
650 lowest values of this study are in the same order of magnitude. These differences could arise from the lower
651 concentration of Cu in this study or from the mixture of metals. It is also reported, that the exposure to more than
652 one metal can cause an increased uptake of another metal into the cell (Archibald and Duong, 1984; Martinez-
653 Finley et al., 2012; Bruins et al., 2000; Shafiq et al., 1991). If more Cu is taken up into the cell, well may be that
654 after the usage of Cu as micro-nutrient, more Cu is left over and is actively deposited into the calcite. It is therefore
655 conceivable that one particular metal in our study was effecting a co-uptake of Cu, which lead to an elevated
656 incorporation into the calcite as compared to other studies.

657 The non – essential elements Hg, Cd and Pb are not used in physiological processes and are therefore believed to
658 have a higher toxic potential (Barbier et al., 2005; Raikwar et al, 2008; Ali and Khan, 2019). This in turn lead to
659 the assumption, that these metals are incorporated into the foraminiferal cell to a smaller amount. This could also
660 result in an enhanced removal of these metals, because they are potentially harmful for marine life and could
661 trigger mechanisms that prevent the foraminifera from intoxication as reported for various organisms (benthic
662 foraminifera: Bresler and Yanko, 1995; Yeast: Adle et al., 2007; Bacteria: Shaw and Dussan, 2015; Microalgae:
663 Duque et al., 2019). Furthermore, it could increase the input of these metals into the foraminiferal calcite as a
664 further removing mechanism. The ionic radii of Pb^{2+} in calcite-coordination is 1.19 Å, which is remarkably higher
665 than those of Hg^{2+} (1.02 Å) and Cd^{2+} (0.95 Å), which are comparable to Ca (Fig. 5). This similarity should also
666 favour the incorporation of Cd and Hg into calcite, which holds only partly true, as Cd shows no trends, but Hg
667 correlates in *A. batava* and in *E. excavatum* if phase 3 is not integrated into the calculations. This indicated that
668 the incorporation of Cd is not straight-forward and is indeed depending on more complex factors than seawater
669 concentration of Cd. Nevertheless, Cd is incorporated well above control level in all three species. Because the
670 ionic radius of Pb is bigger than that of Ca a smaller degree of Ca^{2+} substitution following the ionic radius to charge
671 ratio theory after Rimstidt et al. (1998) is expected. This is not the case as Pb emerged as a well-behaved proxy.
672 All three species incorporated Pb with a significant positive trend indicating that the main controlling factor is the
673 seawater concentration of Pb (Fig. 4, Table 4). Calculated D_{Pb} values including phase 3 of this study are around 1
674 for *E. excavatum* ($D_{Pb} = 0.91$), but lower for *A. aomoriensis* ($D_{Pb} = 0.39$) and *A. batava* ($D_{Pb} = 0.52$). When excluding
675 phase 3, D_{Pb} values of *E. excavatum* ($D_{Pb} = 2.0$) and *A. aomoriensis* ($D_{Pb} = 1.6$) are getting even higher, which is
676 may connected to the overload effect. Comparing the D_{Pb} around 8.4 determined by Sagar et al. (2021), who
677 cultured the large benthic foraminifer *Amphisorus hemprichii*, a symbiont-bearing, miliolid species, with varying
678 Pb concentrations ($0.5-80 \mu m^{-1}$) that are comparable to our concentration range ($\sim 0.11-30 \mu m^{-1}$), the D_{Pb} of this
679 study are a little lower. Nevertheless, D_{Pb} values from the present study are partly higher in individual phases (*A.*



680 *aomoriensis* D_{Pb} : individual phases without phase 3 = 1.3-5.14; *A. batava* D_{Pb} : Control System Phase 3 = 2.52; *E.*
681 *excavatum* D_{Pb} : Control System Phase 0-2 and Metal System Phase 1 ($D_{Pb} \geq 3$) and thereby match the values from
682 Sagar et al. (2021) better. For Hg, no partition coefficients are published so far. D_{Cd} values from different studies
683 ranged between 0.7 and 4 (Havach et al., 2001; Tachikawa and Elderfield, 2002; Maréchal-Abram et al., 2004)
684 and are overall lower than D_{Cd} values from the present study (*A. aomoriensis* D_{Cd} = 0.07-18.49, *A. batava* D_{Cd} =
685 0.08-14.42, *E. excavatum* D_{Cd} = 0.06-49.45), but mean D_{Cd} values are of the same order of magnitude. Cd is known
686 to mimic other metals that are essential to diverse biological functions (Martelli et al., 2006; Urani et al., 2015;
687 Losada Ros et al., 2020). Consequently, one may argue that cadmium follows a Trojan horse strategy to get
688 assimilated (Martelli et al., 2006), which could also be the case for Hg. Smith et al. (2020) found a strong
689 correlation between the Cd concentration in the seawater and the foraminiferal shell in the species *Haynesina*
690 *germanica*, *Ammonia tepida*, *Quinqueloculina sabulosa* and *Triloculina oblonga*. This trend was not present in
691 our study, as no species showed a covariance (Fig. 4, Table 4) indicating the influence of more complex
692 mechanisms than simple incorporation of the seawater ion concentration. Investigations on deep water benthic
693 foraminifera like *Cibicides wuellerstorfi*, *Uvigerina peregrina* and *Melonis barleeanum* revealed a smaller
694 range in the D_{Cd} values (e.g., McCorkle et al., 1995; Tachikawa and Elderfield, 2002), which most likely originated
695 from the smaller variability of the Cd concentration in their seawater as compared to this study.

696 The importance of other metals like Sn, Cr, Ag and Ni is not fully understood yet but some of them are believed
697 to have certain biological functions in the cells of animals or plants (Horovitz, 1988; Mertz, 1993; Lukaski, 1999;
698 Pilon-Smits et al., 2009; Hänsch & Mendel, 2009; Chen et al., 2009). For example, Ni is important for plants and
699 bacteria (Poonkothai and Vijayavathi, 2012; Maret, 2016). The ionic radii of these metals in calcite-coordination
700 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
701 calcite, too (Fig. 5).

702 Ni was incorporated with a positive trend in *E. excavatum*, but with no clear trend in the *Ammonia* species (Fig. 4,
703 Table 4). Munsel et al. (2010) found a similar trend in *A. tepida* and their calculated D_{Ni} values (D_{Ni} = 0.4-2.0) are
704 in good agreement with our results when the highest metal phase of this study is not taken into account (*A.*
705 *aomoriensis* D_{Ni} = 0.19-0.94, *A. batava* D_{Ni} = 0.15-0.41, *E. excavatum* D_{Ni} = 0.64-1.35). Ag exhibited a strong
706 positive correlation between seawater and foraminiferal shell in all three foraminiferal species. Partition
707 coefficients for Ag (*A. aomoriensis* D_{Ag} = 0.56, *A. batava* D_{Ag} = 0.17, *E. excavatum* D_{Ag} = 0.47) cannot be
708 compared to other studies as no literature is available, but the general trend, the ionic radius and the D_{TE} values
709 are comparable to other elements in this study e.g., to Pb. Ag and Ni both display a well-behaved proxy for the
710 estimation of seawater concentration of these ions (Fig. 4, Table 4).

711 Cr and Sn, on the other hand, were not incorporated in a higher amount when the concentration of these metals in
712 the culturing medium was raised, except for Cr in *E. excavatum*, which showed a positive correlation. The D_{Cr}
713 values presented in Remmelzwaal et al. (2019) ($D_{Cr} > 107$), based on the tropical, symbiont bearing foraminifera
714 *Amphistegina spp.*, are at least one order of magnitude higher than D_{Cr} values in this study (*A. aomoriensis* D_{Cr} =
715 0.74-10.3, *A. batava* D_{Cr} = 0.4-6.8, *E. excavatum* D_{Cr} = 2.1). For Sn, no comparative studies are available. One
716 possible reason for dynamics of Cr are the comparable low concentrations in the culturing medium and
717 furthermore, the differences between the phases are also very low (Fig. 3, Fig. B1 and Table 3). It may be that the
718 concentration of Cr needs to be further elevated and the concentration range needs to be extended before the
719 foraminifera are able to incorporate Cr with significant differences between concentrations. We may speculate that



720 the same could apply for Sn. Besides, Remmelzwaal et al. (2019) suggested, that Cr in foraminiferal shells is
721 mainly a result of post-depositional overprinting. Diagenetic processes are very unlikely to play a role in our
722 experiments, which would explain, why we do not recognise a correlation between the concentration of Cr in the
723 culturing medium and in foraminiferal calcite.

724

725 4.3 Interspecies variability

726 The three different species cultured in this study clearly incorporate the same metal in different ways, which is
727 most visible in the overall higher TE/Ca values of *E. excavatum* compared to species from the genus *Ammonia*
728 (Fig. 4, Table 4). Koho et al. (2017) suggested that these differences in the incorporation result from different
729 microhabitats used by different foraminiferal species. This might be true in nature. In our experiments, however,
730 the sediment in the cavities was only a few mm thick and no redox horizon was recognised when recovering the
731 foraminifera after the experiment. Therefore, all foraminifera were living in the same microhabitat.

732 Another possible reason for the difference between *E. excavatum* and *Ammonia* species is their nutrition strategy.
733 As discussed above, D_{TE} values were markedly higher in symbiont-bearing species. *Ammonia* species do not
734 harbour endosymbionts (Jauffrais et al., 2016), whereas at least five intertidal *Elphidium* species were husbanding
735 diatom chloroplasts, including *E. excavatum* (e.g., Pillet et al., 2011; Cesborn et al., 2017). However, an earlier
736 study could not corroborate the assumption that *Elphidium* species living at greater water depth in the Baltic Sea
737 may contain endosymbiotic zooxantellae (Schönfeld and Numberger, 2007). In our experiment, dead
738 *Nannochloropsis* were fed, which is certainly not the preferred food source for *E. excavatum* (Pillet et al., 2011).
739 This could lead to a slower growth and *E. excavatum* built on average only 1 chamber during the individual
740 culturing period of 21 days while *Ammonia* species built more than four chambers. Furthermore, *E. excavatum* did
741 not reproduce, even though the culturing period is close to the generation time of this species (Haake, 1962). When
742 growth is slower it could be possible that a higher amount of a metal is incorporated into the shell, which would
743 lead to higher TE/Ca values in this species. Another possibility is the timing of chamber formation. As *E.*
744 *excavatum* formed on average one new chamber, it is possible that this chamber was formed during the high peak
745 in the metal concentration during the beginning of the culturing phases (Fig. B1, Table A1). This could in turn
746 lead to a higher uptake of the metals and higher D_{TE} values. Both *Ammonia* species on the other hand, formed
747 more chambers, which makes it most likely that not only the first high concentration influences their overall D_{TE}
748 value. Unfortunately, it is not possible to constrain exactly when the specimens formed their new chambers.

749

750 4.4 Application of TE/Ca values in the foraminiferal shell

751 **Table 5:** Comparison of the heavy metal concentrations in different regions of the world to values used for the
752 culturing experiments in this study. It is indicated whether the values of this study are comparable to environmental
753 values or if values from this study are higher or lower. (EPA = Environmental Protection Agency, USA)

Element	Study area	Concentration in $\mu\text{g l}^{-1}$	Comparable?	Reference
Ag		0.06-4.61		This study
	EPA Recommended Values (acute)	1.9	yes	Prothro, 1993



	Restronguet Creek, U.K. + Adriatic Sea	0.0025-0.03	yes	Barriada et al., 2007
	Ibaraki coast + Watarase river	0.014-0.03	yes	Shijo et al., 1989
		0.14-30.61		This study
	EPA Recommended Values (chronic)	7.9	yes	Prothro, 1993
	Suva, Fiji	150-250	no, low	Arikibe and Prasad, 2020
	Black Sea in Rize, Turkey	1-3	yes	Baltas et al., 2017
	Gulf of Chabahar, Oman Sea	0.15-0.19	yes	Bazzi, 2014
	Gulf of Kutch, Arabian Sea	200-1580	no, low	Chakraborty et al., 2014
	East London + Port Elizabeth harbours, U.K.	200-72600	no, low	Fatoki and Mathabatha, 2001
Cd	Yalujiang Estuary, China	0.83-1.33	yes	Li et al., 2017
	Gulf San Jorge, Argentina	0.01-0.09	yes	Muse et al., 1999
	Alang–Sosiya ship scrapping yard, Gulf of Cambay, India	34-560	yes	Reddy et al., 2005
	Kamal estuary, Jakarta	0.01-0.02	no, high	Putri et al., 2012
	Jakarta Bay	0.04-0.104	yes	Williams et al., 2000
	Kepez harbor of Canakkale, Turkey	19-73800	yes	Yilmaz and Sadikoglu, 2011
		0.1-14.0		This study
	EPA Recommended Values (chronic)	50	no, low	Prothro, 1993
	Gulf of Chabahar, Oman Sea	20.16-21.46	yes	Bazzi, 2014
	Gulf of Kutch, Arabian Sea	260-3010	no, low	Chakraborty et al., 2014
Cr	Yalujiang Estuary, China	0.113-0.14	yes	Li et al., 2017
	Gulf San Jorge, Argentina	0.04-0.5	yes	Muse et al., 1998
	Jakarta Bay	0.511-5.25	yes	Williams et al., 2000
	Alang–Sosiya ship scrapping yard, Gulf of Cambay, India	35-765	no, low	Reddy et al., 2005
		0.6-6.2		This study
	EPA Recommended Values (chronic)	3.1	yes	Prothro, 1993
	Suva, Fiji	880-10290	no, low	Arikibe and Prasad, 2020
	Black Sea in Rize, Turkey	30-242	no, low	Baltas et al., 2017
	Gulf of Chabahar, Oman Sea	3.37-5.74	yes	Bazzi, 2014
	Gulf of Kutch, Arabian Sea	1350-1850	no, low	Chakraborty et al., 2014
Cu	East London + Port Elizabeth harbours, U.K.	500-42600	no, low	Fatoki and Mathabatha, 2001
	Yalujiang Estuary, China	1.8-4.7	yes	Li et al., 2017
	Gulf San Jorge, Argentina	0.02-0.65	yes	Muse et al., 1998
	Jakarta Bay	0.405-4.04	yes	Williams et al., 2000
	Alang–Sosiya ship scrapping yard, Gulf of Cambay, India	32-3939	yes	Reddy et al., 2005
		0.00035-0.273		This study
	EPA Recommended Values (chronic)	0.94	yes	Prothro, 1993
	South Florida Estuaries	0.0034-0.0074	yes	Kannan et al., 1998
Hg	Guadalupe River and San Francisco Bay, California	0.0017-0.135	yes	Thomas et al., 2002
	Vembanad, India	0.0024-0.206	yes	Ramasamy et al., 2017
	Kamal estuary, Jakarta	0.1-0.2	yes	Putri et al., 2011
	Yalujiang Estuary, China	0.006-0.049	yes	Li et al., 2017
Mn		320-549		This study



	Black Sea in Rize, Turkey	3-14	yes	Baltas et al., 2017
	Gulf of Chabahar, Oman Sea	15.43-24.76	no, high	Bazzi, 2014
	Gulf of Kutch, Arabian Sea	13000-18000	no, low	Chakraborty et al., 2014
	East London + Port Elizabeth harbours, U.K.	300-23900	yes	Fatoki and Mathabatha, 2001
	Alang-Sosiya ship scrapping yard, Gulf of Cambay, India	31-4920	yes	Reddy et al., 2005
		2.3-24.3		This study
	EPA Recommended Values (chronic)	8.2	yes	Prothro, 1993
	Suva, Fiji	230-800	no, low	Arikibe and Prasad, 2020
	Black Sea in Rize, Turkey	0.006-0.036	yes	Baltas et al., 2017
Ni	Gulf of Chabahar, Oman Sea	16.42-17.14	yes	Bazzi, 2014
	Gulf of Kutch, Arabian Sea	190-330	no, low	Chakraborty et al., 2014
	Jakarta Bay	0.058-5.25	yes	Williams et al., 2000
	Alang-Sosiya ship scrapping yard, Gulf of Cambay, India	32-944	yes	Reddy et al., 2005
		0.11-28.35		This study
	EPA Recommended Values (chronic)	5.6	yes	Prothro, 1993
	Suva, Fiji	880-1770	no, low	Arikibe and Prasad, 2020
	Black Sea in Rize, Turkey	6-130	yes	Baltas et al., 2017
	Gulf of Chabahar, Oman Sea	4.24-4.25	yes	Bazzi, 2014
	Gulf of Kutch, Arabian Sea	20-120	yes	Chakraborty et al., 2014
	East London + Port Elizabeth harbours, U.K.	600-16300	no, low	Fatoki and Mathabatha, 2001
Pb	Yalujiang Estuary, China	0.4-1.8	yes	Li et al., 2017
	Gulf San Jorge, Argentina	0.1-0.5	yes	Muse et al., 1998
	Alang-Sosiya ship scrapping yard, Gulf of Cambay, India	30-2036	yes	Reddy et al., 2005
	Kamal estuary, Jakarta	1.3-4	yes	Putri et al., 2011
	Jakarta Bay	0.485-3.62	yes	Williams et al., 2000
	Kepez harbor of Canakkale, Turkey	49-9390	yes	Yilmaz and Sadikoglu, 2011
		0.86-3.95		This study
Sn	estuarine seawater, Galicia Coast, Spain	0.53-1.23	yes	Bermejo-Barrera et al., 1999
	U.S. and European rivers	0.0001-0.1	yes	Byrd and Andreae, 1982
		30.0-226.9		This study
	EPA Recommended Values (chronic)	81	yes	Prothro, 1993
	Suva, Fiji	80-1450	yes	Arikibe and Prasad, 2020
	Black Sea in Rize, Turkey	38-178	yes	Baltas et al., 2017
	Gulf of Chabahar, Oman Sea	18.01-22.62	yes	Bazzi, 2014
	Gulf of Kutch, Arabian Sea	11000-31000	no, low	Chakraborty et al., 2014
Zn	East London + Port Elizabeth harbours, U.K.	500-27600	yes	Fatoki and Mathabatha, 2001
	Yalujiang Estuary, China	9.2-19.6	yes	Li et al., 2017
	Gulf San Jorge, Argentina	0.01-0.55	no, high	Muse et al., 1998
	Jakarta Bay	2-30.1	yes	Williams et al., 2000
	Alang-Sosiya ship scrapping yard, Gulf of Cambay, India	33-5832	yes	Reddy et al., 2005



755 During the past years, many studies were performed to assess the pollution level of seawater. The range of heavy
756 metal concentrations in the culturing medium of this study are compared to the metal concentrations in polluted
757 environments measured over the past 40 years in different regions all over the world (Table 5). The data
758 demonstrate that the concentration range from our study is in good agreement with threatened environments in
759 San Francisco Bay, California (Thomas et al., 2002), the Black Sea, Turkey (Baltas et al., 2017), the Gulf of
760 Chabahar, Oman Sea (Bazzi, 2014), the Restronguet Creek, U.K., the Adriatic Sea (Ag; Barriada et al., 2007), the
761 Yalujiang Estuary, China (Li et al., 2017), the Gulf of San Jorge, Argentina (Muse et al., 1999), Vembanad and
762 the Gulf of Cambay, India (Ramasamy et al., 2017; Reddy et al., 2005), Kepez harbor of Canakkale, Turkey
763 (Yılmaz and Sadikoglu, 2011), Jakarta (Williams et al., 2000; Putri et al., 2012) and with polluted U.S. and
764 European rivers (Byrd and Andreae, 1982; Kannan et al., 1998; Thomas et al., 2002). Furthermore, the maximum
765 metal concentration as recommended by the EPA is the lower boundary of the concentration range from this study
766 (Prothro, 1993). A lower concentration than the EPA value is also covered by our study during the control phase
767 or in the control system. This enables us to assess metal levels at the very beginning of the harmful pollution phase
768 in environments, which could be used as an early-warning system for the ecological status of an area (Sagar et al.,
769 2021). Furthermore, it allows to assess the effectiveness of contamination reducing measures. This advantage will
770 be important in the future for the possibility to intervene or to apply more promising measures within an adequate
771 time frame. In some regions of the world, seawater heavy metal concentrations are higher than in this study.
772 Examples are Suva, Fiji (Arikibe and Prasad, 2020), the Gulf of Kutch, Arabian Sea (Chakraborty et al., 2014) or
773 the East London and Port Elizabeth harbours, U.K. (Fatoki and Mathabatha, 2001) (Table 5). These areas seem to
774 be extremely polluted, which would make it necessary to apply a higher metal concentration to the cultured
775 foraminifera if a reconstruction covering these values should be made. However, this study clearly indicates a
776 reduced uptake of metals of interest, when the concentration of these metals in the seawater is exceeding a certain
777 threshold value (here between phase 2 and 3). This will make it generally difficult to model extreme high pollution
778 levels. Indeed, it is possible to distinguish between a heavy and a moderate pollution level. Overall, the
779 concentration of heavy metals in seawater should be decreasing all over the world due to a rigorous legislation for
780 reduction of the heavy metal input into the environment, and due to various emission reducing measures that are
781 applied already. This means that the concentration range of metals covered by this study is adequate for future
782 research and monitoring of polluted systems.

783

784 **5 Conclusion**

785 The aim of this study was to assess the incorporation of heavy metals into the foraminiferal calcite as a function
786 of their concentration in the seawater the foraminifera calcified in. Culturing experiments with different
787 foraminiferal species (*A. aomoriensis*, *A. batava* and *E. excavatum*) that were exposed to a mixture of ten different
788 metals (Cr, Mn, Ni, Cu, Zn, Ag, Cd, Sn, Hg and Pb) at varying concentrations (Table 3, Fig. 3, Fig. B1) were
789 carried out to gain further insights into the uptake of heavy metals. Laser ablation ICP-MS analysis of the newly
790 formed calcite revealed species-specific differences in the incorporation of heavy metals. Nevertheless, all metals
791 used in this study were incorporated into the foraminiferal calcite of all three species (Fig. 4, Table 4). Some
792 elements showed a behaviour inferring that the uptake of these metals mainly depends on its concentration in
793 seawater, which was indicated by strong positive correlations between the metal concentration in seawater and in
794 the foraminiferal calcite. All three species showed a strong positive correlation between Pb and Ag in the water



795 and their calcite. *A. aomoriensis* further revealed a slightly weaker correlation for Mn and Cu. *A. batava* holds a
 796 strong positive correlation for Mn and Hg. *E. excavatum* depicts a strong positive correlation for Cr and Ni. Other
 797 elements like Cd and Zn showed a more complicated behaviour indicating that factors other than seawater
 798 concentration are effecting the uptake of these metals, which is demonstrated by no clear correlation between
 799 seawater and calcite metal values. The reasons for this different behaviour are yet unclear. D_{TE} values of Ni, Zn,
 800 Cd, Hg and Pb decrease with increasing metal concentration in the seawater, which is most prominent in the
 801 highest metal treatment in all species. This could be due to an overload effect arising when the concentration of
 802 the metals is exceeding a certain threshold and could potentially be harmful or even lethal for the organism. This
 803 in turn could lead to a removal or a prevention from uptake of the metal (Nardelli et al., 2016). The results of this
 804 study facilitate a reconstruction of the heavy metal concentration in seawater for those elements showing a
 805 correlation between TE/Ca ratios in calcite and seawater. Such estimates can be based on foraminiferal samples
 806 from sediment cores and recent surface sediments, and facilitate a monitoring of anthropogenic footprints on the
 807 environment today and in the past. The presented D_{TE} 's allow a direct quantification of metal concentrations in
 808 polluted and pristine areas. The foraminiferal species considered prevail in nearly all coastal environments
 809 worldwide, except polar latitudes. This in combination provides a powerful tool for monitoring the ecosystem
 810 status in various areas of interest.

811

812 Appendix

813 Appendix A: Additional Tables

814

815 **Table A1:** TE/Ca_{seawater} values from single weeks during the culturing period of the metal system. Measurements
 816 were carried out with ICP-MS. This values are the basis for the calculations of the mean TE/Ca values in Table 3
 817 and for figure B1.

Metal System	Sampling date		Cr/Ca	Mn/Ca	Ni/Ca	Cu/Ca	Zn/Ca	Ag/Ca	Cd/Ca	Sn/Ca	Hg/Ca	Pb/Ca	
	Phase	Day	$\mu\text{mol mol}^{-1}$	nmol mol ⁻¹	$\mu\text{mol mol}^{-1}$								
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

818



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

Reference Materials	Cr	Mn	Ni	Cu	Zn	Cd	Pb
SLRS-6	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	

825

826 **Table A3:** Average concentration, RSD (1 σ in %), literature values, accuracy in comparison to literature values
 827 and number of measurements of the reference materials NIST SRM 610, NIST SRM 614, JCI-1, JCP-1, MACS-3
 828 and ECRM752-1 measured with LA-ICP-MS. Please note that for the ECRM752-1 no reported values for the
 829 elements of interest are available, which is also the case for some elements in other reference materials. It is
 830 important to note that the Hg/Ca values in the NIST glasses are not reliable as Hg is volatile and most likely
 831 volatilized during the glass formation. Average concentration, RSD and accuracy values displayed here are
 832 averaged from single measuring days.

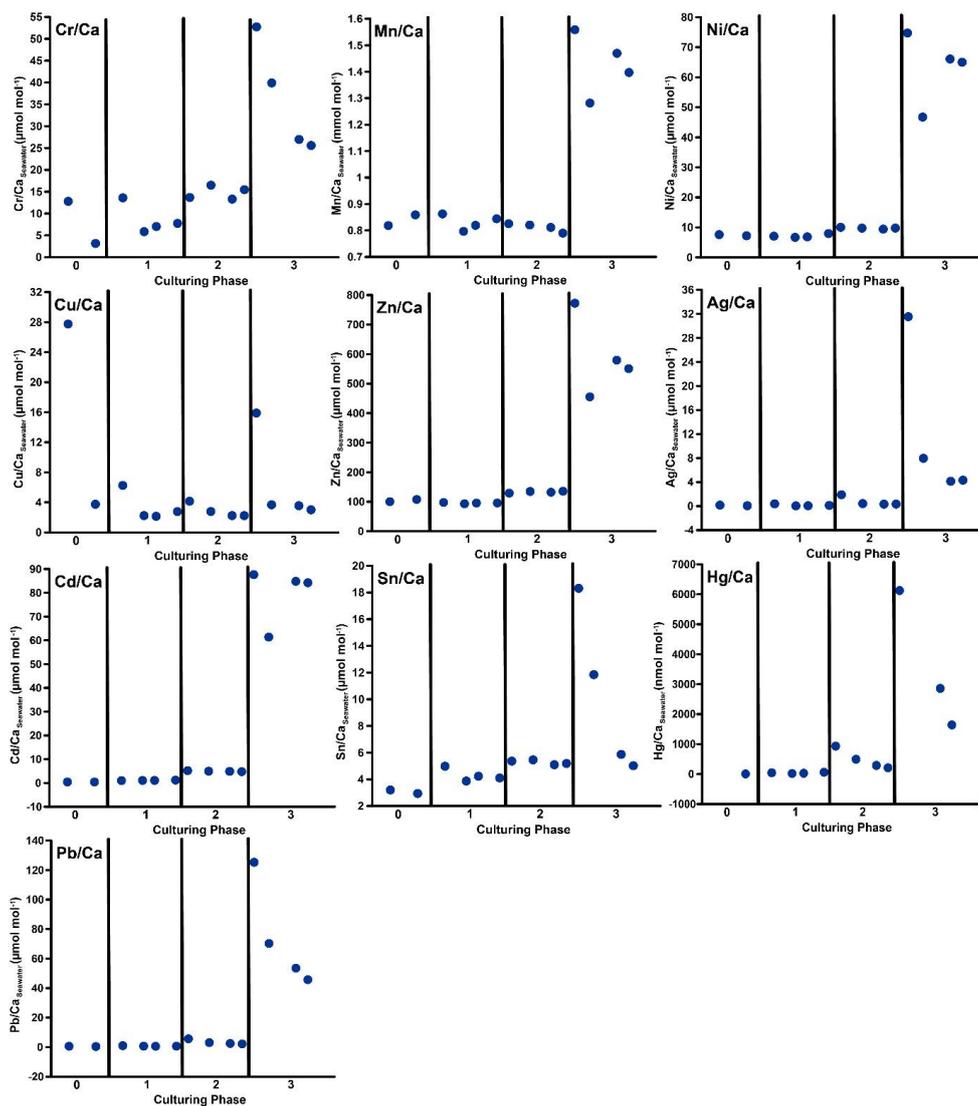


Reference materials	Cr/Ca	Mn/Ca	Ni/Ca	Cu/Ca	Zn/Ca	Ag/Ca	Cd/Ca	Sn/Ca	Hg/Ca	Pb/Ca
NIST SRM 612	mmol mol ⁻¹	μmol mol ⁻¹	mmol mol ⁻¹							
Mean value	0.33	0.34	0.33	0.29	0.37	0.08	0.12	0.15	2.26	0.10
RSD%	3.49	2.09	2.30	4.05	2.97	5.01	3.90	2.08	26.32	2.52
Jochum et al., 2011	0.33	0.33	0.31	0.28	0.28	0.10	0.12	0.15		0.09
Number of spots	69	75	75	75	73	73	75	75	41	75
NIST SRM 614	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	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	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	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	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

833

834 **Appendix B: Additional Figures**

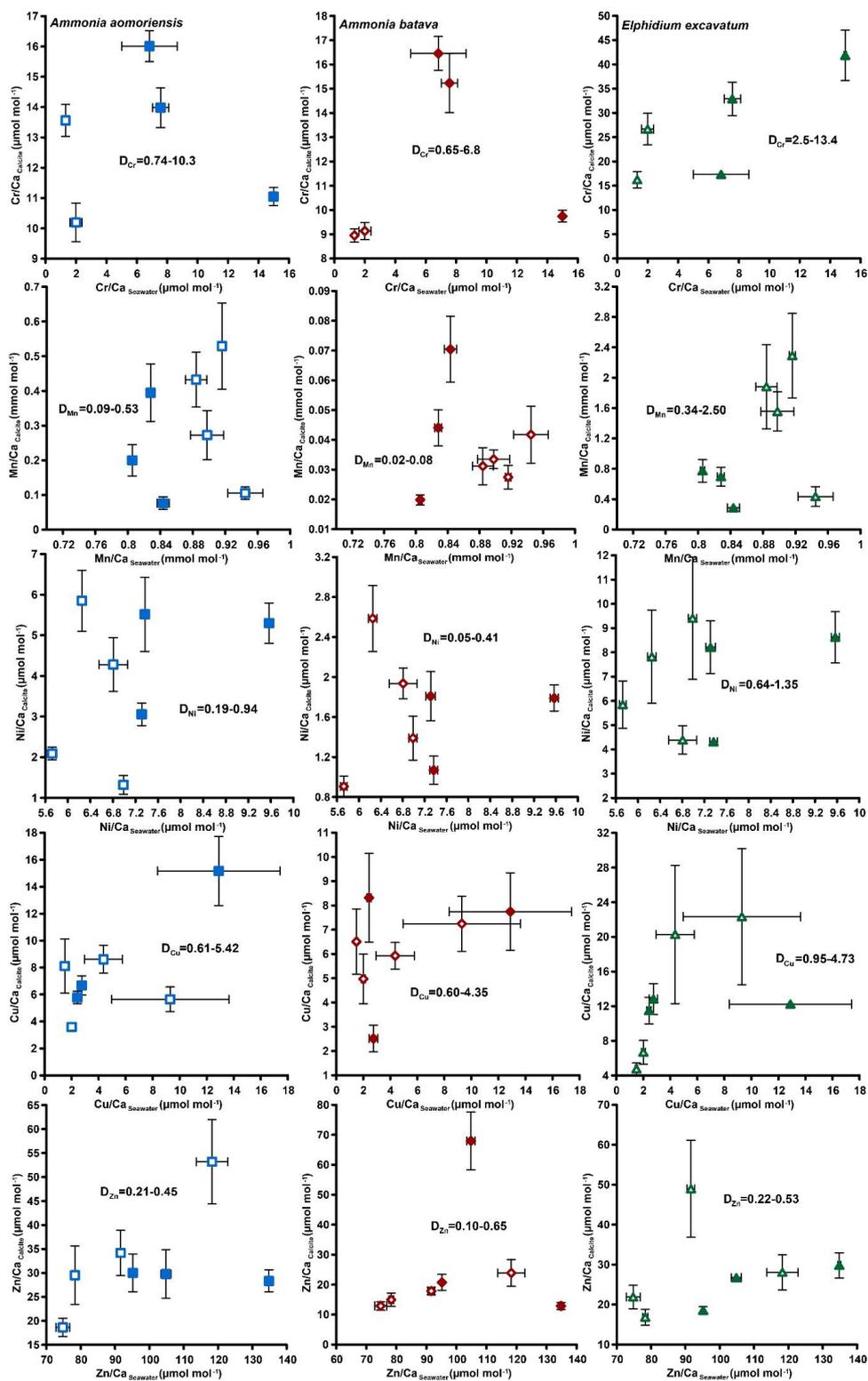
835

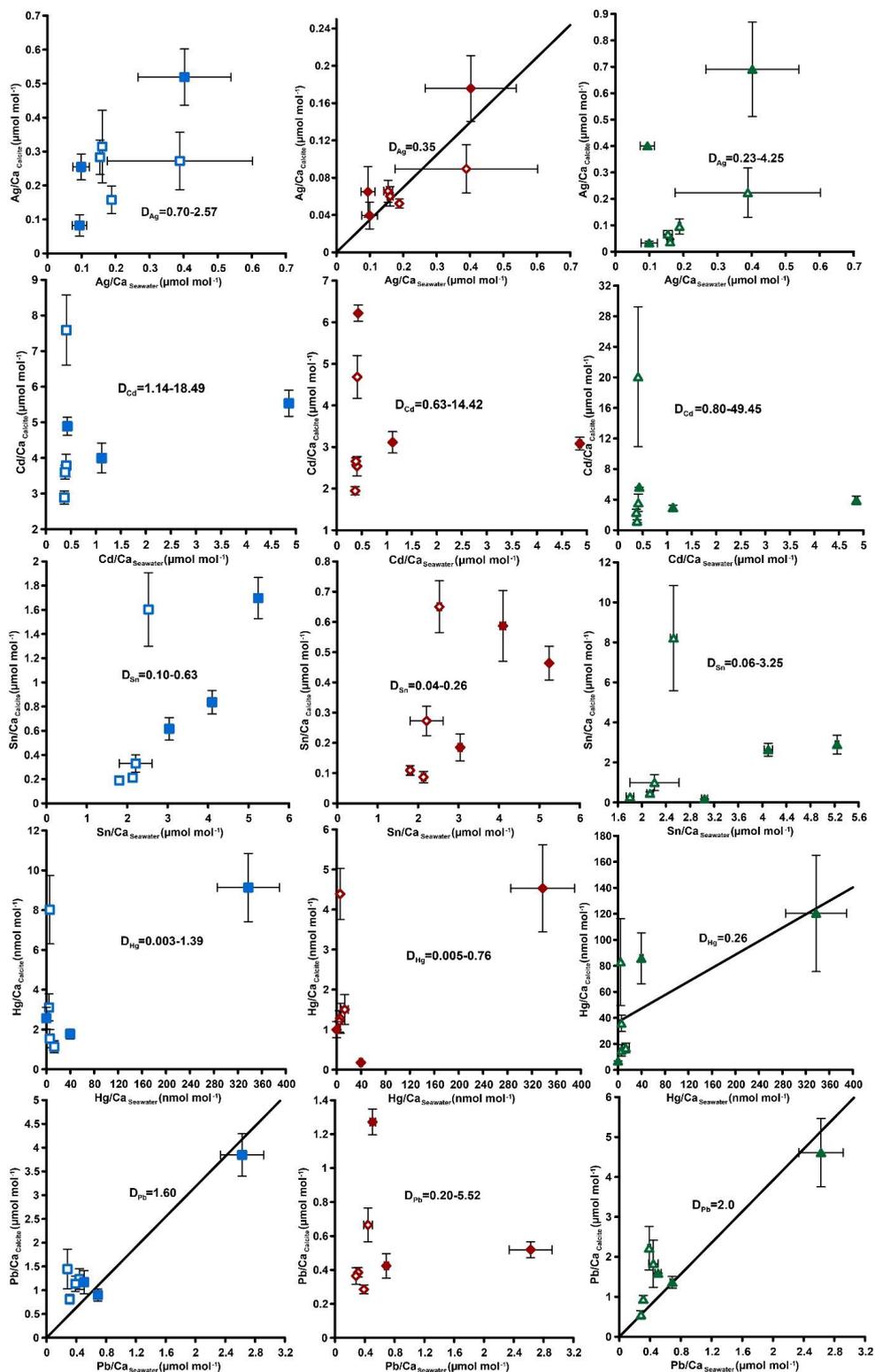


836

837 **Figure B1:** TE/Ca values in the culturing medium of the metal system in $\mu\text{mol mol}^{-1}$ or nmol mol^{-1} divided by
838 individual culturing phases. In this system, phase 0 is the control phase without any extra added metals and for
839 phase 1 to 3, the heavy metal concentration in the culturing medium was elevated. The data the figure is based on
840 can be found in Table A1.

841







844



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

853 Supplements

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

862 Data availability

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

865 Author contribution

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

872 Competing interests

873 The authors declare that they have no conflict of interest.

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878 Gosnell performed the Hg measurements in the water samples and Ulrike Westernströer set up and helped with
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883

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