Impact of seawater Mg^{2+}/Ca^{2+} on Mg/Ca of asterozoan skeleton – evidence from culturing and the fossil record

Dorota Kołbuk^a, Philippe Dubois^b, Jarosław Stolarski^a, Przemysław Gorzelak^{*a}

a - Institute of Paleobiology, Polish Academy of Sciences, Twarda 51/55, PL-00-818 Warsaw, Poland (*pgorzelak@twarda.pan.pl)

b - Université Libre de Bruxelles, Faculté des Sciences, Laboratoire de Biologie Marine, CP 160/15, av. F.D. Roosevelt, 50 B-1050 Bruxelles, Belgium

Abstract

Skeletal Mg/Ca in echinoderms is thought to be related to ambient seawater Mg^{2+}/Ca^{2+} ratio, which prompts the use of well-preserved fossil echinoderms as paleoseawater Mg^{2+}/Ca^{2+} proxies. Nonetheless, experimental studies testing the effect of seawater Mg^{2+}/Ca^{2+} ratio on echinoderm skeleton are limited to one echinoderm class, i.e., sea urchins (echinoids). Here, we investigated the effect of decreased seawater Mg^{2+}/Ca^{2+} ratio on skeletal composition (Mg/Ca ratio) of a sea star *Asterias rubens* and a brittle star *Ophiocomina nigra*. Specimens were tagged with manganese and then cultured under three different Mg^{2+}/Ca^{2+} molar ratios (~5.2, ~2.5, ~1.5 mol/mol) in order to simulate variations of ambient seawater Mg^{2+}/Ca^{2+} ratio that existed throughout the Phanerozoic. Decreased Mg^{2+}/Ca^{2+} in seawater did not affect the respiration rates or inhibit calcification of sea stars, which suggests that they were not significantly stressed by the treatment. It did, however, clearly affect growth and mortality in brittle stars, for which reliable geochemical data could not be obtained. Under decreased seawater Mg^{2+}/Ca^{2+} sea stars produced a skeleton with decreased Mg/Ca ratio. This is consistent with the hypothesis that the skeletal chemistry in echinoderms is influenced by seawater chemistry and, consequently, that fossil echinoderms may preserve a

record of paleoseawater $\text{Mg}^{2+}/\text{Ca}^{2+}$. Although our data from well-preserved fossil sea stars from the Middle Jurassic and Miocene are consistent with low $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio of the Jurassic „calcite sea” and high $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio of the Miocene „aragonite sea”, the accuracy of paleoseawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ reconstructions from fossil echinoderms is limited by the fact that Mg partition coefficients vary significantly between different echinoderm species.

Keywords: echinoderms, biomineralization, magnesium, culturing, seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$

1. Introduction

To date, studies investigating the effect of seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ on the skeletal Mg/Ca in echinoderms focused on sea urchins. It has been experimentally shown that under lowered $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio certain species of echinoids (including cidaroids: *Eucidaris tribuloides*, *Prionocidaris baculosa*, and euechinoids: *Paracentrotus lividus*, *Psammechinus miliaris*) produce skeleton with decreased Mg/Ca ratio (Ries, 2004; Kołbuk et al., 2019, 2020). Accordingly, it has been suggested that Mg/Ca ratio in well-preserved fossil echinoderms constitutes a reliable proxy for the seawater composition ($\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio) in the Phanerozoic era (Dickson, 2002, 2004; Ries, 2004; Hasiuk and Lohmann, 2010). However, experimental studies on some sea urchins showed that magnesium in their skeletons can also originate from diet (Asnaghi et al., 2014; Kołbuk et al., 2019, 2020), imposing constraints on the application of fossil echinoderms as a reliable paleoseawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ proxy. Furthermore, the skeletal Mg/Ca ratio in echinoderms can be also affected by temperature, salinity (Chave, 1954; Weber, 1969, 1973; Borremans et al., 2009; Hermans et al., 2010) and physiological (vital) effects (Hermans et al., 2011).

46 Asterozoans, i.e., a clade comprising asteroids (sea stars or starfish) and ophiuroids (brittle
47 stars), have been more extensively studied in the context of their remarkable regeneration
48 abilities (e.g., Dubois and Jangoux, 1990; Sköld and Rosenberg, 1996; Ben Khadra et al.,
49 2015; 2018 and literature cited therein; Czarkwiani et al., 2016) rather than their skeletal
50 chemistry. Some notable exceptions include field-collected specimens studied by Clarke and
51 Wheeler (1922), who examined MgCO_3 content in 29 asteroid and 16 ophiuroid species from
52 different latitudes. These authors observed a correlation between seawater temperature
53 and skeletal magnesium content in their skeletons. Chave (1954) examined Mg
54 concentration in a few more asterozoan specimens (9 asteroids and 6 ophiuroids from
55 different or unidentified species) from various locations and confirmed a temperature–
56 magnesium level dependency in their skeletons. Weber (1969, 1973) further explored the
57 effect of environmental control (temperature) and physiological factors on skeletal
58 composition of echinoderms on different levels: taxonomic (from class to species), within
59 and between populations, and within single individuals. These studies showed that,
60 compared to echinoids, within-population and within-individual variations in asterozoans are
61 small or insignificant. However, higher taxonomic levels, including genera and orders, show
62 considerable variation with respect to skeletal magnesium concentration. In recent years, a
63 few field studies enabled to better document the chemical composition of asterozoans from
64 various locations (e.g., McClintock et al., 2011; Lebrato et al., 2016; Iglukowska et al., 2017;
65 Duquette et al., 2018), which showed that even under similar environmental conditions,
66 skeletal magnesium concentration can vary strongly between species. Overall, these findings
67 suggest that echinoderms can, at least to some degree, exert control over Mg incorporation.
68 Surprisingly few experimental studies analysed skeletal chemistry and growth dynamics of
69 asterozoans under varying environmental conditions other than ocean acidification (the

effect of the ocean acidification was tested e.g., by Gooding et al., 2009 and Wood et al., 2010). For instance, Borremans et al. (2009) detected a salinity effect on skeletal Mg/Ca and Sr/Ca ratios in the sea star *Asterias rubens*. Donachy and Watabe (1986) found that decreasing calcium concentration and salinity reduced regeneration rate in brittle star *Ophiotrix angulata*.

The effect of lowered ambient Mg^{2+}/Ca^{2+} ratio on skeletal chemistry (Mg/Ca ratio) has not been tested so far on the asterozoan skeleton. Thus, the main goal of this study was to investigate the effect of decreased seawater Mg^{2+}/Ca^{2+} ratio on the chemical composition (Mg/Ca ratio) of starfish *Asterias rubens* and brittle star *Ophiocomina nigra*. Furthermore, skeletal Mg/Ca ratios obtained from both extant and well-preserved fossil asteroids were compared to existing data on echinoids in order to determine whether phylogenetically distant echinoderm species should be treated as equally reliable proxies of ancient seawater Mg^{2+}/Ca^{2+} ratio.

2. Methods

2.1. Collection and handling of animals

Adult sea stars *Asterias rubens* (mean arm length: 41.9 mm $\pm$ 5 SD) (Figure 1A) and brittle stars *Ophiocomina nigra* (mean disc diameter: 13.3 mm $\pm$ 0.9 SD) (Figure 2A) were collected from the North Sea (Belgian coast) and obtained from Roscoff Marine Station (France), respectively, and transported to Laboratoire de Biologie Marine (Université Libre de Bruxelles, Belgium).

Firstly, the specimens were labeled with manganese. Despite being widely used in biomineralization studies (e.g., Hawkes et al., 1996; Barbin et al., 2008; Lartaud et al., 2010), manganese only recently gained attention as a reliable labeling tool for echinoderms

(Gorzalak et al., 2017; Kołbuk et al., 2020). Nonetheless, its efficiency in tagging asterozoan skeletons has not yet been determined. A low concentration of manganese (3 mg/L) was used so as not to disturb the motoric behaviour (which was previously observed at a much higher Mn concentration, i.e., 12 mg/L; Sköld et al., 2015). The solution was prepared by mixing seawater with dissolved $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (Sigma-Aldrich). Specimens of both species were cultured in the marking solution in separate 1 L beakers for the next 24 h.

2.2. Experimental setup

After the labeling, 18 specimens of each species were randomly allocated to separate 1 L beakers with three different $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratios [mol/mol]: ~ 5.2 , ~ 2.5 , and ~ 1.5 (6 specimens of each species per treatment), with molar sums of Mg^{2+} and Ca^{2+} held constant. Both Mg^{2+} and Ca^{2+} concentrations are considered to have concomitantly changed during the Phanerozoic (e.g., Wilkinson and Algeo, 1989), therefore decreased $\text{Mg}^{2+}/\text{Ca}^{2+}$ molar ratios were obtained through lowering Mg^{2+} and increasing Ca^{2+} concentration (see Higuchi et al., 2017). Natural seawater from the English Channel was mixed with artificial Ca^{2+} -enriched seawater without Mg^{2+} (prepared by dissolving NaCl, Na_2SO_4 , CaCl_2 , KCl, KBr, SrCl_2 , NaF, H_3BO_3 , and NaHCO_3 (Sigma-Aldrich) in ultrapure water). Individuals were kept under experimental conditions for 21 days, except for 6 brittle stars cultured under the lowest $\text{Mg}^{2+}/\text{Ca}^{2+}$, which died during the first week of the experiment.

Specimens (18 sea stars and 12 brittle stars) were kept under constant temperature ($\sim 17.8^\circ\text{C}$), salinity (~ 32.9 psu), pH (~ 8), and $\text{Mg}^{2+}/\text{Ca}^{2+}$ molar ratios (~ 5.2 , ~ 2.5 , ~ 1.5 [mol/mol]; Table 1) in separate 1L aerated beakers. Seawater parameters (T, salinity, pH) were checked three times a day with a WTW Multi 340i multimeter, equipped with a conductivity cell and an integrated temperature sensor, and a Metrohm pH-meter (826 pH mobile) equipped with

a combined glass electrode Metrohm 6.0228.010. The pH electrode was calibrated every day with Merck CertiPUR buffer solutions pH 4.00 and 7.00. All pH measurements were converted to total scale using standard buffers TRIS/AMP method (Del Valls and Dickson, 1998). Seawater in each beaker was renewed every day. Seawater samples (50 ml from each beaker; filtered through 0.45 μm membrane filters and stabilized with nitric acid) were collected every day and Mg^{2+} and Ca^{2+} concentrations in each sample were analysed with inductively coupled plasma-optical emission spectrometry (Thermo Scientific iCAP 6500 Duo ICP-OES spectrometer; detection capability <1 ppb) at the Central Chemical Laboratory of the Polish Geological Institute– National Research Institute (Warsaw). Stability of parameters (temperature, salinity, pH, and three levels of $\text{Mg}^{2+}/\text{Ca}^{2+}$) was additionally verified with one-way ANOVA and post-hoc Tukey's HSD tests (Table 1).

2.3. Respiration rate

Brittle stars appeared to be more stress-sensitive than sea stars. Thus, the measurements of respiration rate were not attempted on *Ophiocomina nigra* due to high mortality observed during culturing under the lowest $\text{Mg}^{2+}/\text{Ca}^{2+}$ treatment. Respiration measurements were carried out on 7 random individuals of *Asterias rubens* after 7, 14 and 21 days of exposure in order to examine their metabolic response to potentially stressful conditions. Sea stars were placed in Plexiglas respiratory chambers (diameter: 5.5 cm) with an optode oxygen sensor (PreSens, Regensburg, Germany), which were filled with seawater from their respective beaker. Chambers were placed on magnetic stirring units in order to homogenize the oxygen content. Oxygen saturation was measured every five minutes for one hour with a Fibox 3 PC-controlled fiber-optic oxygen meter and registered via Fibox 3 software v602 (PreSens). After the measurements, the animals were wet weighted. The oxygen uptake rate was calculated using the slope of the linear regression of seawater oxygen concentration over time.

Calculated value was corrected by seawater volume (based on the seawater density calculations by Millero and Poisson, 1981; Millero and Huang, 2009) and divided by the wet weight of the animal. The respiration rate was expressed in μmol of O_2 per hour and grams of wet weight.

2.4. Termination of the experiment, geochemical analysis, data handling

After 21 days of experiment, specimens were dissected and dried at 50°C for 48 h. Arms of *A. rubens* and *O. nigra* were selected and fixed and dehydrated in graded ethanol series (70% for 3 hours, 90% for 1 hour and 100% for 1 hour). Standard thin sections from the biological material embedded in epoxy resin were prepared through a series of diamond suspensions and carbon-coated.

Microscopic observations were performed at the Institute of Paleobiology of the Polish Academy of Sciences in Warsaw. To detect the presence of manganese labeling, thin sections were observed under Lumic HC5-LM cathodoluminescence (CL) microscope (hot cathode; electron energy 14 keV, beam currents 0.1-0.15 mA, exposure time 5-8 s).

Spot geochemical analyses on newly grown parts of the skeleton in each individual were conducted using CAMECA SX100 electron microprobe at the Micro-Area Analysis Laboratory, Polish Geological Institute – National Research Institute in Warsaw (accelerating voltage 15 kV, beam current 5 nA for calcium, 20 nA for magnesium, beam diameter $\sim 5 \mu\text{m}$, standard used: „NIST”, serial number: 12570. Mean Mg/Ca ratios in the newly grown stereom for sea stars were determined from spot measurements in proximal adambulacra, and the most distal parts of the arms (Figure 1). Selected thin sections were additionally imaged with CAMECA SX100 electron microprobe in order to obtain WDS maps of Mg distribution. Few spot analyses were also performed on the stereom grown before the experiment (the

163 manganese labeling demarcated the pre-experimental growth and the growth during
164 culturing). Brittle stars, cultured under control (~ 5.2 mol/mol) and decreased (~ 2.5 mol/mol)
165 $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratios, were examined for Mg/Ca ratio through spot measurements in the most
166 distal arm vertebrae.

167 Skeletal Mg/Ca ratio in *Asterias rubens* ($n = 14$) was analysed through one-way ANOVA with
168 seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio level as a fixed factor. Remaining sea stars ($n = 4$), which displayed
169 no post-tagging growth, were not considered in the statistical analysis. Due to limited
170 growth (Figure 2) and therefore lack of reliable data, brittle stars ($n = 12$) were also not
171 analysed statistically. Respiration rate in *A. rubens* as a function of time was analysed with
172 repeated measures nested ANOVA (specimen, random factor, nested into $\text{Mg}^{2+}/\text{Ca}^{2+}$, fixed
173 factor, and time, repeated factor). Analyses were performed in the Statistica software, with
174 significance level set to 0.05.

175 In order to estimate the reconstruction error, we compared the actual seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$
176 ratios used in the experiment with seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ values calculated with an algorithm
177 based on echinoderm skeletal Mg/Ca ratio. To do so, we inserted the experimentally
178 obtained values of skeletal Mg/Ca ratios in *Asterias rubens* from each treatment to the
179 following equation by Ries (2004): $\text{Mg}/\text{Ca}_c = S(0.000719T + 0.0292)\text{Mg}/\text{Ca}_{\text{SW}}^{0.668}$, where
180 SW—seawater, C—calcite (skeleton), T—temperature, S—species coefficient (*sensu* Ries,
181 2004, i.e. actual skeletal Mg/Ca of this species living in the wild divided by the theoretical
182 Mg/Ca at selected temperature and seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio; species coefficient calculated
183 herein for *A. rubens* $S = 0.994$). It should be noted, however, that Ries' equation is typically
184 applied to echinoid test plates. Calculations performed on asteroid-derived material should
185 be therefore treated with caution.

2.5. Fossil specimens

Several well-preserved fossil asteroids were sampled in order to reconstruct paleoseawater Mg^{2+}/Ca^{2+} ratio based on the Ries' algorithm which utilizes skeletal Mg/Ca ratios (see paragraph above) and our equation determined in this study ($Mg/Ca_c = 0.0207 Mg/Ca_{sw} + 0.0191$, see below). The Middle Jurassic (Callovian; Šaltiškiai, Lithuania) ossicles ascribed to the family Astropectinidae (from the "calcite sea"; i.e., $Mg^{2+}/Ca^{2+}_{sw} \sim 1.5$) were provided by Faculty of Natural Sciences, University of Silesia in Katowice. They were collected from fine-grained grey sandy clays with rich benthic fauna belonging to the Papartine formation (Salamon 2008). Sedimentology and fossil assemblages of this formation indicate a shallow sea depositional environment, with no evidence of anoxic conditions in the water column (Marynowski and Zatoń, 2010). The Miocene (Badenian; Korytnica, Poland) ossicles belonging to the order Paxillosida ($Mg^{2+}/Ca^{2+}_{sw} \sim 3.5$; aragonite sea conditions) were provided by the Faculty of Geology, University of Warsaw. They were collected from the so-called Korytnica clays. These sediments were formed in a shallow marine bay, semi-isolated from the open sea (northernmost part of the Paratethys), somewhat similar to calm lagoons in Recent coral-reef atolls (Zagorsek et al., 2012).

The ossicles were slightly etched in formic acid and observed under Philips XL-20 Scanning Electron Microscope (Institute of Paleobiology of the Polish Academy of Sciences, Warsaw) to determine the presence of layered stereom, which is indicative of low degree of diagenetic structural alteration (Gorzelać et al., 2016). Additional examination under cathodoluminescence (CL) microscope revealed lack or dull luminescence in the areas selected for geochemical analysis, further confirming a good preservation of stereom (Figure 3). Furthermore, CAMECA SX100 electron microprobe analyses revealed no

detectable Mn or other diagenetic elements (e.g., Fe) and no diagenetic depletion of trace elements (Sr and S) in these ossicles (*cf.* Brand and Morrison, 1987). The measured Mg/Ca ratios in these ossicles were thus used for the reconstruction of paleoseawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratios using three algorithms: (a) partition coefficient: $\text{Mg}/\text{Ca}_c = 0.03182(\text{Mg}/\text{Ca}_{\text{SW}})$ (Dickson, 2004), (b) equation determined in this study ($\text{Mg}/\text{Ca}_c = 0.0207 \text{Mg}/\text{Ca}_{\text{SW}} + 0.0191$, see below) and c) species-normalized Mg partition algorithm as a function of seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio and temperature: $\text{Mg}/\text{Ca}_c = S(0.000719T + 0.0292)\text{Mg}/\text{Ca}_{\text{SW}}^{0.668}$ (Ries, 2004; where SW—seawater, C—skeletal calcite, T—temperature, S—species coefficient). For the latter equation, temperature data were taken from Brand (1986), and Scheiner et al. (2018) for the Callovian and Miocene respectively; species coefficients (*sensu* Ries, 2004) were calculated from the literature data on the skeletal Mg/Ca ratios (converted from $\%\text{MgCO}_3$) of extant relatives of the fossil taxa (Chave, 1954; Weber, 1969).

3. Results

3.1. Skeletal Mg/Ca and growth in *Ophiocomina nigra*

Mortality occurred in the first days of the experiment in all six specimens of *Ophiocomina nigra* cultured under the lowest $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio (~ 1.5 mol/mol). Additionally, some of the other specimens kept under lowered $\text{Mg}^{2+}/\text{Ca}^{2+}$ (~ 2.5 mol/mol) displayed signs of disc perforation (Figure 2B) and/or autotomy, which clearly indicated that they were stressed by the treatment (Dobson et al., 1991). Determination of new growth zones under CL was difficult in most cases due to uneven distribution of the CL-activated markers in the stereom (Figure 2C). The bright orange-red luminescence zones were often dispersed in the entire skeleton.

In two specimens, however, cultured under lowered $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio (~ 2.5 mol/mol) newly grown zones could be identified through abrupt decrease in Mg/Ca ratio (~ 0.08 mol/mol) observable in the most distal parts of the arms. Specimens grown under control $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio (~ 5.2 mol/mol) typically displayed higher skeletal Mg/Ca ratios (0.13-0.14 mol/mol; Table S1 in supplementary files).

However, the scarcity of geochemical data obtained from the specimens cultured under decreased $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio did not allow a reliable statistical assessment of the differences between treatments.

3.2. Skeletal Mg/Ca and growth in *Asterias rubens*

No mortality occurred in *Asterias rubens*. Mn-labeling and post-tagging growth fronts were detected in most of the investigated thin sections, which indicates that the sea stars grew during the experiment, although different orientation of the cut surface or uneven growth in the adjacent ossicles often did not allow the quantitative estimation of the actual growth rates. Geochemical analyses performed on the newly grown skeleton show that sea stars cultured under lowered seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio display lower skeletal Mg/Ca ratios compared with the control specimens ($p_{\text{ANOVA}} \ll 10^{-6}$; Figure 4).

3.3. Respiration rate in *Asterias rubens*

Values measured from starfish specimens are presented in the Table 2. No significant effect of seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio on respiration rate was found ($p_{\text{ANOVA}} = 0.89$). Time of exposure also had no significant effect on the respiration of individuals ($p_{\text{ANOVA}} = 0.86$).

3.4. Reconstruction error

For the experimental starfish, specimens cultured under control (~ 5.2 mol/mol) and lowered to ~ 2.5 (mol/mol) $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratios, the ambient seawater reconstruction error (calculated

from the algorithm involving skeletal Mg/Ca ratio, temperature and species coefficient; Ries, 2004) was very low (less than 2%). For the asteroids cultured under the lowest seawater Mg^{2+}/Ca^{2+} ratio (~1.5 mol/mol), the discrepancy between the ambient and calculated theoretical values was higher, leading to a 39.7% error in the reconstruction (Table 3).

3.5. Fossil specimens

Jurassic specimens are easily distinguishable from the Miocene ones due to their much lower Mg/Ca ratios (Table 4). Reconstructed Jurassic seawater Mg^{2+}/Ca^{2+} ratios range from 1.2 to 1.82 mol/mol depending on the algorithm and specimen, whereas the Miocene Mg^{2+}/Ca^{2+} ratios tend to be much higher (up to 6.48 mol/mol).

4. Discussion

Calcium carbonate polymorphs rich in magnesium dissolve faster in warmer and more acidic seawater, which suggests that magnesium can influence the dissolution rate of the skeleton post mortem in some benthic calcifiers (e.g., Ries et al., 2016). In echinoderms, magnesium participates in various processes in the cell, including skeletogenesis. For instance, it has been suggested that magnesium ions play a significant role in stabilization of amorphous calcium carbonate (ACC), the precursor to skeletal calcium carbonate (Addadi et al., 2003). Decreased magnesium concentration in sea water also affects the expression of skeletogenic genes (Martino et al., 2019) and magnesium concentration in the sea urchin tooth skeleton has an effect on the mechanical properties of calcite (Ma et al., 2008, 2009). Low ambient Mg^{2+}/Ca^{2+} is also known to slow down calcification rates in sea urchins (*Prionocidaris baculosa* and *Psammechinus miliaris*; Kotłuk et al., 2020). Magnesium deprivation was expected to be also harmful for other echinoderms, including asterozoans. Indeed, the

277 brittle star *Ophiocomina nigra* cultured under low ambient Mg^{2+}/Ca^{2+} either died or
278 commonly displayed arm autotomy, disc perforation, and growth impairment.

279 The manganese labeling in brittle stars proved to be less efficient than in asteroids due to
280 the dispersed bright orange-red CL luminescence in the entire skeleton, possibly caused by
281 increased Mn^{2+} levels in the original environment. Even though Recent echinoderms are
282 often non- or blue-luminescent (Barbin, 2000; Gorzelak et al., 2016), some echinoderm
283 species collected from the offshore environments may occasionally display bright orange
284 luminescent spots (e.g., Richter and Zinkernagel, 1981). Interestingly, in two specimens of
285 brittle stars kept under lowered Mg^{2+}/Ca^{2+} ratio, the new growth was observed through an
286 abrupt change in skeletal Mg/Ca in the most distal parts of the arm, where, according to the
287 distalization-intercalation model, new plates are formed during arm regeneration (e.g.,
288 Czarkwiani et al., 2016). Overall, all of the above suggests that brittle stars appear much
289 more sensitive to changes in the seawater composition than sea urchins and asteroids.

290 Although these sparse geochemical data may suggest that skeletal chemistry in brittle stars,
291 as in the case of sea urchins and asteroids, may be also controlled by seawater chemistry,
292 further experiments are needed to fully test this hypothesis.

293 Skeletal growth in sea stars is expressed mainly through the formation of the plates in the
294 most distal parts of the arm (e.g., Fewkes 1888, Verrill, 1914; Ben Khadra et al., 2015), as
295 well as thickening of already existing plates (Hotchkiss, 2012). This pattern was confirmed
296 through manganese labeling (Figure 1). Newly formed skeleton of non-control specimens
297 has significantly lower Mg/Ca ratios, thus corroborating the effect of ambient Mg^{2+}/Ca^{2+}
298 ratio on geochemical composition of the starfish skeleton.

Although the seawater composition has the prevalent role in echinoderm biomineralization, the ultimate skeletal Mg/Ca ratio is a result of a complex interplay between environmental and physiological factors. In echinoids, the Mg/Ca ratio in the stereom differs on several organizational levels, e.g., between orders, families, species, or on an individual scale (i.e., significant variation is detected between different ossicles within a single specimen or even within one ossicle; see Discussion in Gorzelak et al. 2013; Kołbuk et al., 2020). Other echinoderm classes, such as asteroids and ophiuroids, also display a wide range of Mg/Ca ratios, on higher taxonomic levels such as orders and families (Weber, 1969); additional variation can also occur between species (Duquette et al., 2018) from similar environmental conditions. Notably, comparison of Mg/Ca values obtained in a series of experiments on echinoids and asteroids showed that they displayed different magnesium fractionation curves, although it should be underlined that these specimens grew under different temperature conditions (Figure 5; Table S2 in supplementary files). Nonetheless, the skeletal Mg/Ca ratio cannot be solely ascribed to environmental conditions (Hermans et al., 2011; Asnaghi et al., 2014; Kołbuk et al., 2019, 2020). The physiological effect (“vital effect”) involved in magnesium incorporation and distribution in the skeleton can complicate reconstructions of ancient seawater Mg^{2+}/Ca^{2+} ratio, and applying a single algorithm to different fossil echinoderms, without considering their physiology-based variation, may produce a significant reconstruction error (e.g., Table 3).

Interestingly, the chemical composition of seawater (concentrations of Mg^{2+} and Ca^{2+}) of the environment in which echinoderms grow might itself be less stable than it was previously thought. Recently, Lebrato et al. (2020) challenged the long-standing assumption of a limited seawater Mg^{2+}/Ca^{2+} variability. It has been long argued that the seawater Mg^{2+}/Ca^{2+} ratio fluctuated on a temporal scale (in the Phanerozoic between ~ 1 and ~ 5.2 (mol/mol; e.g.

Hardie, 1996)), but not on a spatial one. A uniform $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio is typically assumed for the entire global ocean due to the broad presumption that the residence times of Mg and Ca, are ~13 Ma, and 1 Ma, respectively. However, a vast dataset collected by Lebrato et al. (2020) from various modern marine environments showed a significant variability for seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio with respect to location, ecosystem and depth. For instance, in the upwelling and polar regions, shelves or river-influenced settings the seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio ranges from ~4.4 to 6.4 mol/mol whereas these ratios in the open-ocean environment range from ~4.9 to ~5.3 mol/mol. This variability is explained by a complex system of removal and addition of Ca^{2+} and Mg^{2+} to seawater, that is strongly dependent on the spatiotemporal scale, with additional relation to other geochemical or biological parameters of the environment (e.g., total alkalinity or presence of calcifying communities). It is thus clear that such variability occurred also in the past, which may question the use of calcifying organisms as proxies of “global” $\text{Mg}^{2+}/\text{Ca}^{2+}$ trends in the Phanerozoic. Naturally, not all fossils used in paleoenvironmental studies originated from more stable, “semi-conservative” open-ocean conditions; many specimens come from the areas more prone to $\text{Mg}^{2+}/\text{Ca}^{2+}$ shifts, e.g., coasts, river-influenced areas, or shelves. such as the Jurassic or Miocene sea stars used in this study (which grew in the basins semi-isolated from open waters, i.e., in the epicontinental sea connected to the Tethys ocean or in the northernmost parts of the Paratethys basin, respectively). Herein, the reconstructions of the paleoseawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ based on fossil specimens are generally consistent with low $\text{Mg}^{2+}/\text{Ca}^{2+}$ seawater ratio in the Jurassic (“calcite sea”) and high $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio in the Miocene (“aragonite sea”). The variations in the temperature-normalized reconstructed $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratios are lower than for the sea urchins from the same periods (see table 4 in Kotłbuk et al., 2020), which may be caused by the aforementioned Mg fractionation within sea urchins, observed for instance on

an individual level (between and within ossicles). In asteroids differences in Mg content between ossicles are weaker than in echinoids (Weber, 1973; Borremans et al., 2009) . Differences in control abilities of the Mg^{2+} concentration in the coelomic fluid (CF) between sea stars and sea urchins could also account for the lower variations recorded in asteroids. Indeed, sea urchin species significantly differ in their abilities to control magnesium concentration in their CF, with some species maintaining an almost constant concentration whatever the sea water Mg^{2+} concentration (Santos et al., 2013). On the contrary the CF Mg^{2+} concentration in sea stars is much closer to the sea water Mg^{2+} concentration in studied species (Diehl and Lawrence, 1984). This would point to a higher control capacity in some sea urchin species. Interestingly, this is similar to the acid-base balancing abilities of sea urchins, some being able to compensate their extracellular pH (and this parallels the abilities to control the CF Mg^{2+} concentration) (Collard et al., 2013, Di Giglio et al., 2020). On the contrary, sea stars studied so far are unable to compensate their extracellular pH when facing low pH sea water (Dupont and Thorndyke, 2012; Collard et al., 2013). In experimental studies, the intrapopulation variation in Mg content also appears to be larger in echinoids than in asteroids (Figure 5). Comparison of reconstructions based on asteroids and echinoids from the same geological period and using the same algorithms reveals significant discrepancy in paleoseawater Mg^{2+}/Ca^{2+} ratio, which is visible especially in the case of the Miocene specimens, obtained from the same geologic formation deposited in the same part of the basin. For instance, the temperature-normalized reconstructed paleoseawater Mg^{2+}/Ca^{2+} ratio based on skeletal Mg/Ca in asteroids is around 4.5 mol/mol, while the ratio reconstructed from sea urchins is generally much lower (~3-3.3 mol/mol; Kołbuk et al., 2020).

Notwithstanding the effects of other environmental controls (temperature, salinity) and physiology (vital effect) or diet, which may affect skeletal Mg/Ca ratio in echinoderms, geochemical data obtained from fossil echinoderms from a specific location may give an insight into the local environmental conditions at that particular time and place. Regardless, mean skeletal Mg/Ca values calculated from a number of well-preserved fossil species from a given period might still reflect “global” conditions, and confirm secular variation in seawater Mg/Ca during the Phanerozoic (calcite-aragonite seas).

5. Conclusions

In this study we confirmed for the first time the effect of ambient seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio on skeletal Mg/Ca ratio in asteroids. Sea stars cultured under decreased seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio exhibited lower skeletal Mg/Ca ratios compared with the control specimens. However, a comparison of several echinoderm species belonging to different classes showed a significant physiology-based variation in skeletal Mg/Ca ratios on different organizational levels (inter-class, inter- and intra-species, and inter- and intra-individual), producing different magnesium fractionation curves. The presence of the physiological factor (vital effect) imposes limitations on the use of well-preserved echinoderm fossils in paleoseawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio reconstructions, which themselves can be also biased with respect to the local environment.

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619 Figure 1. (A) Sea star *Asterias rubens* in a beaker. Scale bar 1 cm. (B, D, F) CL images (fine
620 dotted lines delineate ossicle margins) and (C, E, G; dashed lines delineate sharp contrast in
621 Mg content) WDS maps of Mg distribution in polished and carbon-coated *A. rubens* ossicles.
622 Specimens were labeled with Mn^{2+} and kept under three Mg^{2+}/Ca^{2+}_{sw} ratios: (B-C) ~5.2
623 mol/mol (control group), (D-E) ~2.5 mol/mol, (F-G) ~1.5 mol/mol. White arrows indicate
624 newly grown skeleton. Scale bar 100 μm . [1.5 column figure]

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627 Figure 2. (A) Brittle stars *Ophiocomina nigra* in beaker. Scale bar 1 cm. (B) Autotomized arms
628 and central disc perforation in *Ophiocomina nigra* cultured under lowest (~1.5 mol/mol)
629 Mg^{2+}/Ca^{2+}_{sw} ratio. Scale bar 1 cm. (C) CL image of *O. nigra* arm with traces of Mn^{2+} -activated
630 luminescence (red-orange spots). White arrow indicates zone of newly grown skeleton
631 (between the dotted line and tip of the arm). Scale bar 0.5 mm. [1.5 column figure]

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635 Figure 3. Examples of CL images showing (A) dark, non-luminescent diagenetically unaltered
636 (DU) fragment of stereom in *Paxillosida* indet. (Miocene, Korytnica) contrasting with Mn-
637 activated bright orange diagenetically altered (DA) region, (B) dark, diagenetically unaltered
638 stereom trabeculae with Mn-activated orange blotchy structure in *Archastropecten* (?) sp.
639 (Middle Jurassic (Callovian), Šaltiškiiai). Preserved fragments of stereom trabeculae showing

(C, D) meshy stereom structure and (E, F) layered growth (white arrows), observed under SEM in (C, E) *Paxillosida* indet. (Miocene, Korytnica), (D, F) *Archastropecten* (?) sp. (Middle Jurassic (Callovian), Šaltišķiai). [2 column figure]

Figure 4. Mean Mg/Ca ratios ($\pm$ SD) of skeletal components of *Asterias rubens* in three seawater $\text{Mg}^{2+}/\text{Ca}^{2+}$ treatments: ~ 5.2 mol/mol ($n = 6$), ~ 2.5 mol/mol ($n = 5$), ~ 1.5 mol/mol ($n = 3$). Asterisks denote statistically significant differences between treatments. Open circles indicate mean skeletal Mg/Ca ratios ($\pm$ SD) obtained from the measurements of the pre-cultured stereom. [1 column figure].

Figure 5. (A) Magnesium fractionation curves and (B) partition coefficients for the ossicles of four echinoderm species kept under different $\text{Mg}^{2+}/\text{Ca}^{2+}$ seawater ratios with the indication of temperature during the experiments: ambital plates (galleried stereom) of *Prionocidaris baculosa* kept in $T = 25.5^\circ\text{C}$ (red squares; Kołbuk et al., 2020), coronal plates of *Eucidaris tribuloides* kept in $T = 25^\circ\text{C}$ (black squares; Ries, 2004), ambital plates (galleried stereom) of *Psammechinus miliaris* kept in $T = 13.7^\circ\text{C}$ (green diamonds; Kołbuk et al., 2020), various arm ossicles of *Asterias rubens* kept in $T = 17.8^\circ\text{C}$ (blue circles; this study). [2 column figure]

Figure 2

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Figure 3

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Figure 4

Figure 5

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Table 1. Seawater parameters (means and standard deviations) in each beaker during the entire experiment (t = 21 days). Temperature, salinity and pH within individual beakers were not significantly different from each other ($p_{\text{Tukey}} > 0.05$). Three different Mg/Ca_{sw} (seawater Mg²⁺/Ca²⁺ ratio) levels were constant throughout the experiment ($p_{\text{Tukey}} > 0.05$)

Species	Nominal Mg/Ca _{sw} [mol/mol]	Mg/Ca _{sw} [mol/mol]	SD	Temp. [°C]	SD	Salinity [psu]	SD	pH	SD
<i>Asterias rubens</i>	5.2	5.15	0.02	17.7	0.45	32.9	0.33	8.1	0.08
<i>Asterias rubens</i>	5.2	5.16	0.02	17.7	0.45	32.9	0.31	8.1	0.08
<i>Asterias rubens</i>	5.2	5.15	0.02	17.7	0.45	32.9	0.29	8.1	0.09
<i>Asterias rubens</i>	5.2	5.16	0.02	17.7	0.43	32.9	0.28	8.1	0.09
<i>Asterias rubens</i>	5.2	5.16	0.02	17.7	0.46	32.9	0.28	8.1	0.08
<i>Asterias rubens</i>	5.2	5.15	0.02	17.7	0.45	32.9	0.31	8.1	0.09
<i>Asterias rubens</i>	2.5	2.44	0.02	17.8	0.49	32.9	0.32	8.0	0.08
<i>Asterias rubens</i>	2.5	2.44	0.02	17.8	0.47	32.9	0.27	8.1	0.08
<i>Asterias rubens</i>	2.5	2.44	0.02	17.8	0.48	32.9	0.29	8.1	0.08
<i>Asterias rubens</i>	2.5	2.44	0.02	17.8	0.47	32.9	0.29	8.1	0.08
<i>Asterias rubens</i>	2.5	2.44	0.02	17.8	0.50	32.9	0.29	8.1	0.08
<i>Asterias rubens</i>	2.5	2.44	0.01	17.8	0.48	32.9	0.29	8.1	0.08
<i>Asterias rubens</i>	1.5	1.44	0.01	17.8	0.50	32.9	0.29	8.0	0.07
<i>Asterias rubens</i>	1.5	1.44	0.01	17.9	0.48	32.9	0.25	8.0	0.07
<i>Asterias rubens</i>	1.5	1.44	0.01	17.9	0.49	32.9	0.25	8.0	0.07
<i>Asterias rubens</i>	1.5	1.44	0.01	17.9	0.49	32.9	0.24	8.0	0.07

<i>Asterias rubens</i>	1.5	1.44	0.01	17.9	0.50	32.8	0.24	8.0	0.07
<i>Asterias rubens</i>	1.5	1.44	0.01	17.9	0.48	32.9	0.27	8.0	0.07
<i>Ophiocomina nigra</i>	5.2	5.16	0.02	17.8	0.32	32.8	0.24	8.0	0.08
<i>Ophiocomina nigra</i>	5.2	5.16	0.02	17.8	0.32	32.8	0.25	8.0	0.08
<i>Ophiocomina nigra</i>	5.2	5.16	0.03	17.8	0.34	32.8	0.24	8.0	0.08
<i>Ophiocomina nigra</i>	5.2	5.16	0.02	17.8	0.32	32.8	0.23	8.0	0.08
<i>Ophiocomina nigra</i>	5.2	5.15	0.06	17.8	0.33	32.8	0.24	8.0	0.08
<i>Ophiocomina nigra</i>	5.2	5.17	0.03	17.8	0.33	32.8	0.24	8.0	0.08
<i>Ophiocomina nigra</i>	2.5	2.45	0.01	17.8	0.41	32.8	0.23	8.0	0.07
<i>Ophiocomina nigra</i>	2.5	2.45	0.02	17.8	0.42	32.8	0.23	8.0	0.07
<i>Ophiocomina nigra</i>	2.5	2.45	0.01	17.8	0.40	32.8	0.22	8.0	0.07
<i>Ophiocomina nigra</i>	2.5	2.45	0.01	17.8	0.44	32.8	0.23	8.0	0.08
<i>Ophiocomina nigra</i>	2.5	2.45	0.02	17.8	0.42	32.8	0.24	8.0	0.08
<i>Ophiocomina nigra</i>	2.5	2.45	0.01	17.8	0.41	32.8	0.23	8.0	0.08

Table 2. Measured respiration rates of *Asterias rubens* (n = 7) after 7, 14 and 21 days of exposure to three ambient Mg^{2+}/Ca^{2+} ratios (high ~5.2, medium ~2.5, low ~1.5 [mol/mol]). No significant differences according to seawater Mg^{2+}/Ca^{2+} ratio ($p_{ANOVA} = 0.89$) and time ($p_{ANOVA} = 0.86$) were recorded.

Specimen	Seawater	Respiration rate [$O_2\mu mol/(h\cdot g)$]		
	Mg^{2+}/Ca^{2+}			
	[mol/mol]	Day 7	Day 14	Day 21
1	~5.2	0.201	0.219	0.150
2	~5.2	0.201	0.100	0.172
3	~5.2	0.313	0.170	0.174
4	~2.5	0.134	0.106	0.244
5	~2.5	0.248	0.182	0.147
6	~1.5	0.222	0.138	0.124
7	~1.5	0.098	0.349	0.221

Table 3. Measured seawater Mg^{2+}/Ca^{2+} ratios compared with the values reconstructed from skeletal Mg/Ca ratios in *Asterias rubens*. Based on Ries’ (2004) algorithm for echinoid test plates: $Mg/Ca_C = S(0.000719 T + 0.0292)Mg/Ca_{SW}^{0.668}$. SW – seawater, C – calcite, T – temperature, S – species coefficient (calculated from the known value of Mg/Ca of this specimen in the wild and T of the original environment).

T	S	Measured Mg/Ca_C (mean for treatment)	Measured Mg^{2+}/Ca^2 sw ratio (mean for treatment)	Calculated Mg^{2+}/Ca^2 sw ratio (mean for treatment)	Calculated reconstruction error (mean for treatment)
17.7	0.994	0.125	5.16	5.16	0%
17.8		0.075	2.44	2.39	1.9%
17.9		0.043	1.44	1.03	39.7%

Table 4. Reconstructed values of seawater Mg^{2+}/Ca^{2+} ratios for fossil asteroids (n specimens = 6) using different algorithms. Note that only Ries' equation is temperature-normalized. T – temperature, S – species coefficient, DcMg – Mg fractionation coefficient, SD – standard deviation. Temperature data were taken from Brand (1986), and Scheiner et al. (2018) for the Callovian and Miocene respectively, species coefficients were based on the data for *Astropecten andromeda* (from Chave, 1954) and the order Paxillosida (from Weber, 1969).

Taxon	Sampling site	Age	T [C]	S	Skeletal Mg/Ca [mol/mol] ± SD	Seawater Mg^{2+}/Ca^{2+} [mol/mol] (Dickson, 2004)	DcMg (mean skeletal Mg/Ca : sw Mg^{2+}/Ca^{2+})	Seawater Mg^{2+}/Ca^{2+} [mol/mol] (this study)	DcMg (mean skeletal Mg/Ca : sw Mg^{2+}/Ca^{2+})	Seawater Mg^{2+}/Ca^{2+} [mol/mol] including T and S (Ries, 2004)	DcMg (mean skeletal Mg/Ca : sw Mg^{2+}/Ca^{2+})	Source of data for S calculation
Astropectinidae (Archastropecten?)	Šaltiškiai, Lithuania	Callovian, Middle Jurassic	12.5	1.56	0.0567 ± 0.004	1.78	0.032	1.82	0.031	1.2	0.047	Chave, 1954 (<i>Astropecten andromeda</i>)
Astropectinidae (Archastropecten?)	Šaltiškiai, Lithuania	Callovian, Middle Jurassic	12.5	1.56	0.0565 ± 0.004	1.78	0.032	1.81	0.031	1.2	0.047	Chave, 1954 (<i>Astropecten andromeda</i>)
Paxillosida indet.	Korytnica, Poland	Miocene, Neogene	24.3	1.38	0.1533 ± 0.008	4.82	0.032	6.48	0.024	4.75	0.032	Weber, 1969 (order Paxillosida)
Paxillosida indet.	Korytnica, Poland	Miocene, Neogene	24.3	1.38	0.1426 ± 0.010	4.48	0.032	5.97	0.024	4.26	0.033	Weber, 1969 (order Paxillosida)
Paxillosida indet.	Korytnica, Poland	Miocene, Neogene	24.3	1.38	0.1518 ± 0.016	4.77	0.032	6.41	0.024	4.68	0.032	Weber, 1969 (order Paxillosida)
Paxillosida indet.	Korytnica, Poland	Miocene, Neogene	24.3	1.38	0.1445 ± 0.003	4.54	0.032	6.06	0.024	4.35	0.033	Weber, 1969 (order Paxillosida)

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Supplementary file

Supplementary Table S1.docx

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Przemysław Gorzelak