Summary

Echinoderms have a skeleton of mesodermal origin, consisting of many plates with a distinct trabecular structure (the so-called stereom). Their skeleton is made of high-magnesium calcite with a small admixture of organic compounds, amorphous calcium carbonate (ACC) and water, and is produced through biologically controlled mineralization.

Until recently it was believed that calcium and magnesium ions required for the formation of the echinoderm skeleton come exclusively from seawater, and that the skeletal Mg/Ca ratio constitutes a proxy for the secular variation in the concentration of these two elements in the environment. Thanks to geochemical data (more precisely, the Mg/Ca ratio) derived from the skeletons of echinoderms, it is possible to calculate the molar ratio of Mg^{2+}/Ca^{2+} of the seawater in which these echinoderms grew. The potential of such reconstructions was recognized by the researchers who used well-preserved fossil echinoderms to reconstruct the chemical composition of the ancient oceans. Nevertheless, in recent years, several published studies suggested that the skeletal Mg/Ca ratio can be also influenced by environmental (temperature and salinity) and biological factors (resulting from the organism's physiology; the so-called vital effect), which means that the degree of biomineralization controlled by echinoderms is larger than previously thought. Moreover, there is no reliable experimental data from Recent echinoderms suggesting a relationship between Mg/Ca in their skeleton and the seawater Mg^{2+}/Ca^{2+} ratio. The aim of the PhD thesis was to conduct a series of biomineralization experiments to verify the effect of the seawater Mg^{2+}/Ca^{2+} ratio and diet on the chemical composition of the echinoderm skeleton.

As part of the PhD thesis, three biomineralization experiments on various unrelated species of echinoderms were planned. The results of these experiments are summarized in the three chapters/articles listed below.

1) Short-term (14 days) experiment testing the effect of increased Ca^{2+} concentration in the seawater and $MgCO_3$-enriched diet on the chemical composition of regenerating spines in adult specimens of an euechinoid *Paracentrotus lividus*;
2) Extended (21 days) experiment testing the effect of a lowered Mg^{2+}/Ca^{2+} ratio in the seawater (achieved through increasing Ca^{2+} and lowering Mg^{2+} concentration) and MgO-enriched diet on the growth rate, respiration rate, and chemical composition of newly formed test plates, teeth, demipyramids, and spines of two echinoid specimens (cidaroid *Prionocidaris baculosa* and euechinoid *Psammechinus miliaris*);

3) Extended (21 days) experiment testing the effect of a lowered Mg^{2+}/Ca^{2+} ratio in the seawater (achieved through increasing Ca^{2+} and lowering Mg^{2+} concentration) on the chemical composition of newly formed plates in asteroids (*Asterias rubens*) and ophiuroids (*Ophiocomina nigra*).

At the beginning of the first experiment, tips of the echinoid spines were cut to initiate regeneration. After that the echinoderms were kept in a sea water with a control (~ 5.2 mol/mol) or lowered (~ 1.9 mol/mol) Mg^{2+}/Ca^{2+} molar ratio and fed with a diet containing a standard (~0.3 wt%) or increased (~ 11 wt%) magnesium content. At the end of the experiment, the spines were removed and the chemical composition (Mg/Ca) of the regenerated tips was investigated. Spines grown under reduced Mg^{2+}/Ca^{2+} ratio conditions displayed lower Mg/Ca ratio in the regenerated tips, which confirmed the hypothesis about the significant effect of seawater on the chemical composition of the echinoderm skeleton. However, echinoids which fed a magnesium-enriched diet had a higher Mg/Ca ratio in the skeleton. This suggests that seawater is not the only source of ions in the biomineralization process, i.e., some of the magnesium can also originate from the diet. In addition, the results also suggest that the transport pathway of magnesium ions in the biomineralization of regenerating sea urchin spines is more complex than previously thought. More specifically, until now, it was believed that the transport of magnesium ions from seawater to sclerocytes (skeleton-forming cells) is mediated solely by the spine-covering epidermis. The results of the experiment suggest that the magnesium originating from the diet could be transported to calcification sites in the spines with the aid of phagocytes or spherulocytes (the only types of cells capable of migrating from coelom to spines). Overal, this has implications in the context of the use of fossil echinoderms in palaeoenvironmental reconstructions. In particular, it seems that the type of diet can influence the chemical composition of echinoderm skeleton, which in turn may induce reconstruction errors of seawater Mg^{2+}/Ca^{2+} ratio inferred from echinoderm material.

In the experiment described above, the decrease of seawater Mg^{2+}/Ca^{2+} molar ratio was achieved through the addition of Ca^{2+}-containing salt ($CaCl_2$), however in the geological history, the seawater Mg^{2+}/Ca^{2+} variations were related to changes in the concentration of both ions (Mg^{2+} and Ca^{2+}). For this reason, in the next experiment the seawater Mg^{2+}/Ca^{2+} ratio was decreased through the addition of calcium chloride and Mg-free water (obtained by dissolution of NaCl, Na_2SO_4, KCl, KBr, $SrCl_2$, NaF, H_3BO_3, and $NaHCO_3$ in ultrapure water). This time two species of sea urchins with different environmental preferences were used for the study: temperate *Psammechinus miliaris* and tropical *Prionocidaris baculosa*. In *P. miliaris* tips of the spines were cut off in order to induce regeneration (in cidaroid *P. baculosa* spine regeneration does not occur). Additionally, both species were labeled with manganese (1 mg/L) and imaged with a cathodoluminescence microscope. The advantage of Mn-labeling, apart from easy, low-cost preparation and lack of negative effects (at low Mn concentrations) on animals' physiology, is the high resolution enabling precise determination of new growth. After labeling, sea urchins were kept in water with a molar ratio of Mg^{2+}/Ca^{2+}: ~5.2, ~2.5, ~1.5 (mol/mol) and fed with a standard (~0.3 wt%) or magnesium-enriched (~20 wt%) diet. At the end of the experiment, different parts ot the skeleton (i.e. spines, teeth, demipyramids and ambital plates) of each specimen were collected. The prepared material was observed under a cathodoluminescence microscope in order to distinguish the newly formed zones of the skeleton to calibrate the growth rate and determine chemical composition (Mg/Ca). The results showed that sea urchins cultured in seawater with a lowered Mg^{2+}/Ca^{2+} molar ratio grew slower and produced a skeleton with a lowered Mg/Ca ratio. However, the skeletons of individuals which fed the magnesium-enriched diet had a higher Mg/Ca molar ratio than the skeletons of individuals which fed the magnesium-depleted diet. These data support the conclusions of the first experiment suggesting that some of the ions in the biomineralization process can originate from the diet, which may have an impact on the resulting skeletal Mg/Ca ratio. Importantly, the Mg/Ca ratio in the calcite that builds the skeleton of sea urchins can be heterogenous within a single specimen (depending on the ossicle type), or even within a single ossicle (depending on the type of stereom). This means that the reconstruction of the seawater Mg^{2+}/Ca^{2+} ratio based on the geochemical data (Mg/Ca) obtained from the echinoderm skeleton may be affected by errors resulting not only from the diet effect, but also from the intraspecimen variation stemming from vital effects. The chapter discusses errors resulting from such reconstructions, calculated on the basis of experimental data. The results of geochemical studies (Mg/Ca) of well-preserved Jurassic and Miocene sea urchins, which were used to reconstruct past seawater Mg^{2+}/Ca^{2+} ratios, were also discussed. Jurassic echinoids usually display lower Mg/Ca ratios than the Miocene ones, which is consistent with the seawater chemistry at the time ("calcite" Jurassic seas and "aragonite" Miocene seas), however a large geochemical variation r complicates accurate reconstruction of the seawater Mg^{2+}/Ca^{2+} ratio changes in the Phanerozoic based on echinoderm material.

The third part of the dissertation discusses the impact of changes in the chemical composition of seawater on the skeletal Mg/Ca ratio in two less frequently studied groups of echinoderms, i.e., asteroids and ophiuroids. As part of the experiment, individuals belonging to two species (sea star *Asterias rubens* and brittle star *Ophiocomina nigra*) were first tagged with manganese and then placed in aquariums containing seawater with a molar ratio of ~5.2, ~2.5 and ~1.5 (mol/mol). In the case of brittle stars, within the first week most of the specimens cultured under reduced Mg^{2+}/Ca^{2+} conditions experienced high mortality, autotomy, and growth inhibition, which made it impossible to obtain data from most individuals (although in few cases we obtained data indicating lowered Mg/Ca in the skeleton). Sea stars which were cultured for 21 days under lowered Mg^{2+}/Ca^{2+} molar ratio, produced skeleton with a lowered Mg/Ca ratio. The Mg^{2+}/Ca^{2+} ratios applied in the experiment on modern asteroids were compared with the seawater Mg^{2+}/Ca^{2+} ratios reconstructed from fossil asteroids. Reconstructions based on well-preserved Jurassic and Miocene fossil sea stars are consistent with geological data showing a low Mg^{2+}/Ca^{2+} ratio in the Jurassic seas and high in the Miocene seas. However, the accuracy of the reconstruction is strongly limited by a different degree of magnesium fractionation in different species of echinoderms, as well as local environmental conditions, which may additionally affect the skeletal Mg/Ca ratio.