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Supplemental Material

Experimental Methods
Paleobiological Methods
Supplemental Figure S1
Supplemental Figure S2
Supplemental Figure S3
Supplemental Table S1
Supplemental Table S2
Supplemental Table S3
Supplemental References
Associated Data Files

Supplemental Materials for

Calcite-aragonite seas as a driver of echinoderm evolution?

Experimental insight and deep-time decoupling

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Supplemental Figures and Tables

Supplemental Figure S1

Supplemental Figure S2

Supplemental Figure S3

Supplemental Table S1

Supplemental Table S2

Supplemental Table S3

Supplemental References

Associated Data Files

Data files are deposited on Figshare (<https://gsapubs.figshare.com/Geology>).

1. Ophiuroid_data.csv – Arm measurement data from ophiuroid regeneration experiments
2. PBDB_output.txt – Raw echinoderm diversity data from the Paleobiology Database (www.paleobiodb.org)
3. Echinoderm_diversification_results.txt – Measures of echinoderm diversity through time calculated from PBDB_output.txt.

Experimental Methods

Selection and verification of Mg/Ca ratios

The three seawater Mg/Ca treatments used for the regeneration experiment were selected based on the range of Mg/Ca ratios that echinoderms have been exposed to throughout their evolutionary history (Cambrian-Present). The highest ratio of 5.2 mol Mg/Ca reflects the composition of modern seawater and the maximum Mg/Ca ratios experienced by echinoderms throughout their evolutionary history. The low ratio of 1.7 mol Mg/Ca used in the experiment is likely higher than the minimum Mg/Ca ratios experienced by echinoderms, because revised Mg/Ca curves for the Phanerozoic indicate ratios dropped as low as 0.7 mol Mg/Ca during the Cretaceous (Hardie, 1996; Dickson, 2004). However, from preliminary results during experimental setup, we found that seawater Mg/Ca ratios at or below a ratio of approximately 1.5 resulted in considerable stress to test specimens, as evidenced by extensive autotomization of the arms. These results are consistent with those of Kolbuk et al. (2021), who found that the brittle star *Ophiocoma nigr*a experienced arm autotomization and disc perforation when cultured in seawater with a Mg/Ca ratio of ~1.5. As a result, a ratio of 1.7 mol Mg/Ca was selected for the lowest seawater treatment, which did not induce arm autotomization or disc perforation. The intermediate treatment of 2.5 mol Mg/Ca reflects seawater chemistry that falls between endmembers, which was reached at several intervals in echinoderm history such as the mid-Carboniferous, Early Permian, and mid-Jurassic.

Seawater treatments were created using formulas from J.B. Ries (2005; personal communication, 2013) by combining reagent grade chemicals measured by weight with distilled, deionized water (Table S1). Mg/Ca ratios used in the treatments were verified as being within ± 0.2 mol of the target ratio using a Perkin-Elmer Optima 4300DV Inductively Coupled Plasma Optical Emission Spectrometer. Geochemical verification was conducted at The Ohio State University, School of Earth Sciences.

Experimental setup and conditions

Ophioderma cinereum is a common shallow-water ophiuroid distributed throughout the Gulf of Mexico and Western Central Atlantic. Specimens of *O. cinereum* were acquired from a commercial saltwater aquaria supplier. Other than variations in Mg/Ca ratios, all other variables

were held constant for all aquaria and specimens throughout the duration of the experiment, including tank size (10 gallons), temperature ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ measured twice a week), pH (8.1 ± 0.1 measured twice a week), salinity (35 ppt measured twice a week), substrate (2 cm deep aragonite sand), feedings (2-3 fishmeal pellets per individual three times per week), and water changes (10% every two weeks).

After random assignment of individual specimens to the three different experimental treatments, specimens were given a minimum 1-month acclimation period prior to beginning the experimental study. Some specimens had longer acclimation periods (up to ~2 months), but all specimens were randomly assigned to tanks and treatments, such that no tank or treatment had specimens with consistently shorter or longer acclimation periods. As a result, it is very unlikely that any observed differences in regeneration rates were due to acclimation time and/or shock. To simulate sublethal predation, an arm was removed from each individual at the junction between the sixth and seventh rows of arm plates, which allowed the position of arm removal to be held constant across specimens.

Data collection and sensitivity tests of experimental results

Measurements of regenerating arms were collected by photographing specimens on a 1 mm grid while underwater, and regenerated arm length was then determined from photos using ImageJ (Schneider et al., 2012). Specimens were identified by marking the arms with a nontoxic paint marker, and separate growth records were maintained for each individual.

Because it is possible that larger individuals may be able to grow or regenerate lost limbs more quickly compared to smaller individuals, we investigated whether body size was a confounding factor. We evaluated the potential effect of body size on growth rate by comparing disc diameter to the total length of the regenerated arm for each specimen. Spearman's rank correlation between disc diameter and total regenerated arm length detects no statistically significant relationship between body size and growth rate (Spearman's $R_{\text{oh}}=0.29$, $p=0.31$), eliminating the concern that results are biased by differences in body size (Fig. S1).

To evaluate the sensitivity of the ANOVA test results presented in the main text, we also conducted a repeated measures nested ANOVA to account for Mg/Ca ratio treatments replicated across multiple aquarium tanks. Results of this analysis also found statistically significant differences among experimental Mg/Ca ratio treatments ($F=36.26$, $df=2$, and $p=0.0004$), and is

therefore concordant with results and conclusions presented in the main text. In addition to the Bonferroni correction for evaluating differences between experimental treatments (discussed in the main text), we also used a Tukey test of honest significant differences (HSD). Results of the Tukey HSD test found statistically significant differences ($p < 0.00001$) among all pairwise comparisons of experimental treatments.

Paleobiological methods

To evaluate whether changes in Mg/Ca ratios had an impact on echinoderm diversification, we calculated rank-based correlations (Spearman's Rho) across first differences between Mg/Ca ratios and temporal patterns of genus-level diversification, and then used Mann-Whitney U tests to evaluate whether diversification dynamics differ during times of calcite vs. aragonite seas.

All fossil echinoderm occurrence data spanning the Cambrian through Neogene were downloaded from Paleobiology Database (<https://paleobiodb.org>, accessed October 2021), resulting in a raw dataset of 25,037 occurrences. Taxa of uncertain taxonomic ranks or affinities were excluded, resulting in a total of 21,852 occurrences used in our analyses. To ensure our results were not dependent on a specific method or interpretation of echinoderm biodiversity, we used multiple approaches to quantify genus-level diversity dynamics. The first approach used sampled-in-bin-diversity, which simply reflects raw counts of taxa within each time bin and is one of the most commonly-used methods for calculating paleontological diversity (Miller and Foote, 1996; Kocsis et al., 2018). We also applied sampling standardization using shareholder quorum subsampling (SQS; Alroy, 2010a, 2010b), a coverage-based technique that uses a frequency distribution coverage of taxa to draw collections such that a target frequency (i.e., a “shareholder quorum”) is obtained. For the SQS analysis, we used a shareholder quorum of 0.6. In addition to quantifying changes in standing diversity, we also calculated per-capita rates of origination and extinction for each stage (Foote, 1999, 2000) to test whether Mg/Ca ratios are correlated with aspects of diversification dynamics, including extinction and net diversification (defined as the origination rate minus the extinction rate).

As is the case for essentially all taxonomic groups, occurrence data from the Paleobiology Database are incomplete for echinoderm taxa. As a result, the timeseries analyses of echinoderm diversity are subject to potential issues of patchy data entry and/or taxonomic worker bias (e.g.,

taxonomic or temporal asymmetries in efforts to document echinoderm biodiversity and/or enter it into the PBDB), as well as possible differences in the efficacy of various methods for calculating biodiversity patterns and their underlying dynamics themselves (e.g., Kocsis et al, 2018). Nevertheless, the PBDB dataset used here represents the highest-resolution compilation of Phanerozoic echinoderm data that has been constructed to date. We consider these data to be appropriate at the vast temporal and taxonomic scales of the present study, as we are seeking to evaluate broad biodiversity patterns over broad taxonomic and temporal scales spanning the Phanerozoic rather than asking questions about specific interval-to-interval changes or requiring accurate higher-level taxonomic affinities. Regardless of these caveats, we emphasize the results of our study do not depend on how diversity dynamics are calculated, as we find no relationship between Mg/Ca ratios and biodiversity patterns regardless of methodology or whether mass extinction events are included or excluded (Figs. S2-S3)

To evaluate the sensitivity of our results to the specific model of Phanerozoic Mg/Ca ratios presented by Stanley and Hardie (1998), we conducted additional analyses using Phanerozoic Mg/Ca ratios from both Wilkinson and Algeo (1989) and Horita et al. (2002). Results of these analyses also show no relationship between echinoderm biodiversity and Phanerozoic Mg/Ca ratios (Tables S2-S3). Thus, our results are robust across alternative models of Phanerozoic Mg/Ca ratios.

Supplemental Figures and Tables

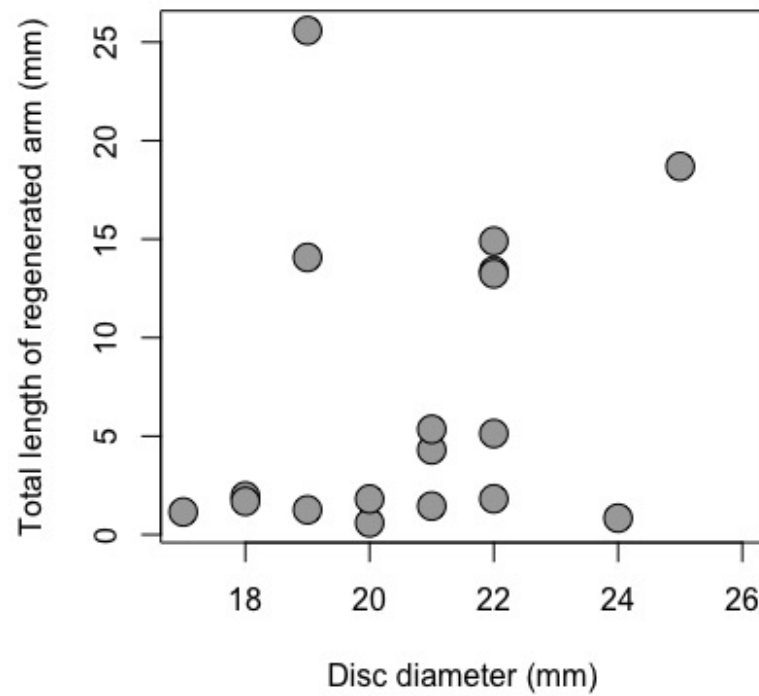


Figure S1. Body size, as measured by disc diameter, does not have a significant effect on total regenerated arm growth in *Ophioderma cinereum* (Spearman's $Rho=0.29$, $p=0.31$).

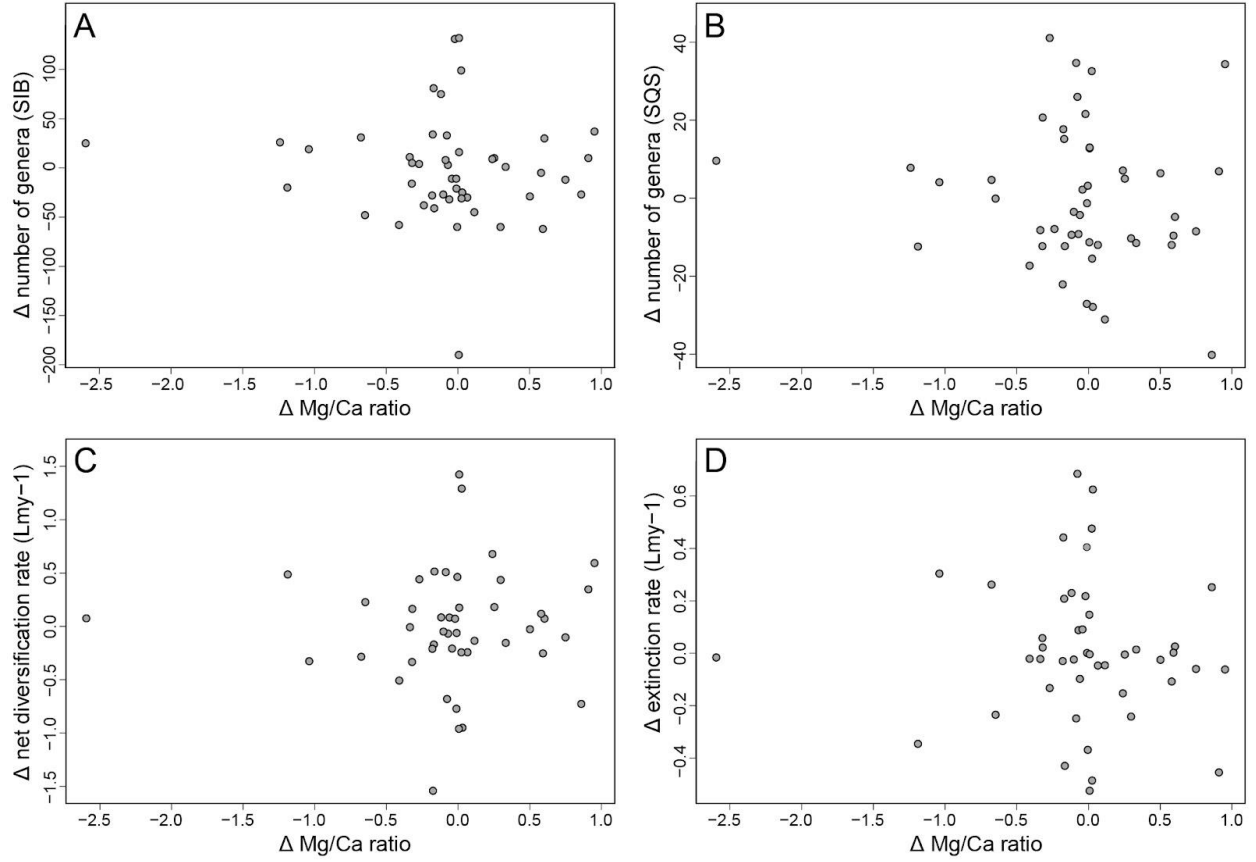


Figure S2. First differences of time series data comparing changes in Mg/Ca ratios to diversification dynamics. (A) Mg/Ca ratios vs. sampled-in-bin (SIB) standing diversity (Spearman's $\rho=-0.111$, $p=0.457$). (B) Mg/Ca ratios vs. shareholder quorum subsampling (SQS) standing diversity (Spearman's $\rho=-0.131$, $p=0.379$). (C) Mg/Ca ratios vs. net diversification (Spearman's $\rho=-0.106$, $p=0.488$). (D) Mg/Ca ratios vs. change in extinction rate (Spearman's $\rho=-0.145$, $p=0.339$).

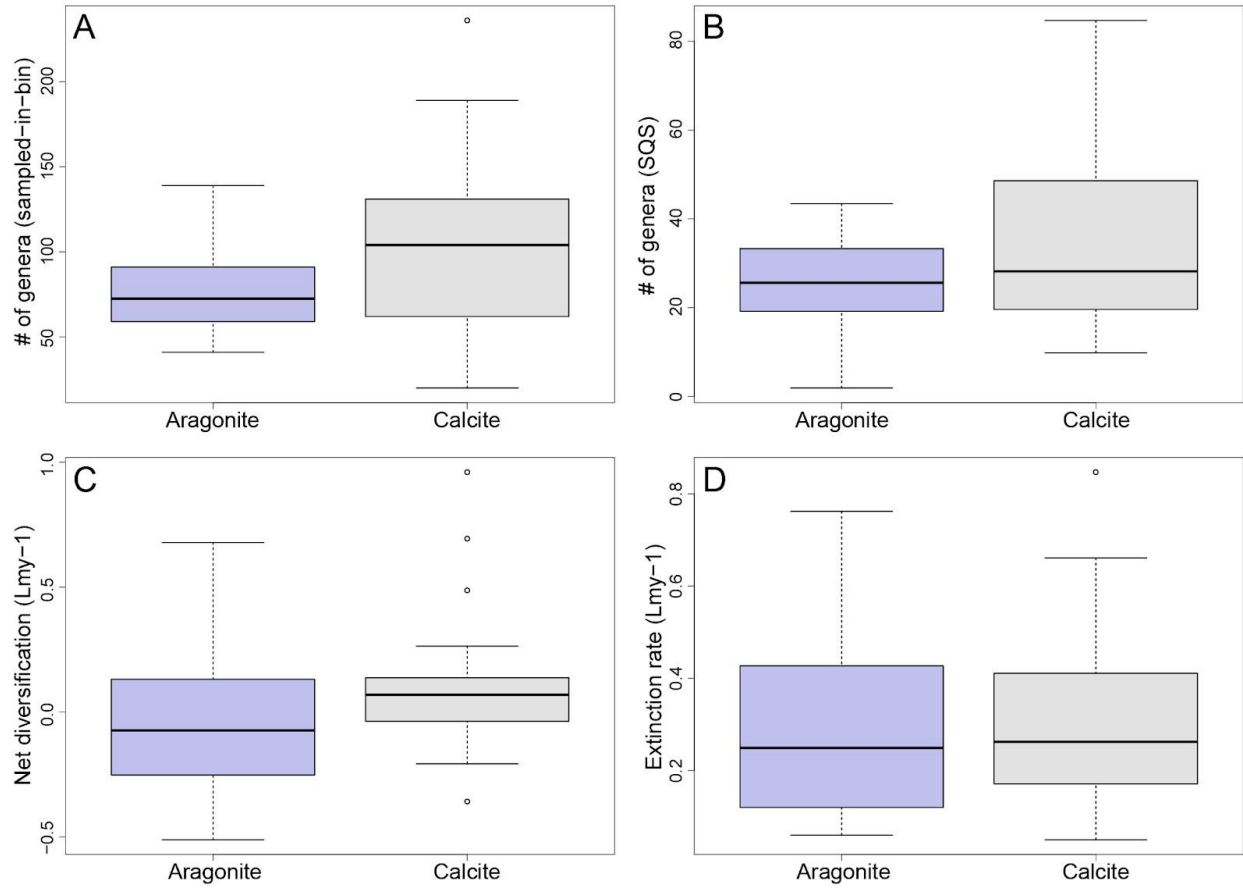


Figure S3. Boxplots comparing measures of standing diversity, net diversification, and extinction in calcite sea intervals versus aragonite sea intervals; Mann-Whitney U tests recover no statistically significant differences in diversity and extinction measures between seawater chemistry regimes. (A) Sampled-in-bin standing diversity in calcite versus aragonite sea intervals (with mass extinctions: $W=154.5$, $p=0.161$, without mass extinctions: $W=134$, $p=0.178$). (B) Shareholder quorum subsampling (SQS) standing diversity in calcite versus aragonite sea intervals (with mass extinctions: $W=156.5$, $p=0.177$, without mass extinctions: $W=131.5$, $p=0.156$). (C) Net diversification per lineage million years (Lmy^{-1}) in calcite versus aragonite sea intervals (with mass extinctions: $W=209$, $p=0.738$, without mass extinctions: $W=122.5$, $p=0.128$). (D) Extinction rate per lineage million years in calcite versus aragonite sea intervals (with mass extinctions: $W=161.5$, $p=0.364$, without mass extinctions: $W=182.5$, $p=1.0$).

Table S1. Weights of reagent grade chemicals used to generate 14 L of each of the three seawater treatments with varying Mg/Ca ratios. Formulas are modified from Ries (2005; personal communication, 2013).

Chemical compounds	1.7 Mg/Ca ratio treatment (grams)	2.5 Mg/Ca ratio treatment (grams)	5.2 Mg/Ca ratio treatment (grams)
NaCl	349.2330	349.2097	349.3710
Na ₂ SO ₄	58.5023	58.4984	58.5254
KCl	9.8817	9.8811	9.8857
NaHCO ₃	2.8609	2.8607	2.8620
KBr	1.4304	1.4304	1.4310
H ₃ BO ₃	0.3795	0.3795	0.3797
NaF	0.0438	0.0438	0.0438
SrCl ₂ • 6H ₂ O	0.3503	0.3503	0.3505
MgCl ₂ • 6H ₂ O	121.8798	133.9959	157.4097
CaCl ₂ • 2H ₂ O	51.8171	38.7586	21.8900
H ₂ O	14000	14000	14000
Total weight (grams):	14596.3788	14595.4083	14602.1487

Table S2. Statistical results for first differences of time series data comparing changes in Mg/Ca ratios to diversification dynamics. SIB = Sampled-in-bin diversity, SQS = shareholder quorum subsampling diversity.

Mg/Ca ratio model	Biodiversity metric	Spearman's rho	<i>p</i> -value
Stanley and Hardie (1998)	SIB	-0.111	0.457
Stanley and Hardie (1998)	SQS	-0.131	0.379
Stanley and Hardie (1998)	Net diversification	-0.106	488
Stanley and Hardie (1998)	Extinction rate	-0.145	0.339
Wilkinson and Algeo (1989)	SIB	0.037	0.803
Wilkinson and Algeo (1989)	SQS	0.361	0.81
Wilkinson and Algeo (1989)	Net diversification	-0.275	0.07
Wilkinson and Algeo (1989)	Extinction rate	0.235	0.12
Horita et al. (2002)	SIB	-0.15	0.315
Horita et al. (2002)	SQS	-0.09	0.547
Horita et al. (2002)	Net diversification	-0.093	0.545
Horita et al. (2002)	Extinction rate	0.052	0.736

Table S3. Statistical results of Mann-Whitney U tests comparing biodiversity metrics in calcite seas versus aragonite seas. SIB = Sampled-in-bin diversity, SQS = shareholder quorum subsampling diversity, ME = mass extinction intervals.

Mg/Ca ratio model	Biodiversity metric	Test statistic (TS)	<i>p</i>-value	TS (ME excluded)	<i>p</i>-value (ME excluded)
Stanley and Hardie (1998)	SIB	154.5	0.161	134	0.178
Stanley and Hardie (1998)	SQS	156.5	0.177	131.5	0.156
Stanley and Hardie (1998)	Net diversification	209	0.738	122.5	0.128
Stanley and Hardie (1998)	Extinction rate	161.5	0.364	182.5	1
Wilkinson and Algeo (1989)	SIB	214	0.556	254.5	0.11
Wilkinson and Algeo (1989)	SQS	226	0.811	201.5	0.871
Wilkinson and Algeo (1989)	Net diversification	201.5	0.871	120.5	0.063
Wilkinson and Algeo (1989)	Extinction rate	221	0.637	196	0.823
Horita et al. (2002)	SIB	149.5	0.234	139.5	0.409
Horita et al. (2002)	SQS	149	0.223	147	0.545
Horita et al. (2002)	Net diversification	140.5	0.278	107	0.19
Horita et al. (2002)	Extinction rate	211.5	0.389	169.5	0.64

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