# INFERRING VARIATIONS IN THE PRODUCTION RATE, COMPOSITION, AND FATE OF ICHTHYOCARBONATE THROUGHOUT THE PHANEROZOIC

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## KEY FINDINGS

- Marine bony fishes acclimated to seawater with lower Mg/Ca ratios and $SO_4^{2-}$ concentrations produce 3× more ichthyocarbonate, with lower mol%$MgCO_3$, and slower dissolution rates than fishes acclimated to modern seawater
- During Greenhouse periods, marine fishes may have been even more important contributors to the global carbon cycle than they are today

## INTRODUCTION

Marine bony fishes are prolific producers of carbonate minerals in the oceans each year, because they drink seawater to stay hydrated (Wilson et al., 2009; Grosell and Oehlert, 2023). Produced in the intestine of all marine bony fishes studied to date, magnesium-rich carbonate minerals called ichthyocarbonate are excreted to the environment nearly continuously. The formation of these precipitates facilitates water absorption and ensures that marine fishes do not suffer from dehydration in the marine environment (Grosell, 2002). The production of ichthyocarbonate by modern marine fish populations contributes substantially to the global inorganic carbon cycle, with estimates suggesting that ichthyocarbonate

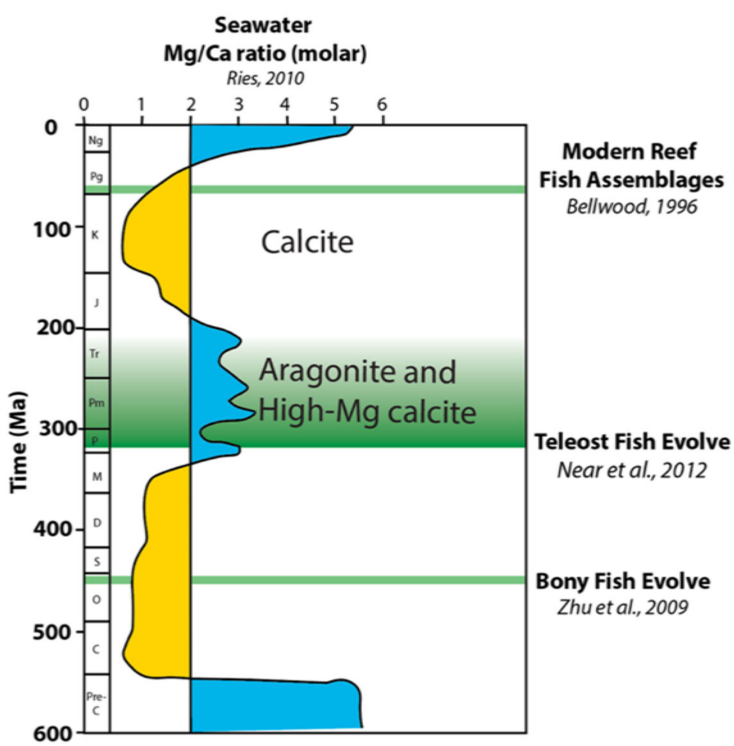


*Figure 1. Changing seawater Mg/Ca ratios throughout the Phanerozoic, as well as major milestones in marine bony fish evolution.*

constitutes 3-33% of new carbonate production in the oceans each year (Wilson et al., 2009; Oehlert et al., 2024a). Research has shown that ichthyocarbonate produced in the marine environment today is composed of carbonate polymorphs including high magnesium calcite (HMC), very high magnesium calcite (vHMC), amorphous calcium magnesium carbonate (ACMC), and minor occurrences of aragonite, brucite or low magnesium calcite (LMC; Salter et al., 2017). Such heterogeneity in carbonate phase composition and high solubility of ichthyocarbonate (Woosley et al., 2012) suggests that ichthyocarbonate is unlikely to accumulate in significant quantities outside of shallow, tropical marine sedimentary environments in modern settings (Perry et al., 2011). However, relatively little information exists about how the composition and sedimentary preservation potential of ichthyocarbonate produced by marine bony fishes may have changed since their evolution more than 400 million years ago (Zhu et al., 2009). Secular changes in seawater chemistry have occurred throughout the Phanerozoic, resulting in mineralogical changes in the composition of inorganic cements precipitated through time. At least three transitions between Aragonite and Calcite Seas have occurred since the rise of marine bony fishes (Fig. 1), indicating that marine fishes have been able to adapt their osmoregulatory strategy, including the production of ichthyocarbonate, to changing seawater chemistry.

## DATASET AND METHODS

We evaluated the effects of changing the Mg/Ca ratios and $SO_4^{2-}$ concentrations in seawater by acclimating Gulf toadfish (*Opsanus beta*) to seawater conditions simulating Mesozoic Greenhouse conditions (Mg/Ca ratios less than 1, and 3× lower $SO_4^{2-}$ concentrations). In this study, we determined ichthyocarbonate excretion rates by measuring the mass of ichthyocarbonate in tanks daily. Mol%$MgCO_3$ was assessed using ICP-QQQ, dissolution rate was measured using a pH-stat approach, total organic carbon content assessed using an elemental analyzer coupled with a continuous flow IRMS, and crystallite morphology assessed using a field emission scanning electron microscope as previously conducted (Folkerts et al., 2024; Grosell et al., 2025).

## RESULTS AND INTERPRETATION

Gulf toadfish acclimated to Mesozoic seawater conditions were found to produce 3.6× more ichthyocarbonate than toadfish acclimated to modern seawater ($p$ <0.001). Fish acclimated to Mesozoic seawater produced ichthyocarbonate with significantly lower mol%$MgCO_3$ (12.4 ± 0.2%) and slower dissolution rates (81.8 μeqv. $g^{-1}$ $h^{-1}$) compared to ichthyocarbonate produced by toadfish acclimated to modern seawater (30.6± 0.2% and 244.2 μeqv. $g^{-1}$ $h^{-1}$, respectively, Fig. 2). In addition, total organic carbon associated with ichthyocarbonate produced by modern fishes (10.1 ± 6.5%) was higher than that found in ichthyocarbonate produced by fishes acclimated to Mesozoic seawater conditions (5.8 ± 3.3%, $p$ = 0.04). The fraction of total rectal base excretion that was found in solid phase versus dissolved bicarbonate also varied significantly between Modern-acclimated and Cretaceous-acclimated fishes ($p$ = 0.01), with Cretaceous-acclimated fishes producing substantially greater quantities of solid phase base excretions (*e.g.*

ichthyocarbonate, 29 ± 8%) than modern-acclimated fishes (7 ± 3%). Preliminary results suggest that the morphology of crystallites in ichthyocarbonate collected from the intestine of fishes in both treatments was similar, and overall particle size may be smaller for ichthyocarbonate excreted by fish acclimated to Cretaceous seas (1.55 mm, *n* =202) than Modern seas (1.98 mm, *n* = 61).

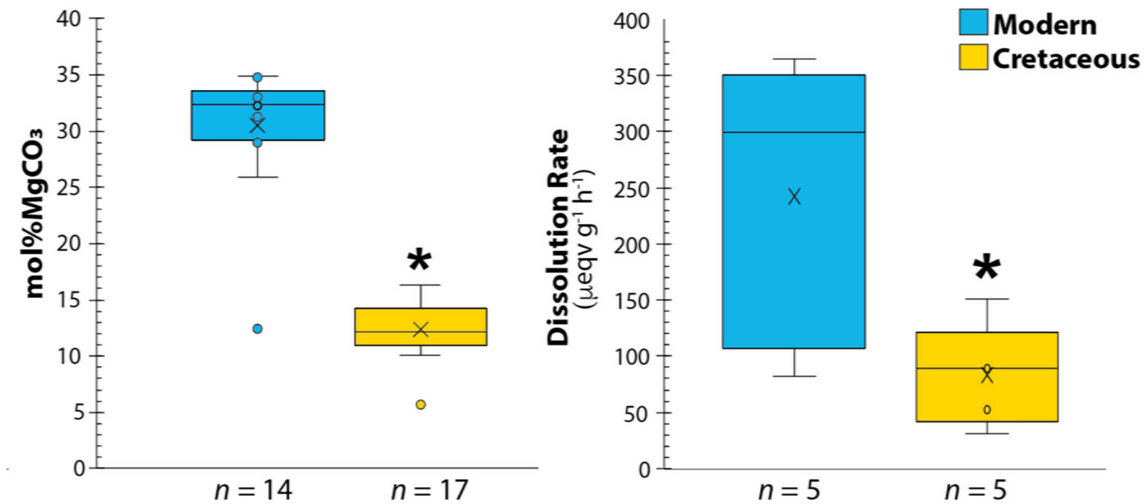


*Figure 2. Mol%MgCO₃ (left) and dissolution rate (right) of ichthyocarbonate produced by Gulf toadfish acclimated to Modern seawater chemistry (blue) and Mesozoic seawater chemistry (yellow).*

## SIGNIFICANCE

Increased production rates coupled with decreased mol%$MgCO_3$ and dissolution rates of ichthyocarbonate produced by fishes acclimated to conditions simulating Mesozoic seawater suggests that ichthyocarbonate produced during the Cretaceous likely deposited in the sediments. Similar crystallite morphology observed in intestinal precipitates suggests that the intestinal physiology responsible for morphology is conserved. Increased sea water temperature, which is proposed to shift the distribution of global fish biomass polewards (Jennings et al., 2008) and potentially result in smaller fishes (Cheung et al., 2013), and increased $pCO_2$, which has been shown to enhanced base excretion by 34% (Heuer et al., 2012, Gregorio et al., 2019; Alves et al., 2020), it is possible that the synergistic effects of changing seawater chemistry, temperature, and carbonate chemistry resulted in an even more important role for marine fishes in the global carbon cycle during Greenhouse intervals than in modern Icehouse intervals.

# REFERENCES


Alves, A., Gregório, S.F., Ruiz-Jarabo, I. and Fuentes, J., 2020. *Intestinal response to ocean acidification in the European sea bass (Dicentrarchus labrax)*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 250, p.110789.

Cheung, W.W.L., Sarmiento, J.L., Dunne, J. Frölicher, T.L., Lam, V.W.Y., Palomares, M.L.D., Watson, R., Pauly, D. (2013) *Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems*. Nature Climate Change. 3, 254-258.

Folkerts, E.J., Oehlert, A.M., Heuer, R.M., Nixon, S., Stieglitz, J., and M. Grosell (2024). *The role of marine fish-produced carbonates in the oceanic carbon cycle is determined by size, specific gravity, and dissolution rate.* Science of The Total Environment, 916: 170044.

Gregorio, S.F., Ruiz-Jarabo, I., Carvalho, E.M. and Fuentes, J., 2019. *Increased intestinal carbonate precipitate abundance in the sea bream (Sparus aurata L.) in response to ocean acidification.* PLoS One, *14*(6), p.e0218473.

Grosell, M. (2002). *Intestinal bicarbonate secretion by marine teleost fish- why and how?* Biochim. Biophy. Acta, 1566, 182-193.

Grosell, M., & Oehlert, A. M. (2023). Staying hydrated in seawater. Physiology, 38(4), 178-188.

Grosell, M., B. Marek, S. Walls, C. Pope, C. Sam, R.M. Heuer, and A.M. Oehlert (2025). *Osmoregulation by the gastro-intestinal tract of marine fish at depth - implications for the global carbon cycle.* Journal of Experimental Biology, 228 (14) jeb249834.

Heuer, R.M., Esbaugh, A.J. and Grosell, M. (2012). *Ocean acidification leads to counterproductive intestinal base loss in the gulf toadfish (Opsanus beta).* Physiological and Biochemical Zoology, 85(5), pp.450-459.

Jennings, S., F. Melin, J.L. Blanchard, R.M. Forster, N.K. Dulvy, and R.W. Wilson, *Global-scale predictions of community and ecosystem properties from simple ecological theory.* Proc.Biol Sci., 2008. 275(1641), 1375-1383.

Oehlert, A. M., Garza, J., Nixon, S., Frank, L., Folkerts, E. J., Stieglitz, J. D., Lu, C., Heuer, R.

M., Benetti, D. D., & Del Campo, J. (2024). *Implications of dietary carbon incorporation in fish carbonates for the global carbon cycle*. Science of the Total Environment, 916, 169895.

Perry, C.T., M.A. Salter, A.R. Harborne, S.F. Crowley, H.L. Jelks, and R.W. Wilson (2011). *Fish as major carbonate mud producers and missing components of the tropical carbonate factory.* Proc Natl Acad Sci U S A, 108(10): 3865-3869.

Salter, M.A., A.R. Harborne, C.T. Perry, and R.W. Wilson (2017), *Phase heterogeneity in carbonate production by marine fish influences their roles in sediment generation and the inorganic carbon cycle.* Sci Rep, 7(1): p. 765.

Wilson, R., Millero, F., Taylor, J., Walsh, P., Christensen, V., Jennings, S., & Grosell, M. (2009). *Contribution of fish to the marine inorganic carbon cycle*. Science, 323(5912), 359-362.

Woosley, R.J., F.J. Millero, and M. Grosell (2012), *The solubility of fish-produced high magnesium calcite in seawater.* Journal of Geophysical Research: Oceans, 2012. 117(C4): C04018.

Zhu, M., W. Zhao, L. Jia, J. Lu, T. Qiao, and Q. Qu (2009) *The oldest articulated osteichthyan reveals mosaic gnathostome characters.* Nature, 458(7237): 469-74.