

DETERMINING THE INFLUENCE OF FISH FEEDING STATE ON ICHTHYOCARBONATE COMPOSITION AND FATE

Caroline L. Tran, Vivian Merk^{1,2}, Sydney M. Cloutier, Bret Marek³, Cameron Sam, Sarah Walls³, Martin Grosell³, and Amanda M. Oehlert

¹⁾ Department of Chemistry and Biochemistry, Florida Atlantic University, Boca Raton, FL, USA

²⁾ Department of Ocean and Mechanical Engineering, Florida Atlantic University, Boca Raton, FL, USA

³⁾ Department of Marine Biology and Ecology, RSMAES, University of Miami, FL, USA

KEY POINTS

- Ichthyocarbonate produced by fed fish is smaller and more complex in shape than that produced by fasted fish.
- Fed fish produce ichthyocarbonate that is more mineralogically variable than that produced by fasted fish.
- Ichthyocarbonate excreted in the ocean may have slower sinking rates and be more variable in solubility than previously estimated.

INTRODUCTION

Marine teleost fish are among the most significant contributors to marine carbonate production through the formation and excretion of ichthyocarbonate, magnesium-rich calcium carbonate precipitates formed in the intestine as a product of osmoregulation (Oehlert et al., 2024a; Wilson et al., 2009). The production and excretion of ichthyocarbonate is an important component in the global carbon cycle, and the degree to which marine fish contribute is determined by the fate of their ichthyocarbonate. As ichthyocarbonate sinks through the water column after excretion, it contributes to oceanic alkalinity budgets through dissolution and to the flux of carbonate to the deep ocean, potentially resulting in seafloor deposition (Folkerts et al., 2024; Hashim et al., 2025; Oehlert et al., 2024b; Saba et al., 2021; Woosley et al., 2012). Studies predicting the fate of ichthyocarbonate employ techniques to characterize mineralogy, morphology, and grain size to inform models estimating the contributions of fish to the global carbon cycle (Folkerts et al., 2024; Grosell et al., 2025; Oehlert et al., 2024b). However, most studies analyzing the effect of ichthyocarbonate mineralogy on fate have been conducted using ichthyocarbonate produced by fasted fish, resulting in estimates that may not be representative of the actual contribution of wild fish to the carbon cycle.

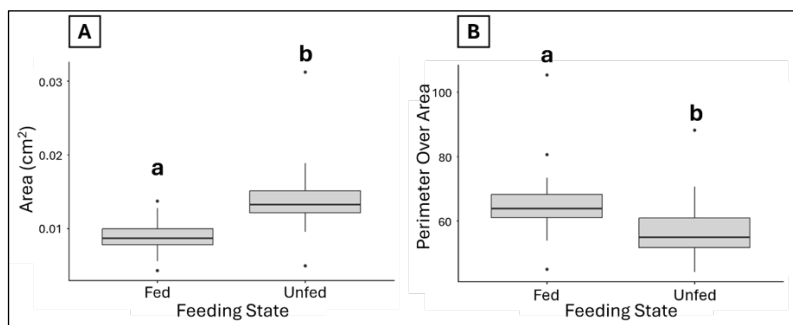


Figure 1: (A) Average area (cm²) of ichthyocarbonate grains produced by fed and fasted toadfish. (B) Average perimeter over area values of ichthyocarbonate grains produced by fed and fasted toadfish. a and b represent statistically significant differences.

METHODS

To fill knowledge gaps about the influence of mineralogy on ichthyocarbonate fate, the mineralogy, crystallite morphology, and grain sizes of ichthyocarbonate produced by fed and fasted fish were characterized. An experiment was conducted using two groups of small to medium Gulf toadfish (*Opsanus beta*). Two tanks of fish were fed twice weekly, and two tanks were fasted for the duration of the experiment. Excreted ichthyocarbonate was sampled from each tank bottom daily for 12 days and photographed in seawater with a scale bar for grain size analysis. Two to three grains from each sample were preserved with 2% glutaraldehyde in filtered seawater buffered with 0.05 mol l⁻¹ sodium cacodylate for scanning electron microscopy (SEM).

Grain size analysis was performed using Binary Morphometrics Analyzer, a Python-based application developed to quantify object morphometrics from images (Cloutier, University of Miami, 2026). Measurements of grain area (cm²), perimeter (cm), eccentricity, equivalent diameter (cm), major axis length (cm), minor axis length (cm), and perimeter over area were provided for each identified grain. Averages for each sample were used to conduct statistical tests to evaluate differences between fed and fasted fish.

The mineralogy of the ichthyocarbonate was assessed by X-ray diffraction (XRD) at Florida Atlantic University. Dried, uncrushed ichthyocarbonate samples were sprinkled onto a sample holder with a silicon zero-background insert, and two scans were conducted on each sample. An intermediate scan was performed from 10° to 90° 2θ, using a step interval of 0.01° and a count time of 200 s per step, and a high-resolution scan was performed from 28° to 32° 2θ, using a step interval of 0.01° and a count time of 1800 s per step. The diffractograms from the high-resolution scans were normalized to their maximum intensity and overlaid by treatment group (Fig. 2).

The preserved ichthyocarbonate samples were dehydrated through a graded ethanol series and dried using hexamethyldisiloxane (HMDS). The dried ichthyocarbonate was then stubbed and coated with a gold sputter-coater. Imaging was conducted in a field emission SEM at a working distance between 9.5 and 10.2 mm and a voltage of 3.0 kV. Images were taken at magnifications of 1,000x, 5,000x, and 10,000x. To maintain a double-blind analysis, sample identifiers and treatments were unknown to the researcher conducting imaging and interpretation.

RESULTS

Grain Size

Average areas of ichthyocarbonate excreted by fed fish (0.009 ± 0.002 cm², n = 23) were smaller than those excreted by fasted fish (0.014 ± 0.005 cm², n = 23) by 35.7% (Fig. 1A; Mann Whitney Wilcoxon U test, p < 0.05). A similar pattern was observed for the other characteristics corresponding to size, with fed fish excreting ichthyocarbonate that had smaller perimeters, equivalent diameters, major axis lengths, and minor axis lengths than those excreted by fasted fish (Two-sample t-test, Mann Whitney Wilcoxon U test, p < 0.05 for each). Regarding shape complexity, ichthyocarbonate excreted by fed fish had higher

perimeter over area values than those excreted by fasted fish, resulting in ichthyocarbonate grains that are more complex in shape (Fig. 1B; Mann Whitney Wilcoxon U test, $p < 0.05$). No other metrics exhibited significant differences between treatments.

Mineralogy

Preliminary observations show that there is greater variability among the diffraction patterns of ichthyocarbonate produced by fed fish compared to samples collected from tanks holding fasted fish. The fasted diffraction patterns overlapped closely, with peak maxima ranging from 30.24° to 30.42° 2θ . The fed patterns showed more variability, with peak maxima ranging from 29.91° to 30.46° 2θ . Peak positions were significantly more variable in the fed group compared to the fasted group (Levene's test, $p = 0.034$).

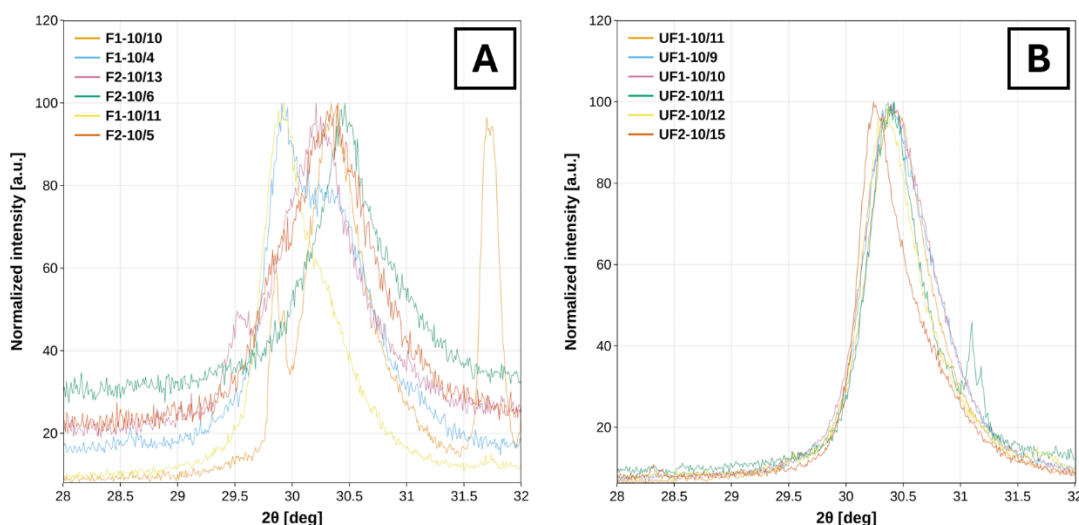


Figure 2: Normalized X-ray diffraction (XRD) patterns from (A) fed toadfish and (B) fasted toadfish.

Morphology

SEM images of the toadfish ichthyocarbonate show highly variable crystallites ranging in size and appearance. They occur in several morphologies including monocrystalline ellipsoids, nanospheres, and aggregates of nanocrystalline material. Interpretation and comparison of crystallite morphologies between ichthyocarbonate excreted by fed and fasted fish are currently ongoing.

SIGNIFICANCE

The observation that fed fish excrete smaller ichthyocarbonate with more shape complexity than ichthyocarbonate produced by fasted fish suggests that the sinking rate of ichthyocarbonate in natural oceanic conditions may be slower than previously published estimates by Folkerts et al. (2024) and Grosell et al. (2025). Slower sinking rates increase the likelihood of carbonate dissolution in the water column rather than export of ichthyocarbonate to sediments in the deep ocean. The effect of feeding on mineralogical variability suggests that ichthyocarbonate excreted in the ocean may be more variable in solubility and dissolution rates

than previously thought, influencing how it is partitioned between dissolution and export carbon flux.

Previous work estimating the contribution of marine fish to the carbon cycle utilizes data from ichthyocarbonate produced by fasted fish in their models, leading to estimates that may not reflect the actual contribution by wild fish in their natural environment. This study addresses this uncertainty by determining how the feeding state of fish influences ichthyocarbonate mineralogy, morphology, and grain size, which will facilitate updates to current models (e.g., Folkerts et al., 2024) and improve predictions of ichthyocarbonate fate. With these findings, we can refine our estimates of the contribution of ichthyocarbonate to oceanic alkalinity budgets and to carbon fluxes to the deep ocean, improving our understanding of the role of marine teleost fish in the global carbon cycle.

REFERENCES

- Cloutier, S.M., 2024. Changes in production rate and composition of ichthyocarbonate produced by the Gulf toadfish as a function of feeding. BSc thesis, University of Miami, p. 1–30.
- Folkerts, E.J., Oehlert, A.M., Heuer, R.M., Nixon, S., Stieglitz, J.D., Grosell, M., 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. <https://doi.org/10.1016/j.scitotenv.2024.170044>
- Grosell, M., Marek, B., Walls, S., Pope, C., Sam, C., Heuer, R.M., Oehlert, A.M., 2025. Osmoregulation by the gastro-intestinal tract of marine fish at depth – implications for the global carbon cycle. *Journal of Experimental Biology* 228, jeb249834. <https://doi.org/10.1242/jeb.249834>
- Hashim, M.S., Conte, M.H., Salter, M.A., Pedrosa-Pamies, R., Weber, J.C., Hayden, M.G., Wilson, R.W., Perry, C.T., Crowley, S.F., Dennis, P.F., Bish, D., Subhas, A.V., 2025. Fish Carbonates in the North Atlantic and Their Potential Role in the Carbon Cycle. *Global Biogeochemical Cycles* 39, e2024GB008387. <https://doi.org/10.1029/2024GB008387>
- 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., Gomez, F.A., Grosell, M., 2024a. Implications of dietary carbon incorporation in fish carbonates for the global carbon cycle. *Science of The Total Environment* 916, 169895. <https://doi.org/10.1016/j.scitotenv.2024.169895>
- Oehlert, A.M., Walls, S., Arista, K., Garza, J., Folkerts, E.J., Vitek, B.E., Tale Masoule, S., Pollier, C.G.L., Duchâtellier, G., Stieglitz, J.D., Benetti, D.D., Heuer, R.M., Ghahremaninezhad, A., Grosell, M., 2024b. Organic Coatings Reduce Dissolution Rate by an Order of Magnitude for Carbonate Minerals Produced by Marine Fish. *Global Biogeochemical Cycles* 38, e2024GB008176. <https://doi.org/10.1029/2024GB008176>
- Saba, G.K., Burd, A.B., Dunne, J.P., Hernández-León, S., Martin, A.H., Rose, K.A., Salisbury, J., Steinberg, D.K., Trueman, C.N., Wilson, R.W., Wilson, S.E., 2021. Toward a better understanding of fish-based contribution to ocean carbon flux. *Limnology & Oceanography* 66, 1639–1664. <https://doi.org/10.1002/lno.11709>
- Wilson, R.W., Millero, F.J., Taylor, J.R., Walsh, P.J., Christensen, V., Jennings, S., Grosell, M., 2009. Contribution of Fish to the Marine Inorganic Carbon Cycle.
- Woosley, R.J., Millero, F.J., Grosell, M., 2012. The solubility of fish-produced high magnesium calcite in seawater. *Journal of Geophysical Research: Oceans* 117. <https://doi.org/10.1029/2011JC007599>