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Feeding-induced variations in ichthyocarbonate production and composition by the Gulf toadfish

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Introduction

Marine fish have a significant impact on the global carbon cycle but are often overlooked. One example of this contribution is their production of ichthyocarbonates (Oehlert et al., 2024). Ichthyocarbonates are Mg-rich calcium carbonate pellets produced by marine bony fish as a byproduct of osmoregulation. Having only been discovered in 1991 (Walsh et al., 1991), much is still unknown about their morphology, geochemical characteristics, and behavior in the natural environment.

Figure 1. Gulf toadfish specimen with excreted ichthyocarbonate precipitates.

Problem Statement

Previous studies of ichthyocarbonate production rate have been conducted using unfed fish. Consequently, while prior results provide an important foundation for understanding the role of marine fish in the carbon cycle, datasets produced from fed fish are lacking. **Here, we investigate the influence of feeding state on production and composition of ichthyocarbonate.**

Methods

The experimental tanks were stocked with Gulf toadfish (*Opsanus beta*) weighing 40 - 100 g. Ichthyocarbonate was collected daily from tank bottoms using disposable pipettes. Days since last feeding were recorded, and samples were divided into two treatments based on prior measurements of Specific Dynamic Action (SDA) for toadfish which indicates toadfish experience elevated metabolic rate from days 0-3 after feeding ("Fed"), with a return to baseline conditions after four days ("Fasted"). All experimental protocols were completed in accordance with University of Miami Institutional Animal Care and Use Committee (IACUC) approved practices.

After collection, four analyses were performed:

1. Measurements of wet weight to calculate **production rate**
2. **Particle morphology** analysis using ImageJ (Fiji) and MATLAB
3. Agilent 8900 ICP-QQQ mass spectrometry for assessment of **mol%$MgCO_3$ and phosphorus** concentration in mineral fraction using MS/MS mode with O_2 gas in the collision-reaction cell (CRC)
4. Zeiss Ultra Plus Field Emission SEM & Apollo 10 EDAX for **crystallite morphology and mol%$MgCO_3$**

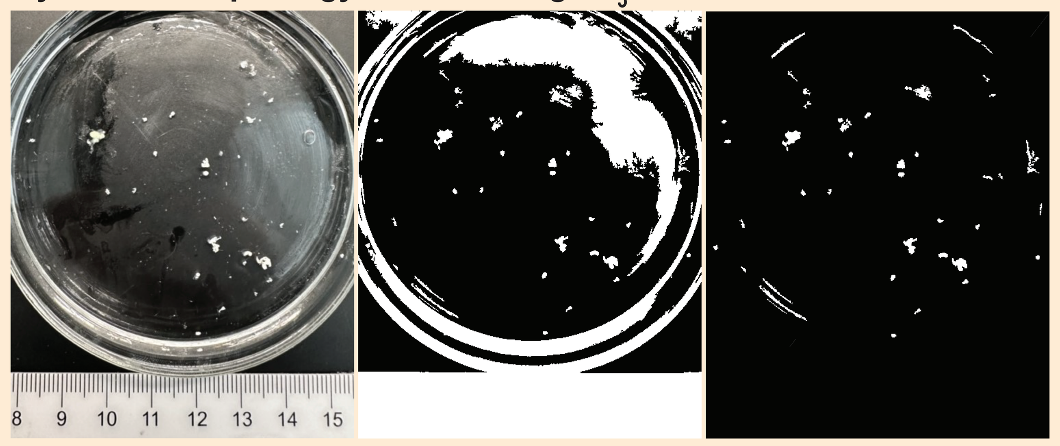


Figure 2. Raw photograph (left), binary (middle) and filtered (right) images of ichthyocarbonate excreted during a 24-hr period. Remaining glare is manually filtered out and particle morphometrics calculated in MATLAB.

Results

1. Production Rate

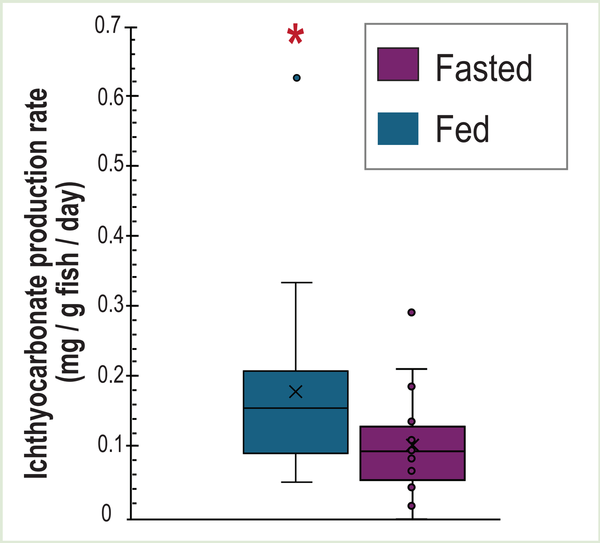


Figure 3. Comparison of ichthyocarbonate production rate in ichthyocarbonate (mg) per mass of fish (g) per day.

2. Particle morphology

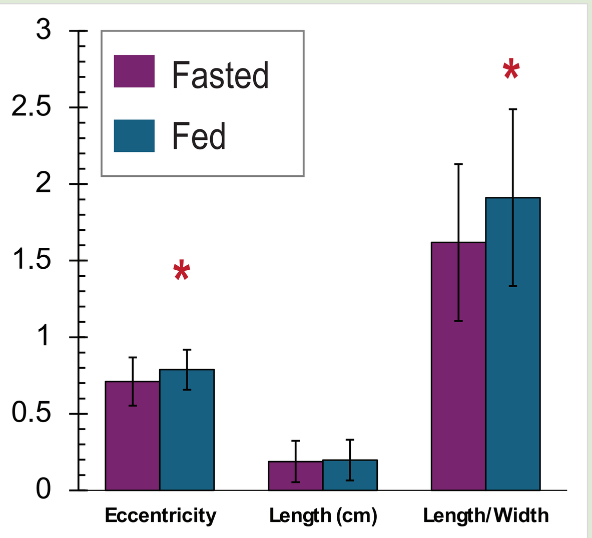


Figure 4. Results of morphometric analysis of ichthyocarbonate. Significant differences between ichthyocarbonate produced by fed (n = 20) and fasted (n = 16) are marked with an asterisk.

Fed Gulf toadfish produce ~ 70 % more ichthyocarbonate per day than fasted fish (p < 0.05).

Ichthyocarbonate produced by fed Gulf toadfish is characterized by higher eccentricity (11 %), and length/width ratios (18 %) compared to ichthyocarbonate produced by fasted fish ($p < 0.05$).

3. Elemental Composition (Mass Spectrometry; ICP-QQQ)

No significant difference was observed in mol%$MgCO_3$ ($p > 0.05$) between ichthyocarbonate produced by fed and fasted Gulf toadfish.

Phosphorus was significantly higher ($p < 0.05$) in ichthyocarbonate produced by fed fish.

Average concentrations:

Fasted: 8.59 ± 22.06 g/kg

Fed: 18.68 ± 26.10 g/kg

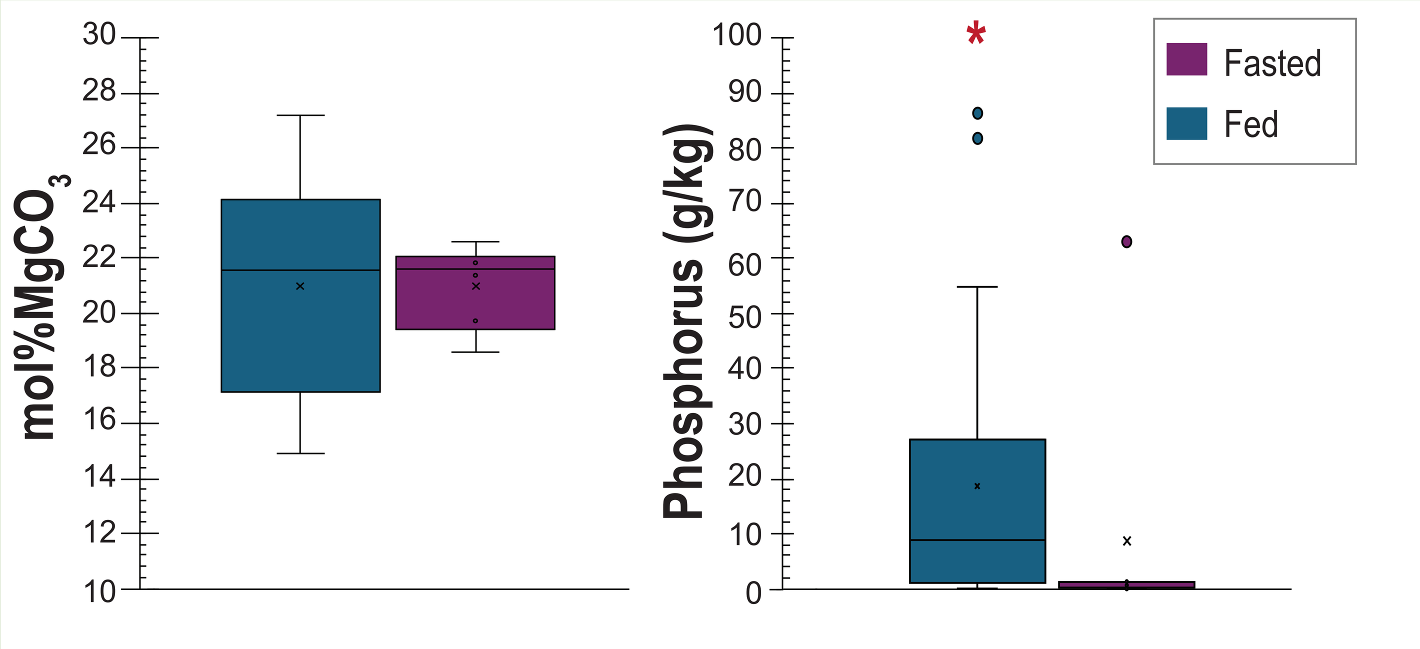
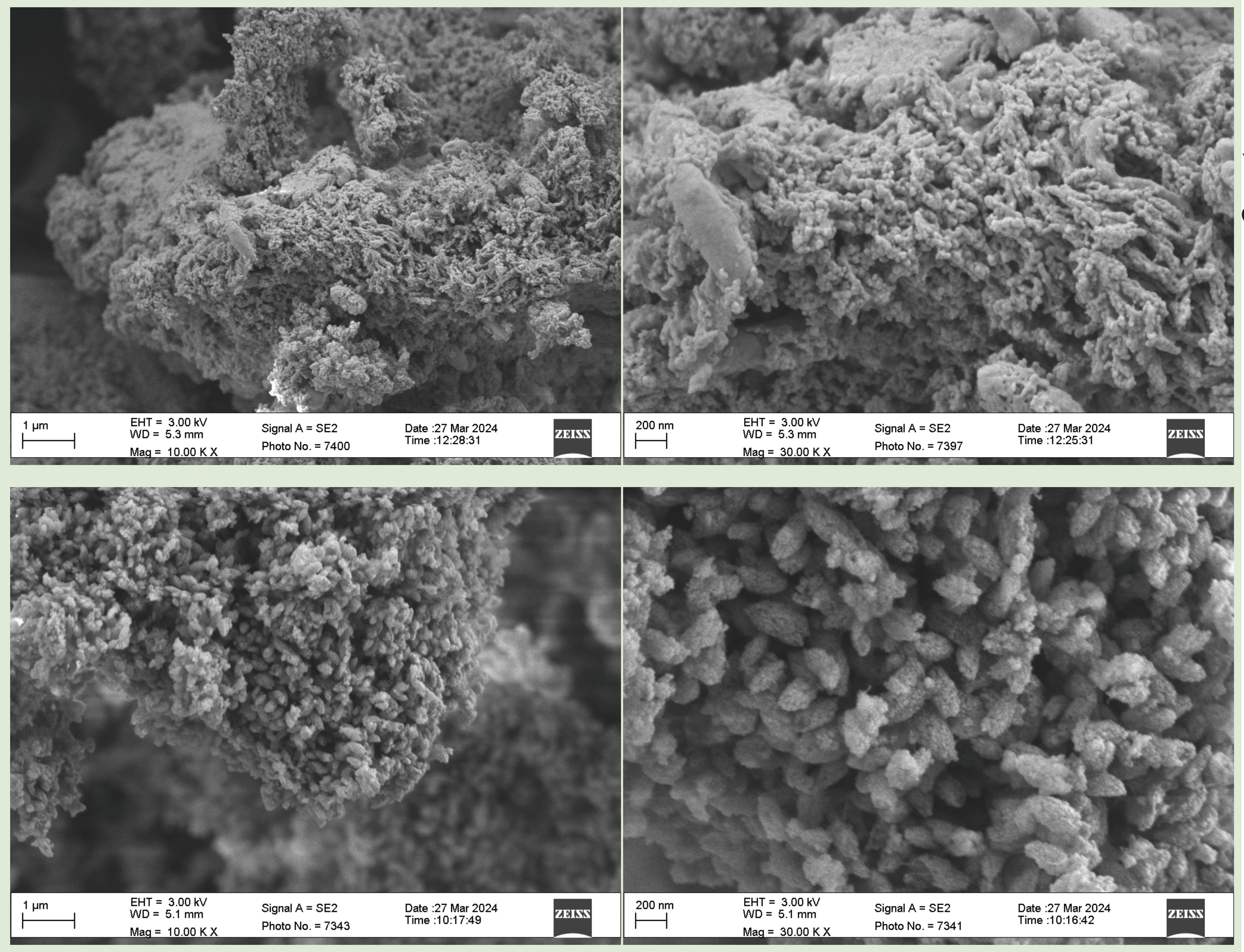


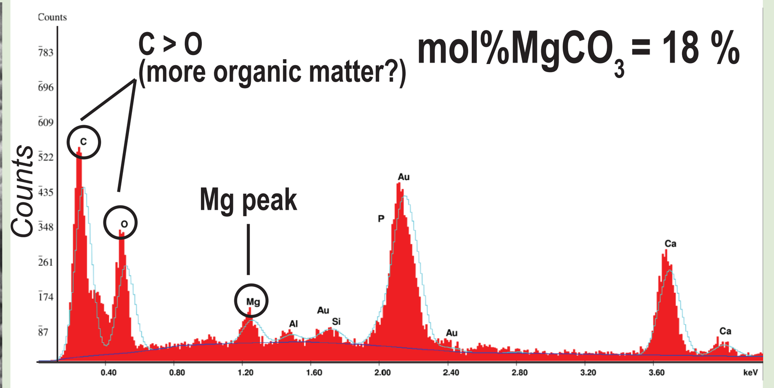
Figure 5. Box and whisker plots showing mol%$MgCO_3$ and phosphorus concentrations in daily sample collections for ichthyocarbonate produced by Gulf toadfish under fed (n = 20) and fasted (n = 6) conditions.

4. Scanning Electron Microscopy and Energy Dispersive Spectroscopy (SEM-EDS)

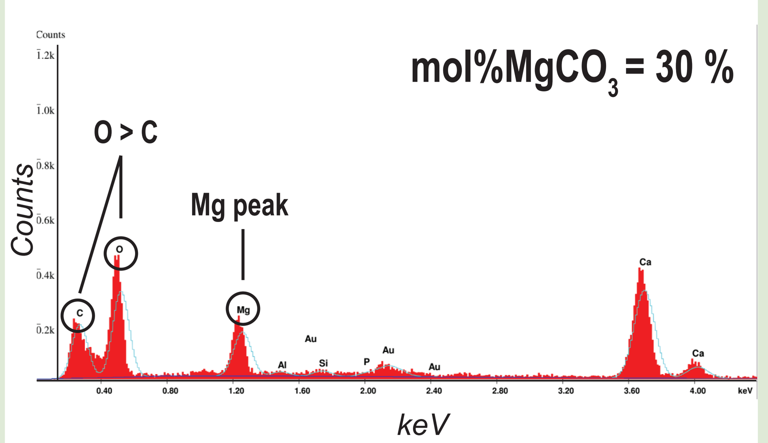
Fasted (12 days post feeding)

Fed (3 days post feeding)





Observations: Crystallites occur in network, with stringy, linear appearance and low mol%$MgCO_3$



Observations: Crystallites were ellipsoidal, ranging from 300 - 600 nm in length, and high mol%$MgCO_3$

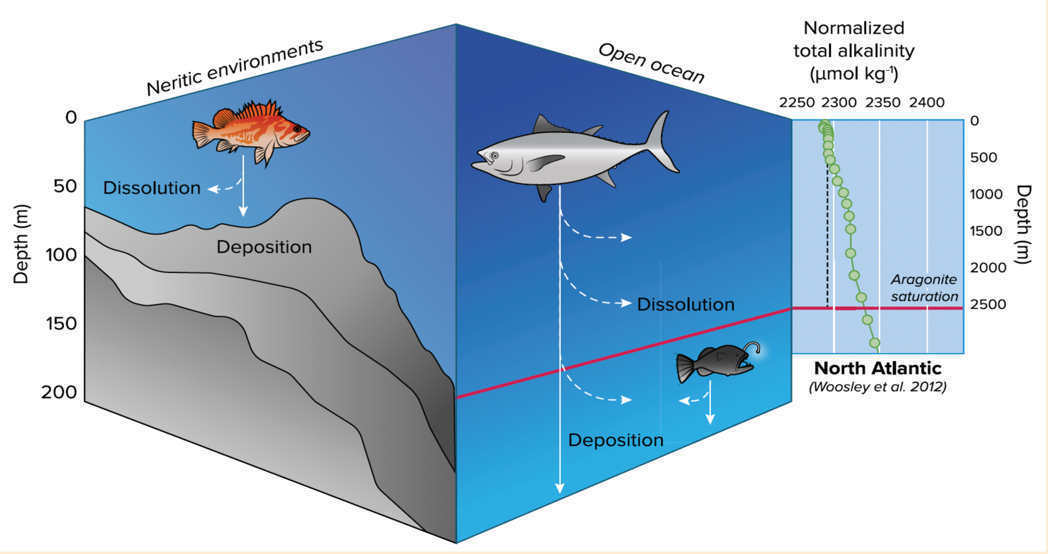
Figure 6. Images of ichthyocarbonates from Scanning Electron Microscopy at 10 K X magnification (left) and 30 K X magnification (middle), with Energy Dispersive X-ray Spectroscopy results (right).

Discussion

As the climate warms, resources such as food may become limited to global fish populations. It is crucial to determine how this will affect ichthyocarbonate production and fate (Grosell and Oehlert, 2023; Oehlert et al., 2024). The results of this investigation showed the following:

1. Production rate by fed toadfish is ~ 70 % higher than fasted fish.
2. Fed fish ichthyocarbonate is more ellipsoidal than fasted fish.
3. Ichthyocarbonate bulk mol%$MgCO_3$ does not change with fasting, but phosphorus content does.
4. Crystallite morphology, size, and mol%$MgCO_3$ were impacted by fasting. EDS measurements suggest lower mol%$MgCO_3$ in crystallites produced by fasted fish, but more replication is needed.

A major finding of this study is that ichthyocarbonate production rate decreased significantly when fasted. Thus, prior estimates of global ichthyocarbonate production rate (Wilson et al., 2009; Oehlert et al., 2024) are likely underestimates. **Compositional and morphometric changes were also shown to occur with fasting**, which has implications for the fate of ichthyocarbonate in the oceans. Ichthyocarbonate fate is thought to be a fundamental determinant of the role of marine fish in the global carbon cycle.



Grosell and Oehlert, 2023

Acknowledgements

We acknowledge Dr. Giacomo Po, Dr. Charles Tomonto, and Matthew Maron at the College of Engineering for access to the sputter coater and SEM-EDS used in crystallite morphology and EDS analysis. I am also grateful for the support of the Rosenstiel School through their Small Undergraduate Research Grant Experience Award and the Geological Society of America's Southeastern Section Student Travel Grant.

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