

ORGANIC MATTER MATTERS: QUANTIFYING THE CONTENT AND COMPOSITION OF ICHTHYOCARBONATE-ASSOCIATED ORGANIC MATTER

Cameron Sam, Bret Marek¹, Caroline Tran, Sarah Walls¹, Roxanne Reed¹, Martin Grosell¹, and Amanda M. Oehlert

²⁾ *Department of Marine Biology and Ecology, RSMAES, University of Miami, FL 33149, USA*

KEY POINTS

- Ichthyocarbonate-associated organic matter content varies, but not predictably, across fish of different lifestyles.
- Marine fish size and feeding state affect the content and composition of ichthyocarbonate-associated organic matter.
- Sinking ichthyocarbonate likely sequesters carbon in the deep ocean and potentially in marine sediments.

INTRODUCTION

Marine fish are noteworthy drivers of carbon fluxes out of the ocean's euphotic zone through the production of organic carbon (fecal material and deadfall) and inorganic carbon (respired CO₂ and excreted HCO₃⁻; Saba et al., 2021). However, marine teleost fish also contribute to the biological and inorganic carbon pumps via ichthyocarbonate production (Grosell and Oehlert, 2023). Ichthyocarbonate is predominantly composed of a high-magnesium calcite mineral produced in the intestine of marine teleost fish as a product of osmoregulation (Wilson et al., 2002). Ichthyocarbonate is associated with substantial quantities of organic matter (OM), ranging from ~3–40%, representing another source of particulate organic carbon (POC) contributed by marine fish to the biological pump (Oehlert et al., 2024b). By shielding ichthyocarbonate from contact with seawater, organic matter plays a vital role in reducing dissolution rate and increasing the depth to which sinking ichthyocarbonate reaches prior to dissolution or deposition (Oehlert et al., 2024b). Likewise, the denser mineral fraction of ichthyocarbonate helps shuttle embedded organic matter to depth ("ballast effect", Armstrong et al., 2002), thereby increasing potential export rates (Oehlert et al., 2024b). Despite the importance of ichthyocarbonate-associated organic matter in the biological pump, knowledge gaps exist regarding the physiological controls on the concentration and composition of ichthyocarbonate-associated organic matter.

To address the knowledge gap of how characteristics like fish lifestyle, fish size, and feeding state affect total organic carbon content (% TOC) and organic matter composition, we measured 1) the effect of fish lifestyle on % TOC in ichthyocarbonate-associated OM, 2) the effect of fish size on $\delta^{13}\text{C}_{\text{org}}$ values and molar C/N ratios in ichthyocarbonate-associated OM produced by gulf toadfish and sergeant majors, and 3) the effect of feeding state on % TOC in ichthyocarbonate-associated OM produced by gulf toadfish. The insights from these studies will improve estimates of organic matter contributions by marine fishes while providing measurements specific to fish with different lifestyles, sizes, and feeding states. Refined estimates of fish-based contributions to ocean

carbon fluxes will further our understanding of ichthyocarbonate fate and its carbon sequestration potential.

METHODS

We examined ichthyocarbonate produced by gulf toadfish (*Opsanus beta*), a benthic and sedentary species, and sergeant major (*Abudefduf saxatilis*), a reef-associated, free-swimming fish. Gulf toadfish were split into fed (< 3 days past feeding) and fasted (> 3 days past feeding) treatments to determine differences in organic matter composition by feeding state. Sergeant majors could not be fasted due to their elevated metabolic demands and thus were excluded from the feeding-state comparison. Ichthyocarbonate was siphoned from tank bottoms holding individuals of each of the two species, separated from any fecal matter, rinsed briefly in Milli-Q water, and allowed to dry at ambient temperatures. We then dissolved ichthyocarbonate samples in 1% nitric acid (HNO₃), collected the remaining organic matter via filtration on a glass microfiber filter, and ran these acid-insoluble residues on an EA Isolink CNS elemental analyzer and DeltaQ continuous-flow isotope ratio mass spectrometer.

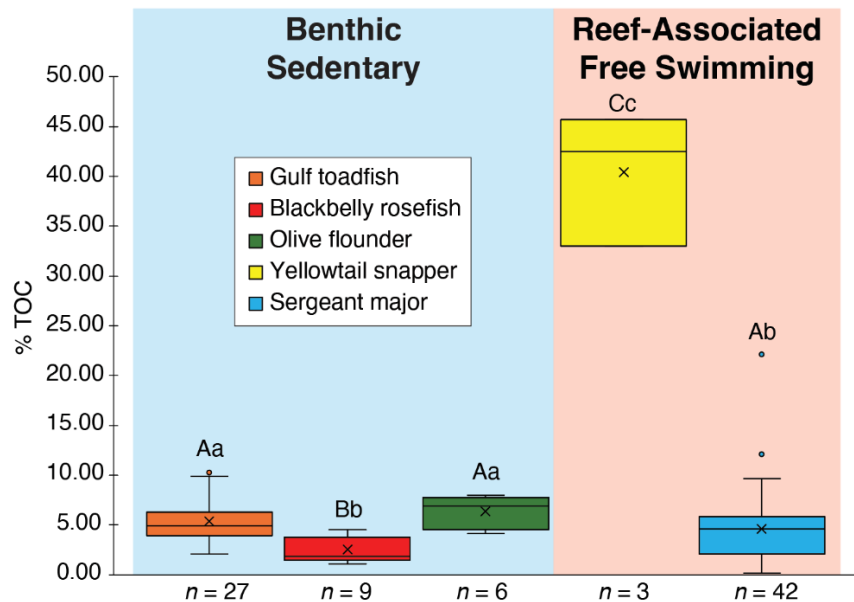


Figure 1: % TOC in ichthyocarbonate-associated organic matter collected from gulf toadfish and sergeant major (this study, Oehlert et al., 2024a), blackbelly rosefish (Grosell et al., 2025), as well as olive flounder and yellowtail snapper (Oehlert et al., 2024a).

RESULTS

Influences of lifestyle on ichthyocarbonate organic matter content and composition

Lifestyle was not found to affect ichthyocarbonate % TOC or molar C/N ratios. Ichthyocarbonate-associated organic matter did not differ in % TOC between gulf toadfish and sergeant majors (Student's t-test, $p = 0.40$). We also compared these new results with those of ichthyocarbonate produced by the blackbelly rosefish (*Helicolenus dactylopterus*; Grosell et al., 2025) and the gulf toadfish,

olive flounder (*Paralichthys olivaceus*), and yellowtail snapper (*Ocyurus chrysurus*) from Oehlert et al. (2024a). Gulf toadfish, blackbelly rosefish, and olive flounder have a benthic lifestyle, while yellowtail snapper and sergeant majors have reef-associated and free-swimming lifestyles. We found that % TOC varies, but not predictably, by fish lifestyle, with no correlation observed between the two factors (Figure 1). Additionally, there was no difference in molar C/N ratios of ichthyocarbonate-associated organic matter between gulf toadfish and sergeant majors (Student's t-test; $p = 0.87$).

Influences of fish size on ichthyocarbonate organic matter content and composition

Fish size exhibited a variable influence on % TOC and C/N ratios. Ichthyocarbonate-associated organic matter produced by small and large gulf toadfish did not differ in % TOC (Student's t-test, $p = 0.43$) or in molar C/N ratios (Student's t-test, $p = 0.85$). For ichthyocarbonate-associated organic matter produced by small and large sergeant majors, % TOC did not differ (Student's t-test, $p = 0.97$), but molar C/N ratios were higher for small sergeant majors (10.14 ± 0.87 ; $n = 9$) than for large individuals (5.23 ± 0.71 ; $n = 25$; Student's t-test, $p < 0.05$, Figure 2). The significant difference remained even after accounting for differences in the C/N ratios of the diets fed to the two experimental groups (Student's t-test, $p < 0.05$).

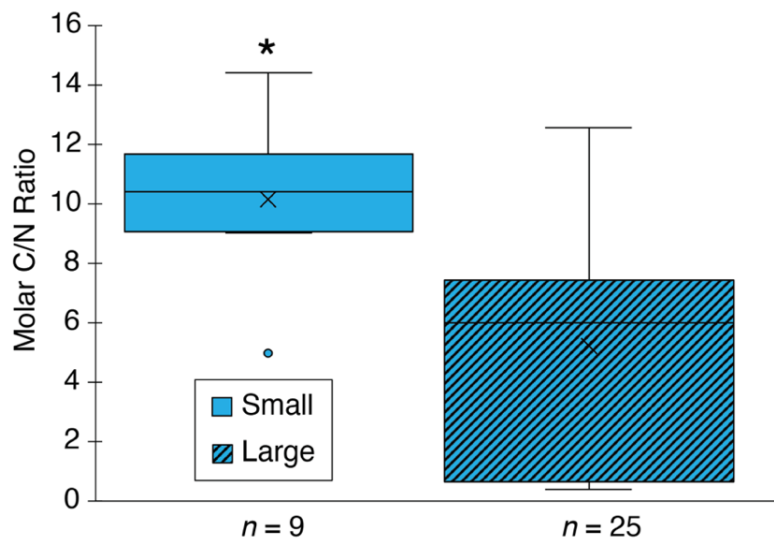


Figure 2: Molar C/N ratios in ichthyocarbonate-associated organic matter collected from small (<40 g) and large (>40 g) sergeant majors.

The $\delta^{15}\text{N}$ values of ichthyocarbonate-associated organic matter did not differ between small ($+13.3 \pm 0.7 \text{ ‰}$; $n = 10$) and large ($+12.3 \pm 0.8 \text{ ‰}$; $n = 11$, Student's t-test, $p = 0.32$) gulf toadfish with an average $\delta^{15}\text{N}$ value across the size classes of $12.8 \pm 0.5 \text{ ‰}$ ($n = 21$). The $\delta^{15}\text{N}$ values of organic matter did not differ between small ($+7.4 \pm 0.6 \text{ ‰}$; $n = 13$) and large ($+6.0 \pm 0.4 \text{ ‰}$; $n = 25$) sergeant majors, with an average $\delta^{15}\text{N}$ value across both size classes of $6.5 \pm 0.3 \text{ ‰}$ ($n = 38$). After adjusting for differences in $\delta^{15}\text{N}$ values of their diets, large sergeant major ichthyocarbonate is characterized by higher $\delta^{15}\text{N}$ values than small sergeant major ichthyocarbonate (Student's t-test, $p < 0.05$). After adjusting for dietary $\delta^{15}\text{N}$ values, there was no difference in values between ichthyocarbonate-associated organic matter produced by gulf toadfish and sergeant majors (Student's t-test, $p = 0.25$).

Influences of feeding on ichthyocarbonate organic matter content and composition

We found that % TOC does not vary by size in fed gulf toadfish (ANOVA, $F_{(2, 29)} = 1.44$, $p = 0.25$) but does increase with size for fasted individuals (ANOVA, $F_{(2, 31)} = 29.99$, $p < 0.05$). These observations suggest that elevated metabolic rates and associated physiological responses driven by recent feeding may influence organic matter production involved with ichthyocarbonate formation.

SIGNIFICANCE

Variations in the amount and composition of the organic matter can impact the fate of ichthyocarbonate (Oehlert et al., 2024b). For example, molar C/N ratios in POC can affect the likelihood that microbes will colonize a sinking ichthyocarbonate grain and determine their ability to remineralize the organic matter (Cuevas-Lara et al., 2024; Geider and La Roche, 2002). Higher molar C/N ratios in ichthyocarbonate-associated organic matter produced by small sergeant majors indicate that the grains they produce may be less likely to remineralize and persist for longer, thus reaching greater depths.

Updated metrics of % TOC, molar C/N ratios, and $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ values will allow researchers to better characterize and quantify ichthyocarbonate organic matter and its effects on ichthyocarbonate fate among different fish populations. Results from this study could be incorporated into the FEISTY model (Petrik et al., 2020), which can be used to predict how future ocean and climate conditions will impact fish biomass and productivity, while accounting for different fish lifestyles and sizes. Implementing results from this study into FEISTY could allow researchers to more accurately estimate projected changes in ichthyocarbonate organic matter production among fish of different lifestyles and sizes. Refined estimates of fish-based contributions to the biological pump and carbonate pump will further our understanding of the understudied role of teleost fishes in marine carbon fluxes which drive climate on submillennial time scales.

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