REFINING ESTIMATES OF DIETARY CARBON UTILIZATION DURING ICHTHYOCARBONATE FORMATION

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KEY FINDINGS

- Marine teleost fish are among the top three modern marine carbonate producers and they incorporate significant amounts of dietary carbon into the carbonate precipitates they form in their intestines.
- The $\delta^{13}C$ values of ichthyocarbonate, intestinal fluid, and muscle samples, along with seawater and dietary carbon sources were measured
- The mineralogical fractionation factor for ichthyocarbonate formation and the assimilation fractionation factor were measured for the first time to refine estimates dietary carbon utilization in ichthyocarbonate formation.

INTRODUCTION

Marine teleost fish are among the top three carbonate producers in the modern oceans through their precipitation of magnesium-rich calcium carbonate, or ichthyocarbonate, which has historically not been factored into global carbonate budgets (Wilson et al., 2009; Oehlert et al., 2024). Unlike foraminifera and corals which mainly incorporate dissolved inorganic carbon (C_{DIC}) from seawater into their shells and skeletons, marine fish incorporate a substantial proportion of assimilated dietary carbon (C_{DIET}) into total rectal base excretions, which include both ichthyocarbonate and bicarbonate ions (Genz et al., 2008; Oehlert et al., 2024). By incorporating both C_{DIET} and C_{DIC} into ichthyocarbonate, marine fish may influence both the biological and carbonate pumps, processes which are important controls on atmospheric CO_2 concentrations (Grosell and Oehlert, 2023). Higher values of C_{DIET} in ichthyocarbonate could indicate that marine fish play an unexpected role in the biological pump (Grosell and Oehlert, 2023). Physiological mixing models suggest C_{DIET} provides 89–95 % of carbon in total rectal base excretions (Genz et al., 2008), and recent Stable carbon isotope ratio ($\delta^{13}C$) mixing models indicate that C_{DIET} constitutes 28–56 %, but up to 81% of ichthyocarbonate (Oehlert et al., 2024). However, to calculate C_{DIET}, several assumptions must be made, including the fractionation factor for the assimilation of carbon from diet, as well as the mineralogical fractionation factor for ichthyocarbonate precipitation (Oehlert et al., 2024).

DATASET AND METHODS

To refine estimates of C_{DIET} in ichthyocarbonate, this study aimed to empirically define $\Delta_{ichthyo\text{-}IF}$, the mineralogical fractionation factor for ichthyocarbonate precipitated from intestinal fluid (IF), and the assimilation fractionation factor. These goals were achieved by measuring the $\delta^{13}C$ values of the diet, muscle, ichthyocarbonate, and IF of the gulf toadfish (*Opsanus beta*).

Previously, $\Delta_{ichthyo\text{-}IF}$ was assumed for ichthyocarbonate based on measurements conducted on high-magnesium calcite (HMC) with varying magnesium contents formed in inorganic precipitation experiments (i.e., Jimenez-Lopez et al., 2006) and natural dolomite (Sheppard and Schwarcz, 1970). Ichthyocarbonate is often predominantly comprised of HMC with significantly higher mol%$MgCO_3$ (<4–~100 %; Salter et al., 2018) than other marine calcifiers such as aragonitic coral (0 %) and the calcite shells of coccolithophores (<1 %; Morse et al., 2007). Based on the mol%$MgCO_3$ of ichthyocarbonate, the predicted range of $\Delta_{ichthyo\text{-}IF}$ is expected to vary between + 0.9 and +2.2 ‰, with increases in $\Delta_{ichthyo\text{-}IF}$ expected with increasing mol%$MgCO_3$ (Fig. 1).

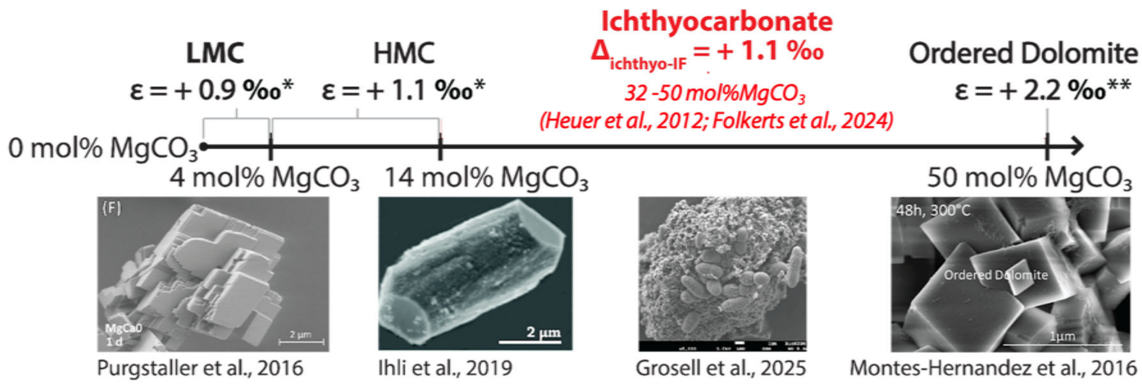

*Figure 1. Known mineralogical fractionation factors (in black) and the ichthyocarbonate mineralogical fractionation factors measured in this study (in red) along with corresponding mol%$MgCO_3$ and SEM images (*Jimenez-Lopez et al., 2006; **Sheppard and Schwarcz, 1970).*

To collect samples, Gulf toadfish were fasted for 3 days, then ichthyocarbonate and intestinal fluid were collected via dissection, along with samples of seawater and excreted ichthyocarbonate. Muscle samples were also collected to fully parameterize the mixing model, and were analyzed on an EA Isolink CNS + Delta Q. The $\delta^{13}C$ values of intestinal fluid, seawater, and ichthyocarbonate were analyzed using the Gasbench Plus + Delta Q. By subtracting the $\delta^{13}C$ values of intestinal fluid from the $\delta^{13}C$ values of ichthyocarbonate, we calculated for the first time the fractionation factor for ichthyocarbonate formation. Using values for $\Delta_{ichthyo\text{-}IF}$, the assimilation fractionation factor and $\delta^{13}C_{DIC}$, we parameterized the IsoError mixing model (Phillips & Gregg, 1991) adapted for ichthyocarbonate (Oehlert et al., 2024) and estimated C_{DIET} and C_{DIC}.

RESULTS AND INTERPRETATION

The average $\delta^{13}C$ values of ichthyocarbonate and intestinal fluid collected from Gulf toadfish were -3.6 ‰ (± 0.3 S.E., n =6) and -4.2 ‰ (± 0.4 S.E., n =6), respectively (Fig. 2). Values for $\Delta_{ichthyo\text{-}IF}$ ranged from -0.6 ‰ to +1.4 ‰, with an average of +0.6 ‰ (± 0.3 S.E.). Two paired samples presented negative values of $\Delta_{ichthyo\text{-}IF}$ which contrasts with known mineralogical fractionation of HMC (Jimenez et al., 2006) so also considered the mineralogical fractionation factor excluding these samples and obtained an average value for $\Delta_{ichthyo\text{-}IF}$ of +1.1 ‰ (± 0.2 S.E.). The average $\delta^{13}C$ value of the fish's diet, which were fed a 50:50 squid-shrimp mix, was -16.2 ‰ and the $\delta^{13}C$ value of the fish's muscle was -

15.2 ‰. These values allowed us to estimate the fractionation factor for the assimilation of carbon from the fish's diet into muscle as +1.0 ‰ (± 0.1 S.E., n = 10). Additionally, the $\delta^{13}C$ value of DIC in seawater ($\delta^{13}C_{DIC}$) collected from the tanks was -0.5 ‰ (± 0.1 S.E., n = 8). Using values for $\Delta_{ichthyo\text{-}IF}$, the assimilation factor, and $\delta^{13}C_{DIC}$, we estimated C_{DIET} to be 0.65 (± 0.1 S.E.) and C_{DIC} to be 0.35 (± 0.1 S.E.). These results suggest that marine fish incorporate substantial proportions of dietary carbon into ichthyocarbonate, confirming their unique role as a link between the marine biological and carbonate pumps. However, physiological mass balance indicates C_{DIET} to be between 89-95 % (Genz et al., 2008). Discrepancy between these estimates may indicate there are additional unknown factors influencing the mineralogical fractionation for ichthyocarbonate precipitation, or that C_{DIET} is partitioned unequally between solid and dissolved phases of total rectal base excretion.

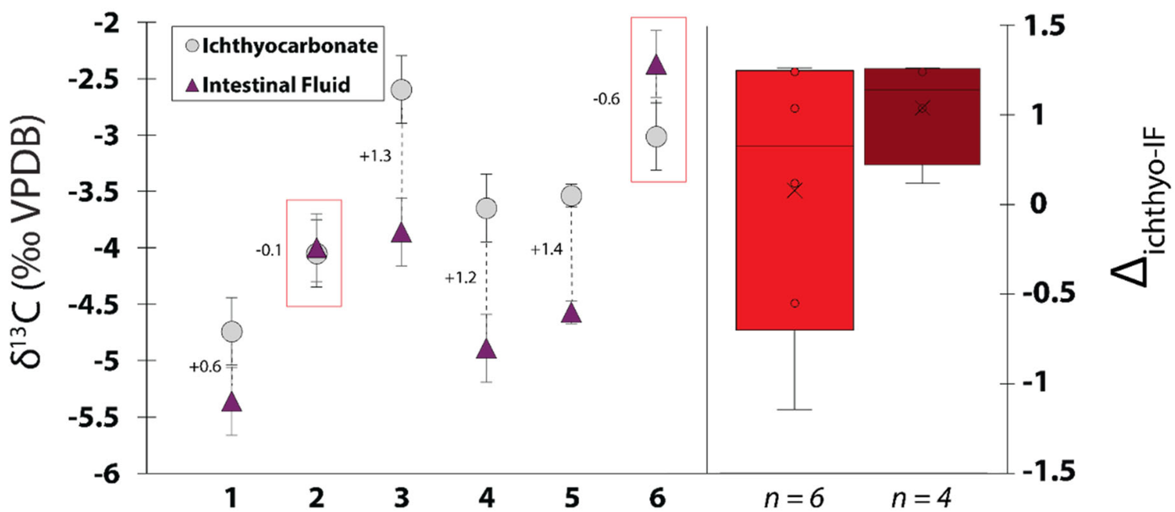

Figure 2. The $\delta^{13}C$ values of paired samples (n = 6) of ichthyocarbonate (gray circles) and intestinal fluid (purple triangles) among 6 fish dissected in this study (left); $\Delta_{ichthyo\text{-}IF}$ values of all paired samples (red) and excluding those pairs with negative mineralogical fractionation factors (dark red, right).

SIGNIFICANCE

We performed the first measurements of paired ichthyocarbonate and intestinal fluid $\delta^{13}C$ values, while developing novel methods for the processing and analysis of IF. These measurements allowed us to define for the first time the mineralogical and assimilation fractionation factors relevant to estimating the proportion of assimilated dietary carbon in ichthyocarbonate. Values for $\Delta_{ichthyo\text{-}IF}$ and C_{DIET} will allow researchers to better estimate the role of marine fish in the global carbon cycle and will provide insight into fractionation factors associated with the formation of biogenic HMC more generally.

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