Calcium carbonate production by fish in temperate marine environments

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Abstract

Marine bony fishes are an important source of calcium carbonate with relevance to sediment production and inorganic carbon cycling. However, knowledge of the production and fate of these carbonates is based primarily on data from warm-water reef fishes, with efforts to assess global-scale implications constrained by assumptions that this small cross section of the global fish community is widely representative. Here we test the extent to which temperature influences fish carbonate mineralogy and morphology by comparing products from temperate settings (20 species at temperatures spanning 10–18°C) against existing data (23–27°C). Overall, carbonate products were mineralogically, compositionally, and morphologically similar throughout the thermal range, and in most cases, we observed no differences within species (18 vs. 24°C) or families (10 vs. 25°C). Confirmation of within-family consistency over large thermal gradients is significant because: (1) it facilitates a substantial range expansion over which fish carbonate production models can be constructed, even where family-level product data are geographically limited; and (2) it implies that the solubility of products from any given fish family varies only due to local carbonate saturation states at excretion (and not crystallographic differences). The only exception was in two members of the Labridae (wrasses), which produced low-Mg calcite (LMC) and minor amorphous calcium–magnesium carbonate (ACMC) at 10°C; the inverse of products from congeneric species at 25°C. This finding could have significant implications for understanding the role of fish carbonates globally because AMC is a highly unstable carbonate polymorph, whereas LMC is very stable.

Continuous and prolific production of calcium carbonate by marine calcifying organisms is a fundamental component of carbonate sedimentary regimes and inorganic carbon cycling (Milliman 1993; Iglesias-Rodriguez et al. 2002). As products of numerous and diverse taxa, these carbonates exhibit a wide range of mineralogies, compositions, and morphologies, and they consequently have contrasting postproduction transport and dissolution potentials (e.g., Fabry 1990; Milliman et al. 1993; Wilson et al. 2009); differences that can be further amplified by thermal and chemical conditions of the environments in which they are produced (Mucci 1983). Accordingly, carbonates of different biogenic and geographic origins can make markedly different contributions to the cycling of sediments and marine inorganic carbon. The need to better understand this system has motivated much research directed at quantifying and characterizing carbonates produced by different taxa in attempts to elucidate their respective roles (e.g., Fabry et al., 1990; Broerse et al. 2000; Rees et al. 2007; Langer 2008; Lebrato et al. 2010; Basso 2012; Perry et al. 2019).

One of the more recently described sources of carbonate sediment is teleost (bony) fishes, which continuously excrete calcium carbonate as a by-product of normal physiological functioning (Walsh et al. 1991; Wilson et al. 2002) and are conservatively estimated to contribute 3–15% of total global marine carbonate production (Wilson et al. 2009). On the basis of initial identification of these carbonates as predominantly high-Mg calcite (HMC), fish carbonates have been invoked as a source of metastable carbonate potentially responsible for at least part of the apparent widespread and unexplained carbonate dissolution taking place in the upper water column (Milliman et al. 1999; Wilson et al. 2009). Subsequent studies corroborated findings that many fish species produce HMC, but they have also documented family-specific production of numerous other carbonate polymorphs, including low-Mg calcite (LMC) and HMC, amorphous calcium–magnesium carbonate (ACMC), aragonite, and monohydrocalcite (Perry et al. 2011; Salter et al. 2012, 2017, 2018). Thus, the role of fish carbonates in the wider marine environment is now hypothesized to be more complex than initially thought.

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However, despite recent expansions of the fish carbonate dataset, which now encompasses 67 fish species and 34 fish families, coverage remains limited both in geographical and ecological scope: most existing data relate to subtropical reefal habitats, where high temperatures and high fish biomass concentrations promote carbonate production rates locally in excess of 100 gCaCO₃ m⁻² yr⁻¹ (Salter et al. 2018). The paucity of data from other settings is especially conspicuous in the extensive temperate marine regions which are thought to account for significant proportions of the global fish carbonate budget, such as the Humboldt Current System and Patagonia Shelf in South America; the northeast and northwest seabords of North America; the North Sea in Europe; and the Yellow Sea, Sea of Okhotsk, and Sea of Japan in Asia (Wilson et al. 2009). A key question thus arises: Are existing fish carbonate compositional and mineralogical data from warm-water regions broadly applicable in temperate regions?

It is widely acknowledged that magnesium uptake in many biogenic calcites correlates positively with temperature (Chave 1954; Dwyer et al. 1995; Weinbauer and Velimirov 1995; Lea et al. 1999; Elderfield and Ganssen 2000; Halfar et al. 2000). Given that carbonate precipitation in the piscine intestine proceeds at environmental temperatures (because most marine teleosts have body temperatures close to ambient water temperatures; e.g., Block et al. 1993), an appealing hypothesis is that: (1) MgCO₃ contents of calcitic fish carbonates will be lower in temperate settings than in warm-water settings; and (2) in consequence, their solubility will also be lower (e.g., Bischoff et al. 1987), with implications for how we interpret their contribution to the inorganic carbon cycle. However, temperature effects on magnesium uptake are not always clear (so-called “vital effects”, sensu Urey et al. 1951), solution pH (Lea et al. 1999), and the ratio of Mg to Ca in solution (Mewes et al. 2014). Within the piscine intestine, proteins capable of regulating magnesium uptake, precipitation rate, and precipitate morphology, at least in vitro, offer a potential organic control (Schauer et al. 2016, 2018; Schauer and Grosell 2017), while somewhat variable and often high values of pH, [Mg]²⁺, and Mg/Ca are likely important inorganic controls (Wilson et al. 1996; Grosell et al. 2001). Intriguingly though, the sole available data point for temperate fish carbonates is consistent with a temperature effect: 10 mol% MgCO₃ in HMC produced at 13°C (Wilson et al. 2009) being low compared with typical values of 20–40 mol% from warm-water settings (20–27°C—Walsh et al. 1991; Perry et al. 2011; Heuer et al. 2012; Salter et al. 2012, 2017, 2018). Both the paucity of temperate data and marked among-family differences in carbonate properties, however, mean that additional data supporting family-level (or lower) product comparisons across large thermal gradients are necessary to: (1) substantiate any apparent relationships and (2) encompass other fish carbonate polymorphs.

Further to this issue, the diversity of carbonate forms produced by different fish families necessitates the cataloguing of products from numerous families and species in order to facilitate construction of local- and regional-scale production models that resolve for mineralogy. While considerable progress has been made in warm-water reef-associated habitats (Salter et al. 2017, 2018), the lack of data beyond these settings leaves a large cross section of fish taxa unaccounted for. Here we begin to resolve these issues by: (1) providing the first detailed characterizations of fish carbonates produced in temperate settings, including within-family product comparisons along a thermal gradient spanning 10–25°C; and (2) expanding the range of teleost taxa studied by 15 species (up to 82 in total) and 11 families (45 in total), thus facilitating refinement of production models across a wide range of environmental settings.

**Materials and methods**

**Study animals and sampling procedures**

Carbonates produced and excreted by 20 fish species were sampled at two locations in the Western Pacific during the southern hemisphere winter. In Queensland, Australia, sampling took place during August 2016 at Moreton Bay Research Station (offshore from Brisbane at 27°29′48″S, 153°23′60″E), where fish were held in seawater aquariums connected to a 20,000 L recirculating system controlled at 18.0°C. Seawater was locally drawn and partially refreshed at 2-week intervals. Salinity was maintained between 35 and 36 practical salinity units (PSU) throughout the sampling period, while pH₅₋₉ ranged from 8.10 to 8.18. In Otago, New Zealand, sampling took place during September 2016 at the Portobello Marine Laboratory (Otago Harbour, Dunedin, at 45°49′41″S, 170°38′27″E), where fish were held in a semi-recirculating system continuously drawing seawater from the adjacent bay. Temperature during this sampling period averaged 10.0°C (ranging from 9.6°C to 11.4°C), while salinity was stable at 35.4 ± 0.2 PSU (1 SD) and pH₅₋₉ ranged from 8.06 to 8.18. Study animals were collected from local waters, either by baited hook-and-line or seine net (Moreton Bay), or by hand net, baited trap, or small trawl net (Otago). In addition, six individual latids (barramundi; *Lates calcarifer*) sampled at Moreton Bay were the same farm-reared individuals as sampled in May 2015 for a previous study (Salter et al. 2018). These fish remained under the care of research station staff during the intervening period and were thus fully acclimated to the aquaria system. Similarly, four individual congriopodids (pigfish; *Congiopodus leucopaecilus*) sampled at Otago had been housed in display tanks supplied by locally drawn seawater for some time prior to sampling. The range of fish species targeted at each study location was broadly representative of the local fish communities. Moreover, target species encompassed several taxa that facilitated within-species or within-family comparisons with existing warm-water data, these including members of the following families: Gobiidae (gobies), non-scarine Labridae (wrasses), Latidae, Lutjanidae (snappers), Pinguipedidae (sandperches), Scorpaenidae (scorpionfishes), Sillaginidae (whittings), and Sparidae (breams).
For most study animals, food intake prior to sampling comprised only natural dietary items consumed in the marine environment. However, in some cases fish were given diets of shrimp, squid, or pilchard for several days (and considerably longer for barramundi and pigfish), at approximately 2–5% of tank biomass per day. In either case, food was withheld throughout the carbonate sampling period and for at least 48 h prior, or until the gut was completely voided of ingested items and excreta comprised only mucus-bound carbonate pellets of white, greenish-yellow, or greenish-blue appearance. This routinely employed approach precludes sampling of carbonates from dietary sources (Wilson et al. 2009; Perry et al. 2011; Foran et al. 2013; Salter et al. 2018). Although digestive processes during feeding are likely to influence gut chemistry—an issue that merits further investigation—available evidence indicates that intestinal carbonates produced by fed fish are similar to those produced by the same fish when fasted (Salter et al. 2012, 2017).

Seawater flowing through sampling tanks at both study sites was filtered to 1 μm to ensure no particulate matter could enter. Tanks were also fitted with false mesh bottoms through which excreted carbonates would sink, thus precluding disturbance by fish (e.g., disaggregation through physical contact, ingestion, etc.). Excreted carbonates were collected from tank floors at 12 h intervals (and in some cases 4 h) using either a siphon or a plastic Pasteur pipette. Previous studies have typically employed 24 h sampling intervals (e.g., Salter et al. 2017, 2018), but observations of ACMC dissolution occurring within this time frame (Foran et al. 2013) necessitate an increased sampling frequency, particularly given the lower water temperatures—and thus carbonate saturation states—of the present study. Immediately after collection, samples were briefly rinsed with deionized water before being soaked in sodium hypochlorite (commercial bleach) for up to 12 h to disaggregate organic mucus coatings. Bleach was then completely removed through repeated rinses with deionized water before samples were dried at 50°C and stored ahead of further analysis.

In order to investigate the possibility that carbonate properties are influenced by temperature, new data derived from these temperate samples were used to make family- or species-level comparisons with either existing subtropical data from Australia and The Bahamas (Perry et al. 2011; Salter et al. 2012, 2014, 2017, 2018) or new subtropical data derived from archived samples used in those studies. It is relevant here to note that the 18°C sampling temperature at Moreton Bay Research Station was approximately 3°C below local ambient seawater from which fish were collected. Consequently, the possibility of a delay between acute temperature transfer and the manifestation of any effects on carbonate properties must be considered. Because no changes were observed throughout the sampling period (48–168 h after transfer to 18°C, and in some cases up to 288 h), this possibility would appear unlikely. Moreover, barramundi were held at this temperature (6°C below the normal operating temperature of the aquaria) for > 1000 h and carbonate properties remained consistent throughout.

**Sample analysis**

Carbonate particle morphologies were characterized using scanning electron microscopy (SEM; JEOL JSM-6390LV) and their chemical compositions determined using energy-dispersive X-ray spectroscopy (EDX; Oxford Instruments X-MAXN) within a Tescan Vega3 SEM. Spectra were obtained as point scans collected at a working distance of 15 mm, accelerating voltage of 10 kV, and working time of at least 60 s. Although point scans were centered over individual crystals, resulting spectra will in most cases incorporate information from both the subject particle and adjacent particles due to typically small particle sizes (often < 1 μm) and relatively large interaction volumes (on the order of several μm). To overcome this problem, particles with diameters less than 5 μm were analyzed only if surrounded by similar morphotypes. To ensure EDX data were representative, at least 10 randomly selected points were scanned per sample.

Carbonate polymorphs were identified using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. Scans were performed at a resolution of 2 cm⁻¹ using a Nicolet 380 FTIR spectrometer coupled with a Thermo Scientific SMART iTR ATR sampler equipped with a diamond reflecting cell, with final spectra obtained by the co-addition of 32 repeated scans. Analyses were performed on at least three powdered subsamples (each comprising at least three pellets) per individual fish to ensure representative spectra were obtained. Resulting spectra are plotted as infrared absorbance vs. wavelength (conventionally converted to reciprocal length, or wavenumber), with peak positions, shapes, and intensities being a function of the frequency and mode of molecular vibrations within the sample. For inorganic carbonates there are four main vibrational modes: symmetrical and asymmetrical stretching (termed ν1 and ν3, respectively), and in-plane and out-of-plane bending (ν2 and ν4, respectively). The absorbance peaks generated by these vibrational modes are polymorph-specific, facilitating identification of carbonate polymorphs—including for most multiphase samples—through reference to an extensive spectral database (see Salter et al. 2017).

These polymorph assignments were further supported by X-ray diffraction data (XRD; Bruker D8 Advance). Diffraction patterns were obtained using Cu Kα radiation (1.5418 nm) generated at 40 kV and 40 mA, initially employing coarse scan parameters for each sample (step size 0.04° 2θ; step time 0.4 s; scan interval 16–40° 2θ) before higher resolution scans (step size 0.02° 2θ; step time 1.0 s; scan interval 23–32° 2θ) were performed for quantitative analyses. Prior to analysis, samples were lightly ground for 30 s in an agate mortar and then pressed onto a glass slide. Test scans performed after progressive grinding times up to 480 s indicated that 30 s was sufficient to properly homogenize samples, and that the grinding process did not induce polymorphic transformations.

Whereas the use of FTIR spectroscopy to estimate MgCO3 contents in fish-derived calcite has proven problematic (see Salter et al. 2017), XRD patterns provide a means of estimation due to a
tightly constrained relationship between MgCO₃ content and d₁₀₄ peak position of calcite (see Goldsmith et al. 1961). For fish carbonates, this diffraction line frequently exhibits double peaks, shoulders, and/or asymmetry, indicating that overall peaks are a composite of smaller peaks resulting from the presence of two or more distinct calcite phases with different MgCO₃ contents. Peak-fitting software (Fityk 0.9.8; Wojdyr 2010) was used to decompose these peaks using a non-linear least squares approach, using either pseudo-Voigt functions, or—if they provided a better fit—combined Gaussian and Lorentzian functions. The number of component peaks and their starting positions were selected on the basis of visual inspection; their intensities and shapes then being allowed to vary until the best fit was achieved by the peak-fitting algorithm. Final peak positions were corrected for goniometer misalignment by reference to known fixed peak positions of internal standards (either quartz d₀₁₁ or aragonite d₁₁₁) before being used to estimate MgCO₃ contents according to the relationship of Goldsmith et al. (1961). However, despite the strong relationship between MgCO₃ content and d₁₀₄ position, these estimates may carry significant uncertainties (≥ ± 5 mol% MgCO₃; Bischoff et al. 1983) as a result of various issues associated with biogenic precipitates.

By integrating all of these morphological, mineralogical, and compositional data, we were also able to estimate the abundances of the different morphotypes and carbonate polymorphs produced by each fish species following the procedure described by Salter et al. (2017).

Statistical analysis

Magnesium contents of calcites produced at different temperatures were compared by performing statistical tests for five families sampled in both subtropical and temperate settings: Latidae, Lutjanidae, Pinguiipedidae, Scorpaenidae, and Sparidae. Data prepared as mol% MgCO₃ values were calculated from EDX data on the assumption that magnesium and calcium occur only as MgCO₃ and CaCO₃; an assumption supported by the generally close agreement of these MgCO₃ values with those estimated from XRD data. These data were filtered where practical to remove potential species effects, with comparisons for the Latidae, Lutjanidae, and Sparidae thus being monospecific. However, for the Pinguiipedidae and Scorpaenidae it was necessary to use different species at each temperature (Pinguiipedidae: Parapercis queenslandica and Parapercis australis at 25°C vs. Parapercis colias at 10°C; Scorpaenidae: Dendrochirus zebra, Pterois volitans, and Scorpaenopsis diabolus at 25°C vs. Helicolenus percoideus at 10°C), meaning any temperature effects cannot be isolated from potential species effects for these families. Data were further filtered to ensure similar particles were being compared, which in all cases were ellipsoids approximately 1 μm in length. While particle types such as wheatsheafs and rhombohedra were also produced by some families, these were generally scarce or were not present in all samples, so they were omitted from the analyses.

For each family, hierarchical comparisons were performed in R statistical software (R Development Core Team 2008) as nested ANOVAs, following the “aov function” procedure outlined on the R companion website (https://rcompanion.org/rcompanion/d_07.html, last accessed July 2019). Temperature was included as a fixed effect and sampling tank (housing individual or multiple fish) as a random effect. In addition to comparing MgCO₃ contents among temperature categories, these tests indicate whether there is significant intra-familial (or intra-specific) variability within each temperature category and overall.

Results

As in previous warm-water studies, most fish yielded daily carbonate excretions at a broadly constant rate (on the basis of visual assessment) when sampled at 18°C. This pattern was also largely maintained at 10°C, but with several exceptions among some of the larger (0.45–1.90 kg) and less active bottom-dwelling fish: carbonate quantities excreted by the scorpaenid species H. percoideus (n = 20) and the nototheniid (icefish) species Notothenia angustata (n = 6) were highly erratic, while a large individual of the uranoscopid (stargazer) species Genyagnus monopterygius excreted most of its carbonate at approximately 4 d intervals. Nevertheless, carbonate samples were obtained from all fish used in the study, which included 11 previously untested fish families: Atherinidae (hardyheads), Carangidae (jacks and pompanos), Fistulariidae (flutemouths), and Mugilidae (mullets) sampled at 18°C; and Congiopodidae, Gobiesocidae (clingfishes), Moridae (morid cods), Nototheniidae, Plesiopidae (prettyfins), Tripterygiidae (triplefins), and Uranoscopidae sampled at 10°C.

Electron microscopy of carbonates produced by 42 individuals of nine fish species at 18°C and 11 species at 10°C revealed a suite of distinctive particle types including nanospheres (typically < 0.5 μm), ellipsoids and rods (typically 0.5–1.5 μm), wheatsheafs (2–5 μm), rhombohedra (5–100 μm), and spheres and dumbbells (5–100 μm), as summarized in Table 1 and Fig. 1. Particle types were consistently similar among individuals of each species, and also among both species of the Labridae (the only family for which multiple species were sampled). Overall these particles were generally similar in appearance to fish carbonates documented elsewhere from subtropical settings (Perry et al. 2011; Salter et al. 2012, 2014, 2017, 2018), with the exception of a striking dissimilarity in the occurrence pattern of rhombohedral particles. In subtropical settings rhombohedra typically occur as subsidiary particles, often in association with dominant nanospheres and/or material lacking definable form (i.e., ACMC; Salter et al. 2017), and this pattern was observed here for three species sampled at 18°C: Atherinomorus ogilbyi (Atherinidae; Fig. 1b); Atule mate (Carangidae); and a gobid spp. (Gobiidae). In contrast, and uniquely among existing observations, rhombohedra produced at 10°C were the dominant particle type in samples from N. angustata (Nototheniidae; Fig. 1c), and were a dominant component alongside spheres, dumbbells, rods, and ellipsoids in samples from Notolabrus celidotus and Notolabrus fucicola (Labridae; Fig. 1d,e), and
occasionally up to 120 μm. Considerably larger numbers of examples are either at the upper end of this range or are often exceeding 50 μm in length (Fig. 1e) and occasionally up to 120 μm. 

F. nigripenne (Tripterygiidae; Fig. 1f). Furthermore, while many of these rhombohedra are within the size range of comparable morphotypes from subtropical regions (0.5–20 μm; Salter et al. 2014), numerous examples are either at the upper end of this range or are considerably larger—often exceeding 50 μm in length (Fig. 1e) and occasionally up to 120 μm. 

Table 1. Carbonate morphologies and mineralogies from fish used in this study (MB18, OH10) and from the same families where used in subtropical studies (HI25, MB24, CE26; Salter et al. 2017, 2018).

| Site* | Fish species | Fish family | N | Body mass (g) | % CaCO₃ shape† | % CaCO₃ polymorph‡ |
|-------|-------------|-------------|---|---------------|----------------|-------------------|
|       |             |             |   |               |  e  w  r  s  n |  H   L   A      |
| MB18  | L. calcarifer | Latidae     | 7 | 1950–5800     | 100 — — — — | 100 — — — |
|       | L. russelli  | Lutjanidae  | 6 | 43–143        | 100 — — — — | 100 — — — |
|       | S. maculata  | Sillaginidae | 1 | 13            | 100 — — — — | 100 — — — |
|       | A. australis | Sparidae    | 6 | 50–250        | 80 20 — — — | 100 — — — |
|       | Gobid spp.   | Gobidae     | 4 | 0.5–0.7       | — — 15 — 85 | — — 15 85 |
|       | Fistularia commersonii | Fistularidae | 1 | 28            | 70 30 — — — | 100 — — — |
|       | Mugil cephalus | Mugilidae   | 1 | 171           | 10 — — 90 — | 100 — — — |
|       | A. mate      | Carangidae  | 11| 105–220       | — — 40 — 60 | — — 40 60 |
|       | A. ogilbyi   | Atherinidae | 5 | 3.1–4.6       | — — 7 3 90  | — 10 90    |
| OH10  | N. celidotus | Labridae    | 10| 9.5–410       | — — 75 15 10 | 20 80 —     |
|       | N. fucicola  | Labridae    | 2 | 165–430       | — — 80 15 5  | 15 85 —     |
|       | N. angustata | Nototheniidae | 6 | 1140–1900    | — — 100 — — | — 100 —     |
|       | P. bouchus   | Moridae     | 1 | 1010          | 100 — — — — | 100 — — — |
|       | Acanthochilinus fuscus | Plesiopidae | 3 | 0.6–1.3      | 100 — — — — | 100 — — — |
|       | Forsterygion nigripenne | Tripterygiidae | 20 | 0.7–7.4   | 45 20 20 15 — | 65 32 — |
|       | P. colias    | Pinguipedidae | 11 | 170–800     | 97 — 3 — —  | 97 3 — —    |
|       | H. percoides | Scorpaenidae§ | 21 | 480–1320  | 100 — — — — | 100 — — — |
|       | C. leucopcaelius | Congiopodidae | 4 | 82–385    | 100 — — — — | 100 — — — |
|       | G. monopterygius | Uranoepopidae | 3 | 7.7–450 | 100 — — — — | 100 — — — |
|       | Gastroscyphus hectoris | Gobiidae | 3 | 0.8–10    | 100 — — — — | 100 — — — |
| HI25  | Three species | Labridae    | 12| 22–65        | — — 26 74 —  | 17 9 74     |
|       | Three species | Lutjanidae  | 5 | 17–555      | 100 — — — — | 100 — — — |
|       | Two species  | Pinguipedidae | 3 | 23–175    | 100 — — — — | 100 — — — |
|       | Two species  | Scorpaenidae | 2 | 1.9–53     | 100 — — — — | 100 — — — |
|       | Silago sihama | Sillaginidae | 12| 160–430    | 100 — — — — | 100 — — — |
| MB24  | L. calcarifer | Latidae     | 23| 4.5–5800    | 100 — — — — | 100 — — — |
|       | L. russellii | Lutjanidae  | 21| 85–175      | 100 — — — — | 100 — — — |
|       | S. maculata  | Sillaginidae | 4 | 5.8–18     | 100 — — — — | 100 — — — |
|       | Three species | Sparidae    | 23| 13–200    | 85 15 — — — | 100 — — — |
|       | Valenciennnea immaculata | Gobiidae | 1 | 2.8       | 10 — 10 — 80 | 14 6 80     |
| CE26  | Two species  | Labridae    | 43| 1.1–7.7     | — — 20 9 71 | 18 11 71    |
|       | Two species  | Lutjanidae  | 56| 4.6–946   | 100 — — — — | 100 — — — |

*Site codes indicate study location and average temperature: MB18, Moreton Bay, 18°C; OH, Otago Harbour; HI, Heron Island (Australia); CE, Cape Eleuthera (The Bahamas).

†Particle shapes are abbreviated as: e, ellipsoid/rod; w, wheatshad; r, rhombohedron; s, sphere/dumbbell; n, nanosphere/material lacking definable form.

‡Mineralogies abbreviated as: H, high-Mg calcite; L, low-Mg calcite; A, amorphous calcium–magnesium carbonate. Where polymorph % sums to < 100 (e.g., OH10, Tripterygiidae), material not listed is aragonite.

§The genus Helicolenus has been variously assigned to the families Scorpaenidae and Sebastidae. Here we follow the most recent classification, which places the traditional Sebastidae within the Scorpaenidae (Smith et al. 2018).

F. nigripenne (Tripterygiidae; Fig. 1f). Furthermore, while many of these rhombohedra are within the size range of comparable morphotypes from subtropical regions (0.5–20 μm; Salter et al. 2014), numerous examples are either at the upper end of this range or are considerably larger—often exceeding 50 μm in length (Fig. 1e) and occasionally up to 120 μm.

Structural data further indicate that carbonates produced in temperate settings are mineralogically similar to those produced in subtropical settings. Representative data are shown in Figs. 2 and 3 (ATR-FTIR spectra and XRD patterns, respectively) and estimated polymorph abundances are summarized in Table 1. Most species sampled at both study locations produced calcite with a low degree of crystallinity (Fig. 2; Pseudophycis bouchus). In addition, three species produced amorphous carbonate (Fig. 2; A. mate), while only one species produced any aragonite (Fig. 2; F. nigripenne), detectable in approximately a third of samples.
analyzed. Insufficient sample sizes precluded quantitative analysis using XRD, but the typical occurrence of aragonite ATR-FTIR $\nu_2$ peaks as weak shoulders on dominant calcite $\nu_2$ peaks suggest it to be a subsidiary phase where present (Loftus et al. 2015). XRD patterns were only obtained for calcitic samples but results were consistent with ATR-FTIR data in all cases.

Because previously documented rhombohedra have always been a subsidiary particle type not readily isolated from other

**Fig. 1.** Electron microscope images showing the diverse range of carbonate particle morphologies produced by some of the fish species sampled in this study. Morphologies include: (a) intergrown spheres, many of which are hollow (inset); (b–f) different sized rhombohedra (spanning 1 to 120 $\mu$m) typically exhibiting well-defined crystal faces (b–d), although more rounded variants also occur (e, f)—In any case they can be produced alongside amorphous carbonate nanospheres (b), spheres and dumbbells (d), – 1 $\mu$m ellipsoids (f), or as the only component (c-inset); (g) a spectrum of particle types spanning – 1 $\mu$m ellipsoids and rods through – 5 $\mu$m wheatsheafs; (h) well-defined – 1 $\mu$m ellipsoids; and (i) poorly defined < 1 $\mu$m ellipsoids.
particles, structural information beyond identification as typically LMC (Salter et al. 2017) has been limited. Their occurrence as a dominant form here thus facilitates acquisition of improved structural data. ATR-FTIR spectra are characterized by sharp and intense calcite $\nu_2$ and $\nu_4$ peaks (Fig. 2; N. angustata), with lower $\nu_2/\nu_4$ intensity ratios (in the range 2.24–3.62) than those associated with ellipsoidal calcite (spanning 5.22–10.75 across all species) indicating that rhombohedra have a comparatively higher degree of crystallinity (see Politi et al. 2004). XRD patterns generated by these samples

**Fig. 2.** Representative ATR-FTIR spectra for the different carbonate polymorphs produced by fish in this study. In addition to the main $\text{CO}_3^{2-}$ vibrations (shaded regions of spectra; refer to guide at top), samples generated peaks corresponding to water (−1650 and −3350 cm$^{-1}$; indicative of degree of hydration), phosphate (−550 and −1050 cm$^{-1}$; probably due to fish scale fragments), aliphatic hydrocarbons (2854 and 2923 cm$^{-1}$ due to fatty acid side chains), and a $\text{CO}_2$ combination mode (−1795 cm$^{-1}$; $\text{CO}_3^{2-} \nu_1 + \nu_4$). In regions spanning 2500–3800 cm$^{-1}$ spectra are 4x vertically exaggerated to highlight variations in water vibrations. Note also that the relative intensities of $\text{CO}_2$ $\nu_2$ and $\nu_4$ vibrations can provide an indication of degree of crystallinity in calcites (see main text). Several species produced carbonates dominated by hydrated ACMC (a), in some cases accompanied by calcite (b). In contrast, several other species produced carbonates dominated by strongly crystalline calcite (c). In addition, one species produced carbonates comprising a mixture of dominant calcite and subsidiary aragonite (d), and possibly minor ACMC (note relatively strong hydration peaks). However, the majority of species sampled in this study produced weakly crystalline calcite (e).
exhibit $d_{104}$ reflections with Full-Width at Half-Maximum values typically less than half those of ellipsoid-dominated samples (Fig. 3). Because this parameter can be used as a measure of crystallinity (Bischoff et al. 1983; Gehlen et al. 2005), these data appear to support the ATR-FTIR data. However, the degree of line broadening in XRD patterns can also...
Fig. 5. Within-family comparisons of structural data for fish carbonates collected at subtropical (24–25°C; magenta), warm temperate (18°C; green), and cool temperate (10°C; blue) conditions. Representative pairs of ATR-FTIR spectra (a) for carbonates from four different fish families demonstrate similar products (poorly crystallized calcite) at each sampling temperature. See Fig. 2 for interpretations of similar spectra. In regions spanning 2500–3800 cm\(^{-1}\) spectra are 4× vertically exaggerated. Of additional note, a weak peak at 745 cm\(^{-1}\) indicates the presence of vaterite in carbonates from \(D.\ zebra\), but its low frequency of occurrence, very low peak intensities, and an absence of vaterite diffraction peaks in corresponding XRD profiles, suggests it is of little significance in terms of overall product outputs. Representative XRD patterns (b) for two fish families also demonstrate carbonate products to be similar regardless of sampling temperature; predominantly HMC. Small differences in \(d_{104}\) calcite peak positions (labeled “c”) are indicative of different MgCO\(_3\) contents (e.g., \(L.\ russellii\): 26.1 mol% at 18°C vs. 30.2 mol% at 24°C), but these differences are within the range of variability at each temperature and do not represent a thermal influence. Weak shoulders at ~29.5° 2\(\theta\) indicate a subsidiary presence of LMC in many samples from each temperature, although correspondingly low Mg values are not seen in compositional analyses of these samples (see Fig. 6). For reference, the gray diffraction pattern corresponds to reagent grade calcite. Additional peaks correspond to internal quartz standards (q), and hydroxylapatite likely due to fish scale fragments (h).
vary as a function of sample grain size and/or compositional heterogeneity, and a more detailed analysis is required to further explore this issue. Nevertheless, a marked contrast in \( d_{104} \) peak positions between these different forms (Fig. 3) confirms that rhombohedral fish carbonates are predominantly LMC and ellipsoidal fish carbonates are predominantly HMC.

In several instances we compared carbonate products within fish species and/or family across the full thermal range of fish carbonate studies (now spanning 10–27°C), and in most cases temperature appeared to have no effect on morphological properties (Table 1; Fig. 4). For example, carbonates produced at 10°C by the scorpionfish species *H. percoideus* comprised ellipsoids < 0.7 \( \mu \)m in length, similar to those produced by congenerals at 25–27°C (*P. volitans* in The Bahamas and *D. zebra* and *S. diabolus* in Australia; Perry et al. 2011; Salter et al. 2018). While there is some degree of variability within each species (e.g., in addition to dominant ellipsoids < 0.7 \( \mu \)m, ellipsoids > 1.0 \( \mu \)m are common products of all scorpionids), we observe no consistent differences as a function of temperature. Such similarities are also observed among three members of the Pinguipedidae sampled across this thermal gradient, and within species for four other families (*L. calcarifer*, Latidae; *Sillago maculata*, Sillaginidae; and *Acanthopagrus australis*, Sparidae), albeit these were sampled across a smaller thermal gradient spanning 18–24°C.

Comparison of ATR-FTIR and XRD data from these family and species pairs further indicates there are no structural differences across these thermal ranges (Fig. 5). Carbonates from all six families are characterized as weakly crystalline calcite on the basis of ATR-FTIR spectra, and where tested (all families except Sillaginidae) the positions of calcite \( d_{104} \) peaks in XRD patterns indicate they are HMC. MgCO\(_3\) contents estimated on the basis of these peak positions are similar at all temperatures, typically in the range 20–30 mol%—consistent with earlier findings for ellipsoidal calcites from the subtropics (Perry et al. 2011).

Estimates of MgCO\(_3\) content based on XRD data are corroborated by compositional data obtained using EDX spectroscopy (Fig. 6). These compositional data provide strong evidence that there is no correlation between temperature and MgCO\(_3\) content in fish-produced HMC ellipsoids, at least within the stated temperature ranges for the six families tested here. Of the five families compared using statistical tests, only the Scorpaenidae show a significant difference, albeit small (mean MgCO\(_3\) of 32.8 mol% at 25°C vs. 27.8 mol% at 10°C). While this result could be indicative of an isolated temperature effect on magnesium incorporation within this family, several other explanations exist. For example, subtropical scorpionfish carbonates typically contained the magnesium hydroxide mineral brucite (Mg(OH)\(_2\); Salter et al. 2017), but this phase was less abundant in cool temperate samples. It is thus possible that the apparent elevation of MgCO\(_3\) contents at higher temperatures (Fig. 6) is actually an artifact of higher brucite contents contributing to EDX magnesium signals. Such a difference in brucite contents could itself be a temperature effect, but one that has no bearing on MgCO\(_3\) incorporation in HMC.

Alternatively, this difference could be a function of product variability among fish of different sizes (scorpionfish body masses in the subtropics spanned 1.8–380 g vs. 480–1320 g in cool temperate settings), and/or different species.

In contrast to these findings, carbonates collected from members of the Labridae in temperate settings were somewhat different to those produced by their warm-water congeneric.
Specifically, labrids sampled at subtropical locations (25–27°C; \( n = 5 \) species; Salter et al. 2012, 2018) produced carbonates dominated by ACMC with subsidiary LMC spheres and rhombohedra, whereas for those sampled at cool temperate locations (10°C; \( n = 2 \) species) the inverse was observed, with LMC rhombohedra and spheres dominating and ACMC being uncommon or absent (Figs. 1 and 7). These findings were consistent regardless of sampling interval (12 vs. 4 h), suggesting they are unlikely to be an artifact of ACMC loss through dissolution unless this occurred within 4 h of excretion.

Conversely, carbonates produced by a gobiid species sampled at 18°C were similar to those produced by a confamilial sampled at 24°C (Table 1; Salter et al. 2018). Because gobiid carbonates are similar to labrid carbonates produced in the subtopics, this result suggests that any temperature effect on these types of carbonate is likely to be family-specific, or it only operates at temperatures below 18°C.

### Discussion

Evidence from subtropical settings indicates that fish carbonate products are highly consistent at both the species- and family-level within a temperature range of 23–27°C (Salter et al. 2012, 2017, 2018). Our findings similarly indicate species-level consistency where tested in temperate settings (see Table 1—e.g., \( H. \) percoideus, \( A. \) mate, and \( N. \) celidotus, among others), and that this consistency is maintained over thermal gradients—at least for HMC-producing species (e.g., \( L. \) russellii, \( L. \) calcifer, and \( A. \) australis). The extent of general species-level consistency has so far been observed without exception for at least 54 species (Perry et al. 2011; Salter et al. 2012, 2017, 2018; this study), suggesting that even data from species represented by only a single individual (i.e., four species in this study; Table 1) are likely to be broadly indicative of species-level products. Family-level consistency was not extensively tested here, although it was observed in the only case where multiple species were sampled (Labridae). Furthermore, four families sampled in previous studies were represented by species sampled for the first time in this study, of which three produced carbonates generally similar to those of their subtropical confamilials (Gobiidae, Pinguipedidae, and Scorpaenidae; Table 1). Only the newly sampled labrid members produced carbonates potentially differing from those of the subtropical representatives, reasons for which are discussed below.

These findings not only encompass carbonate morphology and mineralogy, but also the incorporation of magnesium into calcite: MgCO\(_3\) contents of LMC and HMC are similar across the full thermal range of the study, contrary to the positive correlations often observed in other calcifying organisms (e.g., Chave 1954). This result implies that the organic and/or inorganic chemistry within the fish intestine must be dominant controls on precipitation processes that override any thermal control, at least within the studied temperature interval. The small but significant lowering of MgCO\(_3\) content within the Scorpaenidae is, however, intriguing. There are various possible explanations for this difference, including effects of species, size, temperature, and non-carbonate phases. We also cannot rule out the possibilities that thermal effects are: (1) more pronounced in some fish families than others; and/or (2) mostly overridden within the studied temperature range but potentially becoming more pronounced at lower temperatures—the onset of this influence perhaps captured in our Scorpaenidae data. Additional work involving lower

![Fig. 7. ATR-FTIR spectra for carbonates produced by different members of the Labridae sampled in subtropical (a-d; magenta) and cool temperate (e-f; blue) conditions. See Fig. 2 for interpretations of similar spectra. In regions spanning 2500–3800 cm\(^{-1}\) spectra are 4x vertically exaggerated. Subtropical labrid carbonates are strongly hydrated (note vibrations at ~1650 and 3350 cm\(^{-1}\)) and typically generate CO\(_2^-\) vibrations characterized by broad low intensity \( \nu_2 \) and \( \nu_4 \) peaks at ~858 and ~711 cm\(^{-1}\) (a-c), features that indicate a predominance of hydrated ACMC. Differences in the sharpness and intensity of the CO\(_2^-\) peaks coupled with shifts in \( \nu_2 \) peak position suggest some variability in calcite contents (d), but a strong hydrated ACMC signal is ubiquitous. In contrast, cool temperate labrid carbonates consistently generate spectra that indicate a predominance of well-crystallized calcite (e, f). Weak hydration peaks suggest some samples may contain subsidiary ACMC (e), but this polymorph is typically absent (f).](https://example.com/f7.png)
temperatures, more fish families, and monospecific comparisons is thus necessary to further explore this issue.

Nevertheless, this expansion of the temperature range across which within-family consistency is known to be maintained has two major implications. First, because MgCO₃ content correlates positively with HMC solubility (e.g., Bischoff et al. 1987), and CaCO₃ saturation states correlate negatively with temperature (e.g., Jiang et al. 2015), a systematic decrease in MgCO₃ contents with temperature as seen in other biogenic calcites (e.g., Chave 1954) would offset these relationships. This means that, depending on the rate of decrease, the stability of HMC from a given fish family would be more or less similar at any latitude. The fact that such a relationship does not exist and MgCO₃ contents are uniform over a large thermal gradient (at least in the range 10–25°C) means that HMC from a given fish family should be less stable at higher latitudes; dissolving more rapidly and/or at shallower depths, and thus playing a different role in the marine inorganic carbon cycle than in warm-water regions.

Given that fish carbonates involve several carbonate polymorphs other than HMC (Salter et al. 2012), and that there remains a large cross section of the global fish community for which no carbonate data exist, ascertaining the relevance of this implication in a global context is challenging. Regional scale models do, however, indicate HMC to be the dominant fish carbonate product in some subtropical settings (Salter et al. 2017). Furthermore, HMC comprises more than 75% of carbonates produced by nearly two thirds of the fish families for which they have been characterized (28 out of 45; Salter et al. 2018; this study). Thus, available evidence suggests HMC is likely to be a major fish carbonate product globally and that this implication is therefore highly relevant to future modeling work.

The second implication of our findings is that they facilitate substantial improvements to fish carbonate production models across large thermal gradients. Construction of carbonate production models for a given fish community has for some time been possible using the fish carbonate production rate—body mass—temperature relationships of Wilson et al. (2009). However, such models lack the capacity to predict carbonate polymorph ratios; information that is critical to predicting the role of fish carbonates in the marine environment. Subsequent production models resolving for polymorph have been constructed at local scales in the subtropics (Salter et al. 2017, 2018), but these were confined to those settings in the absence of data from broader environmental conditions. The findings that fish carbonate properties are mostly insensitive to temperature variations, and that species- and family-level consistency is largely maintained across large temperature intervals, are thus crucial in establishing the potential to extend such models across regions at least spanning the temperature range of this study (10–25°C). This would represent an enormous range expansion for fish carbonate modeling potential compared with capabilities hitherto being confined to subtropical settings, and is highly significant given the large proportion of global fish carbonate production predicted at high latitudes (Wilson et al. 2009).

This advance is again tempered, however, by the limited scope of the existing database of family-level fish carbonate products (Salter et al. 2017, 2018; and this study). Major fish biomass centers of the temperate zones are continental shelf settings that include: the Yellow Sea, the Sea of Okhotsk, and the Sea of Japan in Asia; the Northeast and Northwest seaboards of North America; the seas of Northern Europe; the Humboldt Current System and the Patagonia shelf in South America; and seas around New Zealand (Wilson et al. 2009). The fish communities of these regions are largely characterized by families that are generally poorly represented in the existing fish carbonate database. For example, of the major North Sea fish families (e.g., Daan et al. 1990), carbonate polymorphs can be predicted only for the Pleuronectidae (righteye flounders) and Carangidae; other families, including the Gadidae (codfishes), Clupeidae (herrings and sardines), Scombridae (tunas and mackerels), and Ammodotiidae (sandrines) (sandlances) remain unrepresented. Similarly, of the important Patagonian Shelf fish families (e.g., García et al. 2010), products can be predicted only for the Carangidae; the Merlucciidae (hakes), Percophidae (duckbills), and Congridae (conger eels) being among those that are unrepresented. These high latitude fish communities thus represent an obvious target for future research—particularly given their importance in the fisheries industry (e.g., Watson 2017) and the consequent potential for their overexploitation to result in shifts in carbonate production regimes (Salter et al. 2017).

At more localized scales, however, the existing database is likely to be sufficient for modeling efforts in some cases. For example, although the compositions of fish communities supported by temperate rocky reef habitats differ among biogeographic regions (e.g., Reef Life Survey; https://reelfireshelfsurvey.com, accessed January 2019; Edgar and Stuart-Smith 2014), many are characterized by an abundance of families for which carbonates have been characterized. These include, for example, the Apogonidae (cardinalfishes), Pinguipedidae, Scorpidae, Serranidae (groupers, rockcods, and their allies), Sparidae, and Tripterygiidae, all of which are known to produce predominantly HMC. Some knowledge gaps remain, with important families such as the Kyphosidae (sea chubs), Cheliodactylidae (morwongs), and Pempheridae (bullseyes and sweepers) currently unrepresented in the database. Again, these families are identified as targets for future work.

A more problematic issue, however, concerns families known to produce mainly ACMC in subtropical settings. These include the Gobiidae, Labridae, Microcanthidae (mados and stripeys), and Pomacentridae (damselfishes), which can collectively account for large proportions of total fish carbonate production in subtropical coral reefs (Salter et al. 2018), and biomass data suggest they are likely to be similarly important producers in many temperate rocky reef systems (e.g., Truong et al. 2017). The issue arises because our finding that two members of the Labridae produced mainly LMC when sampled at 10°C is potentially
indicative of temperature sensitivity in the production of ACMC. The significance of this issue lies not only in the fact that these families are important producers across a large thermal gradient, but that highly unstable ACMC and very stable LMC represent either extreme of the carbonate solubility spectrum (Brečević and Nielsen 1989; Morse and Mackenzie 1990; Purgstaller et al. 2019). Thus, a thermally regulated switch from ACMC to LMC production would imply that carbonates from these families are more stable at higher latitudes; the opposite outcome to that already discussed for HMC fish carbonates. Several key questions thus arise: (1) is the observed switch from warm-water ACMC production to cool water LMC production within the Labridae temperature-controlled, or is there another explanation; (2) is the switch widely applicable to all warm-water ACMC-producing fish families; and (3) over what temperature range does any switch from ACMC to LMC take place?

An appealing hypothesis regarding the first question is that the observed switch within the Labridae is a result of longer gut residence times at lower temperatures (Miegel et al. 2010), and is thus indirectly controlled by temperature changes. In this scenario, initial precipitates of amorphous carbonate—which is widely recognized as a precursor phase to many biogenic calcites and aragonites (Politi et al. 2004; Radha et al. 2010; Gong et al. 2012)—would have more time to crystallize to LMC than in subtropical settings where gut residence times are comparatively low. If gut residence time was generally longer in this study than in previous subtropical studies, we find no indication that it influenced precipitation among the HMC-producing families (e.g., Lutjanidae, Scorpaenidae). However, such an effect could explain the apparent lack of ACMC produced by all species sampled at 10°C (Table 1). It is of course possible that temperature changes could regulate a polymorph switch in a more direct manner (e.g., Morse et al. 1997), and such mechanisms should be explored further, but from the available data it is difficult to rule out other explanations that do not invoke a temperature effect.

Compared with previous studies, cooler sampling conditions—and consequently lower carbonate saturation states—invite the possibility that the apparent polymorph switch is an artifact of (or at least exaggerated by) rapid postexcretion dissolution of ACMC. Although seemingly unlikely given that some samples were exposed to seawater for less than 4 h, this issue requires further investigation. Another possibility is that it represents a deviation from within-family product consistency. This would be a surprising outcome given the hitherto strong pattern of within-family consistency (Salter et al. 2018), but it cannot be disregarded—especially because our new labrid data involve two members of the genus Notolabrus; a genus not sampled in the subtropics to provide direct comparison. However, available data from other families indicate that carbonate products are uniform across multiple genera (e.g., Lutjanidae, n = 2 genera; Serranidae, n = 4; Scorpaenidae, n = 4; this study and Salter et al. 2018). Moreover, the same is otherwise true within the Labridae, wherein eight members of four genera sampled in tropical and subtropical regions (Thalassoma, Halichoeres, Cheilinus, and Hemigymnus—Salter et al. 2017, 2018; and M. A. Salter unpubl.) all produce ACMC and subsidiary LMC. Given this available evidence and the absence of any obvious reason why Notolabrus labrids should produce different carbonates (e.g., they share similar dietary and lifestyle traits with labrids sampled in the subtropics; Russell 1983; Sano et al. 1984; Froese and Pauly 2019), a within-family deviation seems unlikely.

Although a temperature effect cannot be confirmed, it is noteworthy that none of the 10 families sampled in cool temperate conditions produced carbonates with ACMC as a substantial component, yet approximately one third of those sampled in warm-water settings did (Salter et al. 2018). In addition, none of the 67 species sampled in the subtropics produced carbonates dominated by LMC rhombohedra, yet this was a major product of 4 of the 11 species sampled in cool temperate conditions. These disparities seemingly support a hypothesis of temperature sensitivity in ACMC production, but the evidence is inconclusive; they are also consistent with sampling bias through rapid dissolution, for example. Additional sampling within species and/or genera is clearly necessary, and data from other families (e.g., Pomacentridae and Microcanthidae) are also needed to establish whether there is a temperature effect on ACMC production; and if it is widely applicable across fish families (thus answering the second question outlined above).

As to the question of the temperature range over which potential changes take place, we can only surmise that ACMC appears to dominate at 25°C and LMC appears to dominate at 10°C, but intermediate data are limited. Thus, detailed work involving a thermally tolerant species known to produce ACMC in warm-water conditions should be conducted to determine the nature and timing of any thermally induced changes. It is worth adding that three families sampled in this study at 18°C did produce significant ACMC. Of these, one (Gobiidae) has previously been sampled at 24°C (Salter et al. 2018) and our analyses indicate products were similar, suggesting no temperature sensitivity in this family over this 6°C gradient. Two other families (Atherinidae and Carangidae) were sampled here for the first time, so we have no basis for comparison. However, both cases involved active swimmers that likely have higher metabolic rates (and thus presumably lower gut residence times) than either the Labridae or Nototheniidae sampled at 10°C, which—in captivity at least—were largely inactive. Thus, if the apparent polymorph switch observed in the Labridae is, as hypothesized, a function of gut residence time, it is likely that fishes with different lifestyles will add another layer of complexity to this issue.

Concluding remarks

This study presents the first detailed analyses of fish-derived carbonate outside of subtropical regions, expanding the fish
carbonate database across a temperature range of 10–25°C to now encompass 82 species (a 22% increase over existing data) and 45 families (a 32% increase). Overall, we find that a similar suite of carbonates is produced throughout this thermal range. In particular, HMC ellipsoids—the most widely produced polymorph within the present fish carbonate dataset—maintain similar morphology, mineralogy, crystal structure, and MgCO₃ in all production settings. Furthermore, we find no evidence of temperature sensitivity in any fish carbonate polymorph produced between 18°C and 25°C. However, a marked polymorph transition in carbonates produced by the Labridae is intriguing and points to the possibility of temperature sensitivity at least in the ACMC—LMC system. These findings have important implications for understanding the roles of these carbonates in sediment production and inorganic carbon cycling because they confirm for the first time that a large proportion of fish carbonate is insensitive to temperature changes over a large thermal gradient, thus facilitating a significant range expansion over which production models resolving for mineralogy can now be constructed. However, we also highlight a need for more data in order to underpin high-resolution production modeling at a truly global scale. In particular, future work should focus on: (1) describing the carbonate products of key fish families that represent large proportions of the global fish biomass; (2) extending fish carbonate studies to temperatures lower than 10°C; and (3) addressing questions regarding possible thermally regulated polymorph switching between ACMC and LMC.

Data availability statement
Data supporting this publication are openly available from the University of Exeter’s institutional repository at https://doi.org/10.24378/exe.1815

References
Basso, D. 2012. Carbonate production by calcareous red algae and global change. Geodiversitas 34: 13–33. doi:10.5252/g2012n1a2
Bischoff, W. D., F. C. Bishop, and F. T. Mackenzie. 1983. Biogenically produced magnesian calcite: Inhomogeneities in chemical and physical properties; comparison with synthetic phases. Am. Mineral. 68: 1183–1188.
Bischoff, W. D., F. T. Mackenzie, and F. C. Bishop. 1987. Stabilities of synthetic magnesian calcites in aqueous solution: Comparison with biogenic materials. Geochem. Cosmochim. Acta 51: 1413–1423. doi:10.1016/0016-7037(87)90325-5
Block, B. A., J. R. Finnerty, A. F. Stewart, and J. Kidd. 1993. Evolution of endothermy in fish: Mapping physiological traits on a molecular phylogeny. Science 260: 210–214. doi:10.1126/science.8469974
Brečević, L., and A. E. Nielsen. 1989. Solubility of amorphous calcium carbonate. J. Cryst. Growth 98: 504–510. doi:10.1016/0022-0248(89)90168-1
Broese, A. T. C., P. Ziveri, J. E. van Hinte, and S. Honjo. 2000. Coccolithophore export production, species composition, and coccolith-CaCO₃ fluxes in the NE Atlantic (34°N 21°W and 48°N 21°W). Deep-Sea Res. II Top. Stud. Oceanogr. 47: 1877–1905. doi:10.1016/S0967-0645(00)00010-2
Chave, K. E. 1954. Aspects of the biogeochemistry of magnesia I. Calcareous marine organisms. J. Geol. 62: 266–283. doi:10.1086/626162
Daan, N., P. J. Bromley, J. R. G. Hislop, and N. A. Nielsen. 1990. Ecology of North Sea fish. Neth. J. Sea Res. 26: 343–386. doi:10.1016/0077-7579(90)90096-Y
Dwyer, G. S., T. M. Cronin, P. A. Baker, M. E. Raymo, J. S. Buzas, and T. Corrège. 1995. North Atlantic deepwater temperature change during Late Pliocene and Late Quaternary climatic cycles. Science 270: 1347–1351. doi:10.1126/science.270.5240.1347
Edgar, G. J., and R. D. Stuart-Smith. 2014. Systematic global assessment of reef fish communities by the Reef Life Survey program. Sci. Data 1: 140007. doi:10.1038/sdata.2014.7
Elderfield, H., and G. Ganssen. 2000. Past temperature and δ¹⁸O of surface ocean waters inferred from foraminiferal Mg/Ca ratios. Nature 405: 442–445. doi:10.1038/35013033
Fabry, V. J. 1990. Shell growth rates of pteropod and heteropod molluscs and aragonite production in the open ocean: Implications for the marine carbonate system. J. Mar. Res. 48: 209–222. doi:10.1357/00222409784984614
Foran, E., S. Weiner, and M. Fine. 2013. Biogenic fish-gut calcium carbonate is a stable amorphous phase in the gilt-head seabream, Sparus aurata. Sci. Rep. 3: 1700. doi:10.1038/srep01700
Froese, R., and D. Pauly [eds.]. 2019. FishBase; 2019 Feb [accessed xxx]. Available from http://www.fishbase.org
Garcia, M. L., A. J. Jaureguizar, and L. C. Protogino. 2010. From fresh water to the slope: Fish community ecology in the Río de la Plata and the sea beyond. Lat. Am. J. Aquat. Res. 38: 81–94. doi:10.4067/S0718-560X2010000100008
Gehlen, M., F. C. Bassinot, L. Chou, and D. McCorkle. 2005. Reassessing the dissolution of marine carbonate: I. solubility. Deep-Sea Res. I Oceanogr. Res. Pap. 52: 1445–1460. doi:10.1016/j.dsr.2005.03.010
Goldsmith, J. R., D. L. Graf, and H. C. Heard. 1961. Lattice constants of the calcium-magnesium carbonates. Am. Mineral. 46: 453–457.
Gong, Y. U. T., and others. 2012. Phase transitions in biogenic magnesian carbonates. Proc. Natl. Acad. Sci. U.S.A. 109: 6088–6093. doi:10.1073/pnas.1118085109
Grosell, M., C. N. Laliberte, S. Wood, F. B. Jensen, and C. M. Wood. 2001. Intestinal HCO₃– secretion in marine teleost fish: Evidence for an apical rather than basolateral Cl⁻ / HCO₃⁻ exchanger. Fish Physiol. Biochem. 24: 81–95. doi:10.1023/A:1011994129743
Halfar, J., T. Zack, A. Kronz, and J. C. Zachos. 2000. Growth and high-resolution paleoenvironmental signals of rhodoliths
(coralline red algae): A new biogenic archive. J. Geophys. Res. 105: 22107–22116. doi:10.1029/1999JC000128
Heuer, R. M., A. J. Esbaugh, and M. Grosell. 2012. Ocean acidification leads to counterproductive intestinal base loss in the Gulf toadfish (Opsanus beta). Physiol. Biochem. Zool. 85: 450–459. doi:10.1086/667617
Iglesias-Rodriguez, D., R. Armstrong, R. Feely, R. Hood, J. Kleypas, J. D. Milliman, C. Sabine, and J. Sarmiento. 2002. Progress made in the study of the ocean’s calcium carbonate budget. EOS Trans. Am. Geophys. Union 83: 365–375. doi:10.1029/2002EO000267
Jiang, L.-Q., R. A. Feely, B. R. Carter, D. J. Greeley, D. K. Milliman, J. D. 1993. Production and accumulation of calcium carbonate in the ocean: Budget of a nonsteady state. Global Biogeochem. Cycles 29: 1656–1673. doi:10.1020/2015GB005198
Langer, M. R. 2008. Assessing the contribution of foraminiferan protists to global ocean carbonate production. J. Eukaryot. Microbiol. 55: 163–169. doi:10.1111/j.1550-7408.2008.00321.x
Lea, D. W., T. A. Mashioita, and H. J. Spero. 1999. Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. Geochim. Cosmochim. Acta 63: 2369–2379. doi:10.1016/S0016-7037(99)00197-0
Lebrato, M., and others. 2010. Global contribution of echinoderms to the marine carbon cycle: CaCO3 budget and benthic compartments. Ecol. Monogr. 80: 441–467. doi:10.1890/09-0553.1
Loftus, E., K. Rogers, and J. Lee-Thorp. 2015. A simple method to establish calcite: Aragonite ratios in archaeological mollusc shells. J. Quat. Sci. 30: 731–735. doi:10.1002/jqs.2819
Mewes, A., G. Langer, L. Jan de Nooijer, J. Bijma, and G. -J. Reichart. 2014. Effect of different seawater Mg2+ concentrations on calcification in two benthic foraminifers. Mar. Micropaleontol. 113: 56–64. doi:10.1016/j.micron.2014.09.003
Miegel, R. P., S. J. Pain, W. H. E. J. van Wettere, G. S. Howarth, and D. A. J. Stone. 2010. Effect of water temperature on gut transit time, digestive enzyme activity and nutrient digestibility in yellowtail kingfish (Seriola lalandi). Aquaculture 308: 145–151. doi:10.1016/j.aquaculture.2010.07.036
Milliman, J. D. 1993. Production and accumulation of calcium carbonate in the ocean: Budget of a nonsteady state. Global Biogeochem. Cycles 7: 927–957. doi:10.1029/93GB02524
Milliman, J. D., D. Freile, R. P. Steinen, and R. J. Wilber. 1993. Great Bahama bank aragonitic muds: Mostly inorganically precipitated, mostly exported. J. Sed. Pet. 63: 589–595. doi:10.1306/D42678B1-2B26-11D7-8648000102C1865D
Milliman, J. D., P. J. Troy, W. M. Balch, A. K. Adams, Y. -H. Li, and F. T. Mackenzie. 1999. Biologically mediated dissolution of calcium carbonate above the chemical lysolcine? Deep-Sea Res. I Oceanogr. Res. Pap. 46: 1653–1669. doi:10.1016/S0967-0637(99)00034-5
Morse, J. W., Q. Wang, and M. Y. Tsio. 1997. Influences of temperature and mg:Ca ratio on CaCO3 precipitates from seawater. Geology 25: 85–87. doi:10.1130/0091-7613(1997)025<0085:IOATMC>2.3.CO;2
Mucci, A. 1983. The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure. Am. J. Sci. 283: 780–799. doi:10.2475/ajs.283.7.780
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: 3865–3869. doi:10.1073/pnas.1015895108
Perry, C. T., M. A. Salter, K. M. Morgan, and A. R. Harborne. 2019. Census estimates of algal and epiphytic carbonate production highlight tropical seagrass meadows as sediment production hotspots. Front. Mar. Sci. 6: 120. doi:10.3389/fmars.2019.00120
Politi, Y., T. Arad, E. Klein, S. Weiner, and L. Addadi. 2004. Sea urchin spine calcite forms via a transient amorphous calcium carbonate phase. Science 306: 1161–1164. doi:10.1126/science.1102289
Purgstaller, B., K. E. Goetschl, V. Mavromatis, and M. Dietzel. 2019. Solubility investigations in the amorphous calcium magnesium carbonate system. CrystEngComm 21: 155–164. doi:10.1039/C8CE01596A
R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing.
Radha, A. V., T. Z. Forbes, C. E. Killian, P. U. P. A. Gilbert, and A. Navrotsky. 2010. Transformation and crystallization energetics of synthetic and biogenic amorphous calcium carbonate. Proc. Natl. Acad. Sci. U.S.A. 107: 16438–16443. doi:10.1073/pnas.1009951017
Rees, S. A., B. N. Opdyke, P. A. Wilson, and T. J. Henstock. 2007. Significance of Halimeda bioherms to the global carbonate budget based on a geological sediment budget for the northern Great Barrier Reef, Australia. Coral Reefs 27: 177–188. doi:10.1007/s00338-006-0166-x
Russell, B. C. 1983. The food and feeding habits of rocky reef fish of north-eastern New Zealand. N. Z. J. Mar. Freshwater Res. 17: 121–145. doi:10.1080/00288330.1983.9515991
Salter, M. A., C. T. Perry, and R. W. Wilson. 2012. Production of mud-grade carbonates by marine fish: Crystalline products and their sedimentary significance. Sedimentology 59: 2172–2198. doi:10.1111/j.1365-3091.2012.01339.x
Salter, M. A., C. T. Perry, and R. W. Wilson. 2014. Size fraction analysis of fish-derived carbonates in shallow sub-tropical marine environments and a potentially unrecognised origin for peloidal carbonates. Sed. Geol. 314: 17–30. doi:10.1016/j.sedgeo.2014.10.005
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: 765. doi:10.1038/s41598-017-00787-4
Salter, M. A., C. T. Perry, R. D. Stuart-Smith, G. J. Edgar, R. W. Wilson, and A. R. Harborne. 2018. Reef fish carbonate production assessments highlight regional variation in sedimentary significance. Geology 46: 699–702. doi:10.1130/G45286.1
Sano, M., M. Shimizu, and Y. Nose. 1984. Food habits of teleostean reef fishes in Okinawa Island, southern Japan. Tokyo: Univ. of Tokyo Press.
Schauer, K. L., and M. Grosell. 2017. Fractionation of the Gulf toadfish intestinal precipitate organic matrix reveals potential functions of individual proteins. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 208: 35–45. doi:10.1016/j.cbpa.2017.03.007
Schauer, K. L., C. M. LeMoine, A. Pelin, N. Corradi, W. C. Warren, and M. Grosell. 2016. A proteinaceous organic matrix regulates carbonate mineral production in the marine teleost intestine. Sci. Rep. 6: 34494. doi:10.1038/srep34494
Schauer, K. L., E. A. F. Christensen, and M. Grosell. 2018. Comparison of the organic matrix found in intestinal CaCO3 precipitates produced by several marine teleost species. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 221: 15–23. doi:10.1016/j.cbpa.2018.03.007
Smith, W. L., E. Everman, and C. Richardson. 2018. Phylogeny and taxonomy of Flatheads, Scorpionfishes, Sea Robins, and Stonefishes (Percomorpha: Scorpaeniformes) and the evolution of the lachrymal saber. Copeia 106: 94–119. doi:10.1643/CG-17-669
Truong, L., I. M. Suthers, D. O. Cruz, and J. A. Smith. 2017. Plankton supports the majority of fish biomass on temperate rocky reefs. Mar. Biol. 164: 73. doi:10.1007/s00227-017-3101-5
Urey, H. C., H. A. Lowenstam, S. Epstein, and C. R. McKinney. 1951. Measurement of paleotemperatures and temperatures of the Upper Cretaceous of England, Denmark, and the southeastern United States. Geol. Soc. Am. Bull. 62: 399–416. doi:10.1130/0016-7606(1951)62[399:MOPATO]2.0.CO;2
Walsh, P. J., P. Blackwelder, K. A. Gill, E. Danulat, and T. P. Mommsen. 1991. Carbonate deposits in marine fish intestines: A new source of biomineralization. Limnol. Oceanogr. 36: 1227–1232. doi:10.4319/lo.1991.36.6.1227
Watson, R. A. 2017. A database of global marine commercial, small-scale, illegal and unreported fisheries catch 1950–2014. Sci. Data 4: 170039. doi:10.1038/sdata.2017.39
Weinbauer, M. G., and B. Velimirov. 1995. Calcium, magnesium and strontium concentrations in the calcite sclerites of Mediterranean gorgonians (Coelenterata: Octocorallia). Estuar. Coast. Shelf Sci. 40: 87–104. doi:10.1016/0272-7714(95)00115-2
Wilson, R. W., K. M. Gilmour, R. P. Henry, and C. M. Wood. 1996. Intestinal base excretion in the seawater-adapted rainbow trout: A role in acid–base balance? J. Exp. Biol. 199: 2331–2343.
Wilson, R. W., J. M. Wilson, and M. Grosell. 2002. Intestinal bicarbonate secretion by marine teleost fish—why and how? Biochim. Biophys. Acta 1566: 182–193. doi:10.1016/S0005-2736(02)00600-4
Wilson, R. W., F. J. Millero, J. R. Taylor, P. J. Walsh, V. Christensen, S. Jennings, and M. Grosell. 2009. Contribution of fish to the marine inorganic carbon cycle. Science 323: 359–362. doi:10.1126/science.1157972
Wojdyr, M. 2010. Fityk: A general-purpose peak fitting program. J. Appl. Cryst. 43: 1126–1128. doi:10.1107/S0021889810030499

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Conflict of Interest
None declared.