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Reef fish carbonate production assessments highlight regional variation in sedimentary significance

Salter et al.

DR Methods

Sample Collection and processing

A total of 44 fish species were collected at the study locations using either baited hook-and-line, seine net, or dip net, and then transferred to aquaria facilities at Heron Island and Moreton Bay Research Stations. In addition, 23 farm-reared individuals of one other species—*Lates calcarifer*—were supplied to Moreton Bay Research Station for the study. A list of all species sampled in the study is provided in Table DR1.

Aquaria were supplied with locally-drawn surface seawater filtered to 1 µm to remove any particulate matter. Seawater conditions were similar at both sites: mean temperatures of 23–25 ˚C; salinity 35.1–36.5 PSU; and pH_{NBS} 8.09–8.18. These parameters were also similar to those of earlier studies (Perry et al., 2011). Fish were fasted throughout the sampling period and for at least 48 hours prior to allow the gut to be voided of previously ingested items. Application of these measures ensured that material excreted during the sampling period comprised only carbonates precipitated within the gut and their associated mucus envelopes. Fitted within tanks were false mesh bottoms through which carbonates would sink after excretion, thus precluding the possibility they would be eaten by fish.

Carbonates were collected from aquaria using a siphon or disposable Pasteur pipette, typically at 24 hour intervals—although 4 hour intervals were employed in a small number of cases involving amorphous carbonate; see section on production rate measurements below. They were then rinsed with deionised water before being soaked in sodium hypochlorite (commercial bleach; <4% available chlorine) for approximately 6 hours to disaggregate organic material (Gaffey and Bronnimann, 1993). Further rinses with deionised water removed all traces of bleach before samples were dried for 24 hours at 50 ˚C.

Sample analysis

Physical properties of particles

Morphological characterisation of samples was achieved using a JEOL JSM-6390LV Scanning Electron Microscope. Dry samples, either in powder or pellet form, were mounted on adhesive carbon tape before application of a 20 nm conductive coating (Au-Pd). Images were acquired at accelerating voltages between 5 and 15 kEV and working distances of 7–12 mm using either secondary electron or backscatter detectors. Observations were made on at least 5 pellets from each fish or group of fish that was sampled, such that morphological data are collectively based on observations of >630 pellets produced by 240 individual fish (summarised in Table DR1).

Compositional data were collected using an energy-dispersive X-ray spectrometer (Oxford Instruments) fitted to a Hitachi S-3200N SEM. Samples were prepared as for morphological analyses with the exception that a carbon conductive coating was used instead of Au-Pd, since the X-ray peaks it generates do not interfere with the elements of interest. Analyses were performed using an accelerating voltage of 20 kEV, a working distance of 15 mm, and acquisition time of >40 s, employing a spot sampling approach to facilitate analysis of each particle morphotype independently. However, owing to the possibility that regions surrounding sampling spots could have contributed to spectra, particularly given the uneven nature of surfaces being analysed, data were only considered representative of a particular morphology if subject particles were surrounded by morphologically similar particles. To ensure data were representative, analyses were performed at a minimum of 6 spot locations on each of at least 5 pellets per sample. In cases of multiple individual fish or groups of fish being sampled per species, this approach was replicated for up to 6 different samples; within-species compositions being found to be broadly consistent.

The rough and often sloping surfaces of samples are problematic because they represent topographic features that can interfere with the passage of X-rays to the EDS detector, with potential to introduce analytical artefacts through shadowing effects and remote excitation of X-rays. To minimise these effects, analyses were performed only on near-horizontal surfaces elevated above other surface topographic features. In addition, the multi-spot analytical approach described above removes the possibility of analytical artefacts being introduced as a consequence of surface topographic features at a particular spot and/or pellet. Nevertheless, compositional data are regarded as semi-quantitative on account of these issues. Employed elsewhere, however, this approach yielded $MgCO_3$ contents consistent with those estimated from X-ray diffraction and liquid ion chromatography data (Salter et al., 2012), so it is considered to be reliable for the purposes of this study.

Carbonate polymorph assessments were made using Fourier Transform Infrared (FTIR) spectroscopy. Powdered samples were mounted in ~0.5 mm thick transparent KBr discs and spectra were obtained by the co-addition of 32 repeated scans obtained at a resolution of 2 cm^{-1} using a Nicolet FTIR spectrometer. To ensure data were representative, spectra were acquired for at least three sub-samples (each comprising 2–3 pellets) from samples produced by each of up to 9 individuals or groups of fish per species. Carbonate polymorphs were then identified by comparing spectra against an extensive spectral database (see Salter et al., 2017).

Finally, polymorph and compositional data were used to assign each particle morphotype to a carbonate mineral category. The relative abundances of different particle morphotypes (and associated mineral categories) produced by each fish species were then estimated by visual assessment of every sample observed using SEM. Because carbonates produced by members of the same family are generally similar (see main text; also Salter et al. 2012; 2017), mineral categories for each species were combined with existing data from Caribbean locations (Salter et al., 2017) and averaged to generate family-level data (Table DR3).

Production rate measurements

Production rates were determined over periods typically ≥72 hours (and in all cases at least 48 hours) by quantifying the carbonate produced by fishes of known mass. Since samples are typically small, and in some cases contain fragments of fish scales and fin rays, they are often not suited to

quantification by weighing. Instead samples were quantified using a double titration approach to determine the amount of carbonate present, as described by Perry et al (2011). Titrations were performed using a Metrohm Titrando autotitrator and Methrohm Aquatrode pH electrode. Titrant concentrations of 0.001–0.1 N (for both HCL and NaOH) were used as appropriate for sample size.

Because study fishes were: i) starved; ii) held in confined spaces that restrict active swimming; and iii) in many cases held individually (precluding social interactions), their metabolic rates will have been depressed compared with those of fish that are feeding, actively swimming, and engaged in activities such as chasing, foraging, and fleeing (i.e., normal natural behaviour). Since metabolic rate correlates positively with carbonate production rate (Wilson *et al*., 2009), this means our measured production rates will be highly conservative. In order to generate more practical real-world outputs, we thus apply a scaling factor to our data to account for the difference between normal metabolic rates and those depressed through removal of feeding and physical activity. Such scaling factors are estimated to be in the range 2.5–3.4 (Kerr, 1982), and the lowest value in that range is adopted here to conservatively adjust measured production rates (see Wilson *et al*., 2009; Salter *et al.*, 2017).

A number of fish species sampled in this study produce carbonates dominated by amorphous calcium carbonate; a phase widely considered to be highly unstable at Earth surface conditions (Brečević and Nielsen, 1989; Clarkson et al., 1992; Beniash et al., 1997; Radha et al., 2010). Indeed, it has been shown that fish-derived amorphous carbonates can begin to dissolve in seawater within 24 hours of excretion (Foran et al., 2013). Consequently, sampling intervals of 24 hours may be insufficient for determining production rates involving amorphous polymorphs due to the potential for sample loss through dissolution. Production rate data based on 24 hour sampling intervals for these fish families (Labridae, Pomacentridae, Blennidae, Gobiidae, Microcanthidae, and Muraenidae) were therefore omitted. However, sampling intervals of 4 hours were employed for 5 indivdual/groups of labrid fishes (n = 7 fish; Table DR1) in order to reduce post-excretion exposure times and thus limit the potential for significant sample loss through dissolution. Production rate data obtained using these samples were comparable to production rates determined for fishes with similar body masses that produce crystalline carbonate polymorphs.

Fish biomass surveys and carbonate production modelling

Reef-scale carbonate production models were generated for 9 coral reef systems in tropical and subtropical regions on the eastern and western seaboards of Australia (Table DR2) by integrating carbonate production data with data on whole fish community biomass structure from the Reef Life Survey (RLS) database. The RLS database represents a compilation of global surveys of shallow rocky and coral reef ecological communities that are conducted according to a standard methodology detailed in an online methods manual (http://reeflifesurvey.com/wp-content/uploads/2015/07/NEW-Methods-Manual_150815.pdf) and described in Edgar and Stuart-Smith (2014) and Edgar et al. (2017). Fish surveys were conducted along paired belt transects 50 m x 10 m, and up to a height of 5 m above the reef substrate, which was typically at depths between 1 and 12 m (but ranging from 0.3 to 22 m). All fishes observed in the transect area were recorded, with abundance estimates made by counting individuals of less abundant species and estimating the numbers of more abundant species. The majority of fishes were recorded to species level, but unidentified fishes were classified at the highest taxonomic resolution possible rather than omitting them. Body lengths (from snout to tip of tail) were allocated to the following size classes: 2.5, 5.0,

7.5, 10.0, 12.5, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 50.0, and 62.5 cm total length. Fishes larger than 62.5 cm were estimated to the nearest 12.5 cm and recorded individually. Fish biomass (in units of $g \cdot 500\ m^{-2}$) was estimated using the abundance and sizes of fishes observed and species-specific length-weight relationships provided in FishBase (Froese and Pauly, 2017). When length-weight relationships were unknown for a species, values were taken from a similarly-shaped congener.

Gross carbonate production (in $\mu mol \cdot hr^{-1}$) by each fish recorded in these biomass datasets was estimated using the production rate–body mass relationship, with the proportion of production represented by each carbonate polymorph being estimated based on family-specific abundance data (for this purpose Western Pacific data from this study were combined with existing Caribbean data; Salter et al., 2017). Overall outputs per 500 m^2 transect were calculated as the sum of estimates for every fish recorded within it. Conversion of these outputs to mass units using the appropriate molecular weight for each mineral phase (ranging from 84.31 g for amorphous Mg-carbonates to 100.09 g for pure calcite and aragonite) facilitates their expression in terms of $g \cdot m^{-2} \cdot yr^{-1}$.

Numerous discrete sites were surveyed within each reef system, with multiple unique surveys being conducted at many of these sites – either as time-series data or as surveys conducted simultaneously along different transects (Table DR2). Site-specific fish biomass data and carbonate production outputs are expressed as the average of outputs from each transect recorded at that site, and the median of these site-specific data (and inter-quartile range) is used to describe reef-scale outputs.

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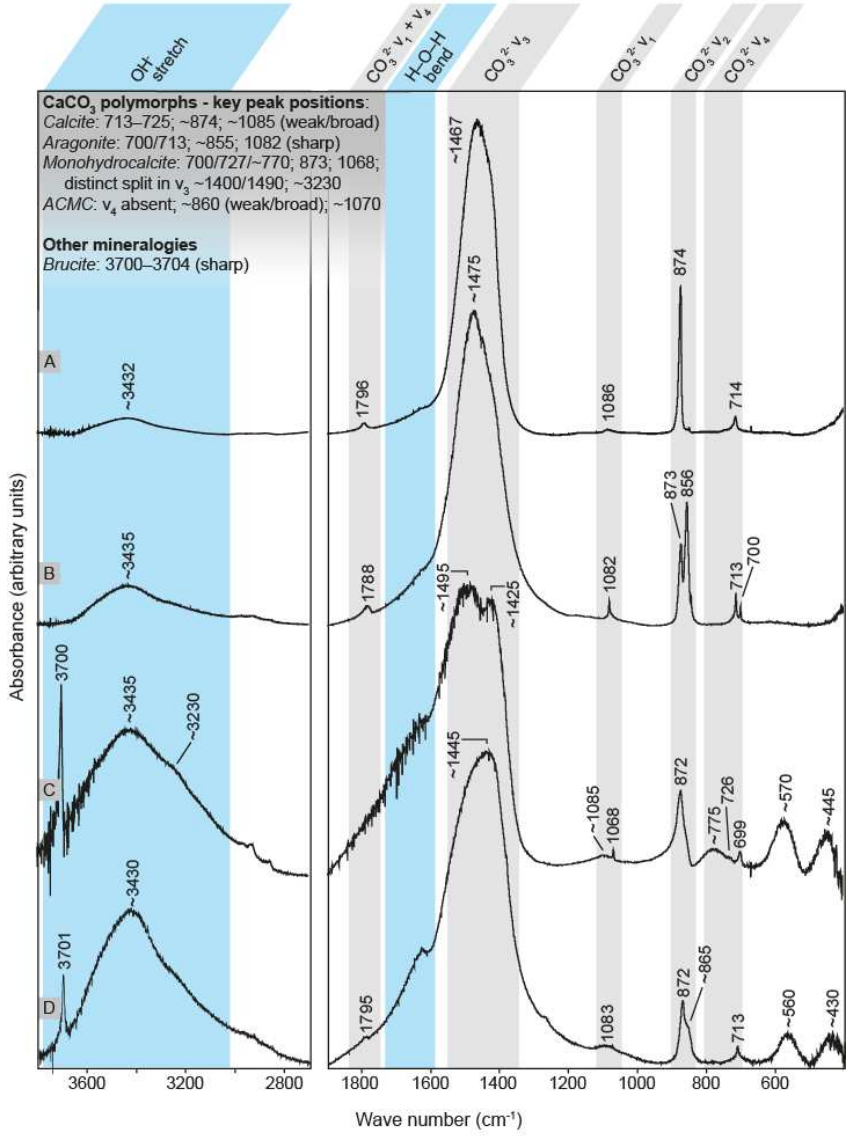

Figure DR1 Representative FTIR spectra for fish carbonate polymorphs sampled at Western Pacific Ocean locations, characterised on the basis of absorption bands associated with vibrational modes of CO_3^{2-} (highlighted grey) and OH^-/H-O-H (blue). A – calcite (produced by *Sillago sihama*); B – calcite and aragonite (*Lethrinus miniatus*); C – monohydrocalcite, amorphous carbonate, and brucite (*Arothron hispidus*); and D – amorphous carbonate, calcite, and brucite (*Thalassoma lunare*).

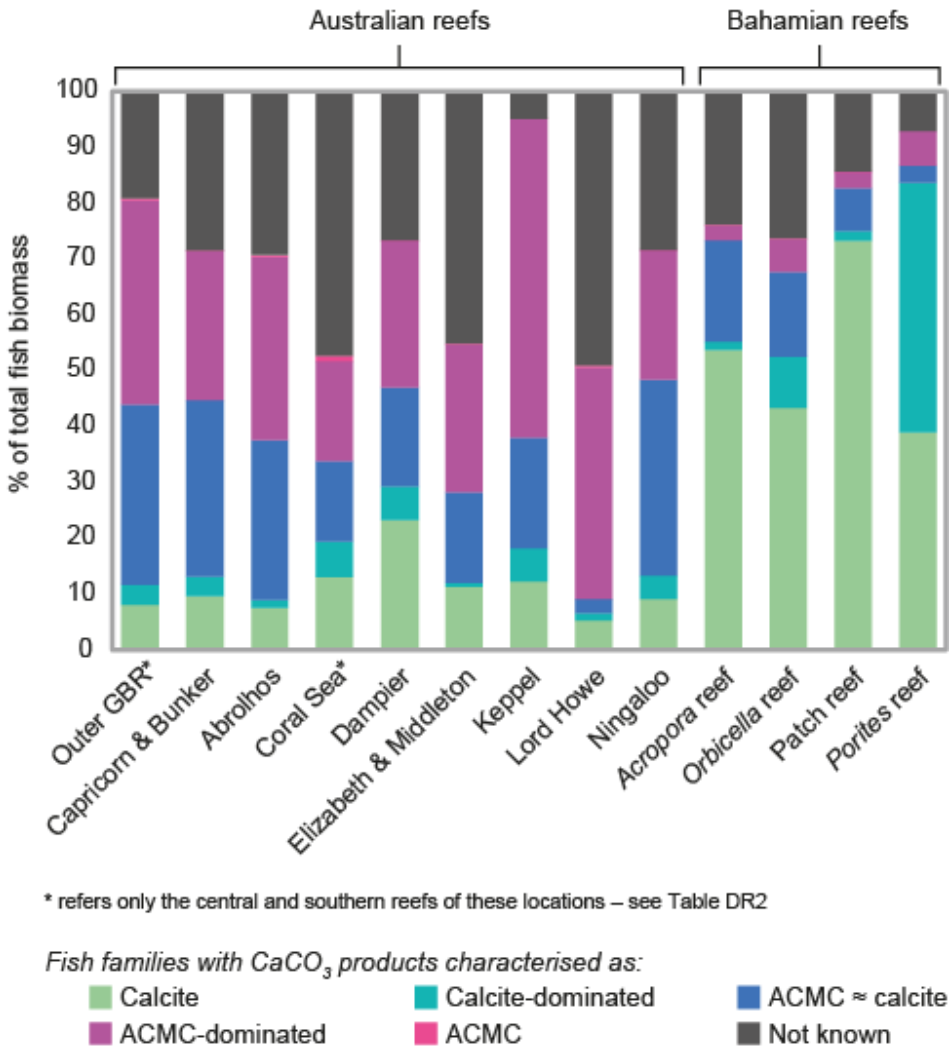

Figure DR2 Fish biomass of Australian and Bahamian coral reefs grouped by fish families with different dominant carbonate products. On the Australian study reefs, families known to produce calcite-dominated carbonates account for 7–29% of total biomass (13–40% of biomass for which products have been assessed), compared with 53–84% on Bahamian reefs (71–90% assessed biomass). By contrast, families known to produce carbonates with a significant amorphous component account for 33–77% of total biomass on Australian study reefs (60–87% of assessed biomass), compared with only 9–21% on Bahamian reefs (10–29% of assessed biomass). Data for Australian study reefs are from the Reef Life Survey (RLS) database. Data for Bahamian reefs are from the biomass datasets described in Salter et al. (2017).

Table DR1 Western Pacific fishes sampled in this study and a summary of carbonate analyses and products

Species	Family	No. of groups^ sampled	No. of fish sampled	Production rate data (no. of groups, fish)	Particle morphotypes †	Precipitate mineralogy ‡
Apogon limenus	Apogonidae	1	1	1, 1	e	H
Blenny spp.	Blennidae	1	4	amorph**	ns>r	AC>L>H
Caesio cuning	Caesionidae	1	1	1, 1	d>ns>r	H>AC>L
Tragulichthys jaculiferus	Diodontidae	1	1	1, 1	s>d>e>ns	H>AC>AR
Echeneis naucrates	Echenidae	1	1	-	d>s>e>w	H>L
Valenciennea immaculata	Gobiidae	1	1	amorph**	a>ns>r>s	AC>H>L
Plectorhinchus picus	Haemulidae*	1	1	1, 1	e	H
Microcanthus strigatus	Microcanthidae	2	3	amorph**	ns	AC
Thalassoma lunare	Labridae*	2	3	1, 1***	a>ns>r	AC>H>L
Halichoeres margaritaceus	Labridae*	1	1	amorph**	a>ns>r	AC>H>L
Halichoeres trimaculatus	Labridae*	6	8	4, 6***	a>ns>r	AC>H>L
Lates calcarifer	Latidae	15	23	12, 20	e>p	H>B
Lethrinus miniatus	Lethrinidae	9	14	9, 14	e>w>d>s>ns	H>AR>AC
Gymnocranius audleyi	Lethrinidae	3	4	3, 4	e>w>d>s	H>AR
Lethrinus genivittatus	Lethrinidae	3	15	3, 16	e>s>d	H
Lethrinus nebulosus	Lethrinidae	3	3	3, 3	e>d>s	H>AR
Lutjanus kasmira	Lutjanidae*	1	1	-	e	H
Lutjanus russellii	Lutjanidae*	10	21	10, 21	e>p	H>B
Lutjanus carponotatus	Lutjanidae*	1	1	1, 1	e>np	H
Lutjanus adetii	Lutjanidae*	2	3	2, 3	e>np	H
Gymnothorax pseudothyrsoideus	Muraenidae	2	2	amorph**	ns	AC
Pentapodus paradiseus	Nemipteridae	1	1	-	e	H
Parapercis queenslandica	Pinguipedidae	2	2	2, 2	e>np	H
Parapercis australis	Pinguipedidae	1	1	-	e	H
Cymbacephalus nematophthalmus	Platycephalidae	1	1	-	e>np	H
Plotosus lineatus	Plotosidae	1	3	-	s>d>e>w	H>L
Amphiprion clarkii	Pomacentridae*	1	1	amorph**	ns	AC
Abudefduf bengalensis	Pomacentridae*	2	2	amorph**	a>ns>r	AC>H>L
Abudefduf septemfasciatus	Pomacentridae*	2	2	amorph**	a>ns>r	AC>H>L
Ogilbyina queenslandiae	Pseudochromidae	2	2	2, 2	e>ns>a	H>AC
Dendrochirus zebra	Scorpaenidae*	1	1	1, 1	e>np>p	H>B
Scorpaenopsis diabolus	Scorpaenidae*	1	1	1, 1	e>p	H
Epinephelus fasciatus	Serranidae*	6	6	5, 5	e>np	H
Epinephelus quoyanus	Serranidae*	1	1	1, 1	np>e	H
Plectropomus leopardus	Serranidae*	3	4	3, 4	e>ns	H>AC
Sillago maculata	Sillaginidae	1	4	1, 4	e	H
Sillago sihama	Sillaginidae	5	12	5, 12	e	H
Pagrus auratus	Sparidae	5	8	5, 8	e>w>np	H
Rhabdosargus sarba	Sparidae	3	6	3, 6	e	H
Acanthopagrus australis	Sparidae	7	9	4, 6	e>w>np	H
Trachyrhamphus bicoarctatus	Sygnathidae	1	5	-	e	H
Hippocampus whitei	Sygnathidae	1	1	1, 1	e>np	H
Pelates sexlineatus	Terapontidae	6	49	4, 29	e>p	H>B
Arothron hispidus	Tetraodontidae*	1	1	-	s>d>e>a	L>H>AC>M
Centropogon australis	Tetrarogidae	4	5	4, 5	e	H
Totals		**126**	**240**	**94, 180**		

***Footnotes*:** Morphotypes and mineralogies are listed in order of relative abundance.

† Morphotype codes: e – ellipsoid; s – sphere; d – dumbbell; w – wheatsheaf; np – nanoparticle (similar to ellipsoid but poorly-defined); ns – nanosphere; a – material lacking definable shape; r – rhombohedra; p – plate

‡ Mineral/polymorph codes: H – HMC; L – LMC; AC – ACMC; AR – aragonite; M – monohydrocalcite; B - brucite

^ Groups refer to the total number of separate groups in which the combined total number of individual fish were housed. I.e., 21 individuals of *Lutjanus russellii* were housed in 10 separate groups (group size in this case ranging from 1 to 3).

* Families also used in The Bahamas.

** Production rates not determined owing to likelihood of sample loss through dissolution of ACMC.

*** Although these fish produce significant ACMC, the potential for dissolution was limited by reducing sampling intervals to 4 hours.

Table DR2 Details of reef systems for which fish carbonate production models were generated.

Reef system	Latitudinal range	Longtudinal range	No. of RLS^ sites	No. of RLS^ surveys	Year(s) RLS^ surveys conducted
Abrohlos Islands	-28.5 to -28.9	113.8 to 114.0	10	25	2008
Capricorn and Bunker group, GBR	-23.2 to -23.9	151.7 to 152.4	29	79	2015, 2017
Coral Sea	-20.9 to -23.6	153.5 to 155.8	56	170	2013, 2016, 2017
Dampier archipelago	-20.5 to -20.6	116.5 to 116.7	16	44	2010, 2013
Elizabeth and Middleton reefs	-29.4to -30.0	159.0 to 159.1	33	66	2013
Keppel Islands	-23.2	150.9 to 151.1	15	42	2010, 2015, 2017
Lord Howe Island	-31.5 to -31.8	159.0 to 159.3	52	534	2009, 2010, 2012, 2014, 2016
Ningaloo Marine Park	-21.9 to -23.3	113.7 to 114.0	35	208	2008, 2010, 2012, 2015, 2016
Central and southern outer GBR*	-16.4 to -22.4	146.0 to 152.5	73	182	2010, 2012, 2015, 2016, 2017

^Reef Life Survey
*Excludes Capricorn and Bunker group

Table DR3 Precipitate mineral abundance estimates for each family sampled in the Western Pacific (this study) and the Caribbean (Salter et al., 2017)

	Precipitate mineral/carbonate polymorph							
	Calcite ($MgCO_3$ content)						Monohy-	
Family	**0-4**	**4-15**	**15-25**	**>25**	**ACMC**	**Aragonite**	**drocalcite**	**Brucite**
Lutjanidae[a]	-	-	25.9	73.7	-	-	-	0.4
Lethrinidae	-	6.3	40.6	47.2	0.9	5.0	-	-
Sparidae	-	-	7.0	93.0	-	-	-	-
Serranidae[a]	-	-	-	92.4	6.7	-	-	0.9
Scorpaenidae[a]	-	-	1.7	93.0	-	-	-	5.3
Pinguipedidae	-	4.0	30.0	66.0	-	-	-	-
Pseudochromidae	-	-	17.5	52.5	30.0	-	-	-
Silliganidae	-	-	28.0	72.0	-	-	-	-
Echenidae	3.0	44.0	53.0	-	-	-	-	-
Latidae	-	-	-	98.0	-	-	-	2.0
Caesionidae	1.8	30.3	28.9	-	39.0	-	-	-
Labridae (non-scarine)[a]	11.7	12.0	6.3	-	70.0	-	-	-
Microcanthidae	-	-	-	-	100.0	-	-	-
Haemulidae[a]	-	-	11.0	89.0	-	-	-	-
Platycephalidae	-	-	19.0	81.0	-	-	-	-
Apogonidae	-	-	-	100.0	-	-	-	-
Nemipteridae	-	0.5	69.5	30.0	-	-	-	-
Tetrarogidae	-	-	-	100.0	-	-	-	-
Syngnathidae	-	-	11.0	89.0	-	-	-	-
Gobiidae	6.0	14.0	-	-	80.0	-	-	-
Muraenidae	-	-	-	-	100.0	-	-	-
Terapontidae	-	-	7.0	92.0	-	-	-	1.0
Pomacentridae[a]	9.4	10.6	-	-	80.0	-	-	-
Diodontidae	-	45.0	13.2	16.8	20.0	5.0	-	-
Tetraodontidae[a]	50.0	15.0	5.0	-	15.0	5.0	10.0	-
Plotosidae	20.0	14.0	49.0	17.0	-	-	-	-
Blennidae	13.0	7.0	-	-	80.0	-	-	-
Labridae (scarine)[b]	35.0	-	-	-	40.0	20.0	5.0	-
Bothidae/Paralichthyidae[b]	-	-	-	100.0	-	-	-	-
Sphyraenidae[b]	3.0	-	37.0	20.0	30.0	10.0	-	-
Belonidae[b]	10.0	45.0	5.0	-	30.0	7.5	-	2.5
Pomacanthidae[b]	95.0	-	-	-	-	5.0	-	-
Ostraciidae[b]	95.0	-	-	-	-	5.0	-	-
Albulidae[b]	-	20.0	30.0	-	35.0	10.0	5.0	-
Gerreidae[b]	2.5	7.5	75.0	-	5.0	10.0	-	-

All mineral abundance estimates are based on new data from Western Pacific fishes, with the following exceptions:
[a]Assessments based on data from both Western Pacific and Caribbean members;
[b]Assessments based on data from Caribbean members only