

S1: Additional methods and data

Methods – collagen demineralization description

Small bone chunks (~0.5g) were cleaned manually using a toothbrush and sandpaper, then sonicated at least three times in Type I water to remove surface contaminants. Bones were initially placed in 10mL of 1M HCl for rapid demineralisation, followed by 10mL of refrigerated 0.5M HCl and placed in the fridge to slow the reaction and prevent collagen loss while the sample continued to demineralize. The acid was replaced with fresh, cold 0.5M HCl every two days until demineralisation was complete and a pseudomorph was present. The samples were then rinsed three times with Type 1 water. The collagen samples were treated with 0.125M NaOH for 1 hour to remove any humic contaminants and then rinsed again three times in Type 1 water, centrifuging between rinses if needed. To solubilise the collagen, 10mL of pH 3 water was added to each sample before being placed in the Vortemp at 70°C for 48 hours. After solubilisation, the samples were filtered using Ezee filters to remove any insoluble pieces and the liquid transferred into weighed glass vials. Ultrafiltration was not considered necessary as the samples were relatively well-preserved and pretreatment with NaOH was used to remove humic contaminants. In the collagen quality assessment below, we provide evidence that this approach was sufficient for these samples. Samples were then frozen at -20°C for 48 hours and then lyophilized for 48 hours, resulting in a white or off-white, cotton-candy-like product.

Methods – sample analysis extra information

These data were produced over two analytical runs, with the first run containing most of the samples (n=16; 1 duplicate) and the second run containing one sample run as a duplicate. Both runs used the same calibration standards (USGS40, USGS41, and EDTA-OAS) (Supplementary Table 1). An internal QC reference material, GLT (gelatin long-term mean values and σ : $\delta^{13}\text{C} - 9.37 \pm 0.01$ ‰, $\delta^{15}\text{N} 5.94 \pm 0.02$ ‰) was also analysed in both runs (n=7 for first run and n=4 for second run).

Supplementary Table 1. Standard reference materials used for calibration of $\delta^{13}\text{C}$ relative to VPDB and $\delta^{15}\text{N}$ relative to AIR.

Standard	Material	Accepted $\delta^{13}\text{C}$ (‰, VPDB)	Accepted $\delta^{15}\text{N}$ (‰, AIR)
USGS40	Glutamic Acid	-26.389	-4.52
USGS41	Glutamic Acid	+37.626	+47.57
EDTA-OAS	Ethylenediaminetetra-acetic Acid	-38.83	-0.71

Collagen quality – justification for samples included

Four samples fit into standard ‘envelopes’ for quality criteria (e.g., Ambrose 1990; DeNiro 1985), but warrant more scrutiny before their inclusion. The Diodontidae sample, LBL009a, has %wtC value higher than expected (~45% in VanKlinken 1999), however the C:N value is acceptable, suggesting no extra carbon contamination. The other Diodontidae sample in this study (LBL022a) had good QC indicators and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of these two samples were very similar, so LBL009a is included in the following analysis. The Mullidae sample, LBL020a, has a low %wtC and %wtN despite a robust collagen yield. However the C:N value was acceptable and the $\delta^{13}\text{C}$ value was consistent with other carnivorous fish analysed in this study. Two samples, LBL010a and LBL014a had C:N values lower than expected (less than 3, as in Guiry and Szpak 2020), and overall lower %wtC and %wtN. However, statistical assessment of all these samples through correlation between the different QC criteria does not show alteration of the stable isotope values of carbon or nitrogen as a result of these lower C:N values (Supplementary Figure 1).

The correlograms presented in Supplementary Figure 1 visualise Spearman’s rank order correlations between the various QC criteria. Orange indicated a strong positive correlation, while blue is a strong negative correlation. The strength of either color indicated the strength of the correlation. Non-significant

tests are marked with an 'X'. For taxa with more than 3 samples, taxa-specific tests were performed (Supplementary Figure 1b-c).

Supplementary Figures and Tables

Supplementary Table 2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and collagen quality indicators of human and faunal material. Samples below the black line failed. Bold indicates the collagen quality control indicators that were not within the acceptable range, and asterisks indicate samples that failed during the demineralization process, so no quality indicators are available.

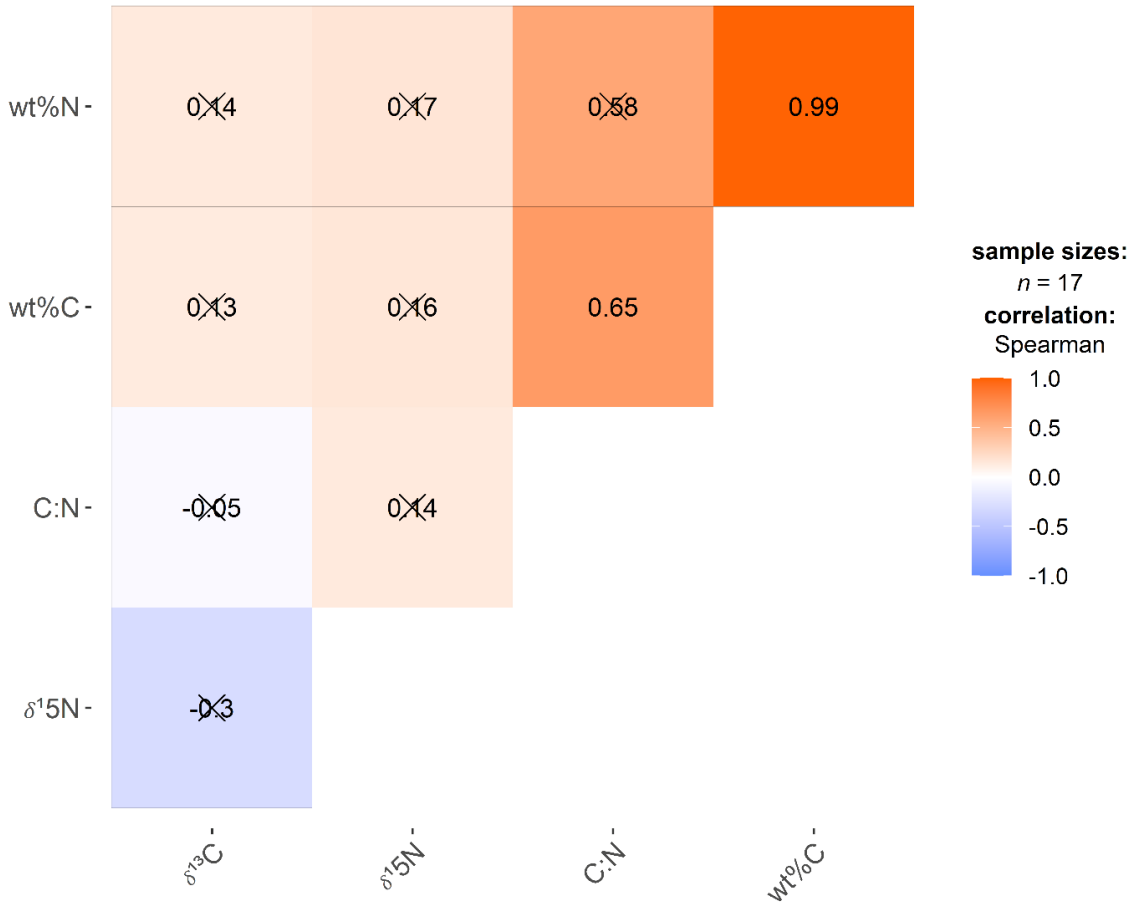
Sample ID	Species	Collagen yield, %	$\delta^{13}\text{C}_{\text{VPDB}}$, ‰	Carbon % w/w	$\delta^{15}\text{N}_{\text{AIR}}$, ‰	Nitrogen % w/w	C:N
LBL001a	Human Y2-25-1	3.64	-15.2	37.7	10.9	14.3	3.07
LBL002b	Human Y2-39 juvenile (10-12 years old)	2.21	-18.5	36.2	7.9	13.8	3.07
LBL003b	Human Y2-39 adult	3.16	-16.5	38.3	10.1	14.6	3.05
LBL004a	Turtle	7.20	-9.9	36.1	4.4	13.9	3.02
LBL007a	Pig	4.38	-16.4	34.4	9.3	13.0	3.07
LBL009a	<i>Diodontidae</i>	11.36	-11.8	47.0	8.2	17.9	3.07
LBL010a	<i>Acanthuridae</i>	9.87	-9.2	18.1	5.2	7.1	2.96
LBL013a	<i>Labridae</i>	6.53	-10.5	42.2	7.2	15.8	3.17
LBL014a	<i>Carangidae</i>	7.89	-11.3	23.5	9.3	9.3	2.95
LBL015a	<i>Carangidae</i>	6.17	-12.8	34.2	9.4	13.1	3.06
LBL017a	<i>Lethrinidae</i>	1.08	-9.7	38.3	10.3	14.1	3.16
LBL019a	<i>Lutjanidae</i>	15.54	-12.9	20.3	8.8	7.7	3.06
LBL020a	<i>Mullidae</i>	6.29	-12.9	14.5	7.4	5.6	3.02
LBL022a	<i>Diodontidae</i>	9.85	-11.8	16.5	8.1	6.3	3.04
LBL023a	<i>Scorpaenidae</i>	9.63	-10.2	40.6	10.1	15.1	3.13
LBL025a	Bird	1.20	-13.6	34.3	6.3	12.2	3.29
LBL002a	Human Y2-39 juvenile (10-12 years old) *	0.26					

Table 12. (continued).

LBL003a	Human Y2-39 adult*	0.89					
LBL005a	Pig*	NA					
LBL006a	Pig	0.40					
LBL008a	<i>Serranidae</i> *	1.18					
LBL011a	<i>Scaridae</i>	5.97	-11.1	63.4	5.3	23.8	3.12
LBL012a	<i>Mullidae</i> *	1.57					
LBL016a	<i>Carangidae</i> *	NA					
LBL018a	<i>Serranidae</i> *	NA					
LBL021a	<i>Carangidae</i>	0.65					
LBL024a	<i>Holocentridae</i> *	NA					

a

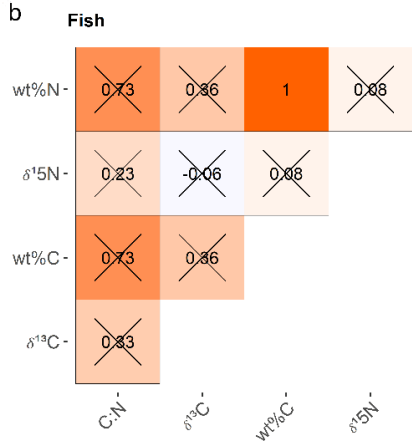
Correlogram for QCs for all collagen samples



X = non-significant at $p < 0.05$ (Adjustment: Holm)

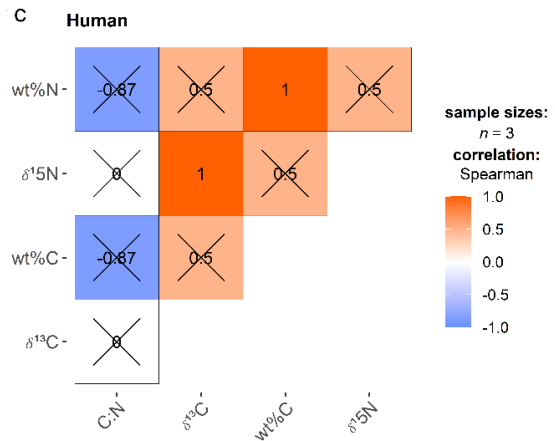
Relationship between QC indicators by taxa

b



X = non-significant at $p < 0.05$ (Adjustment: Holm)

c

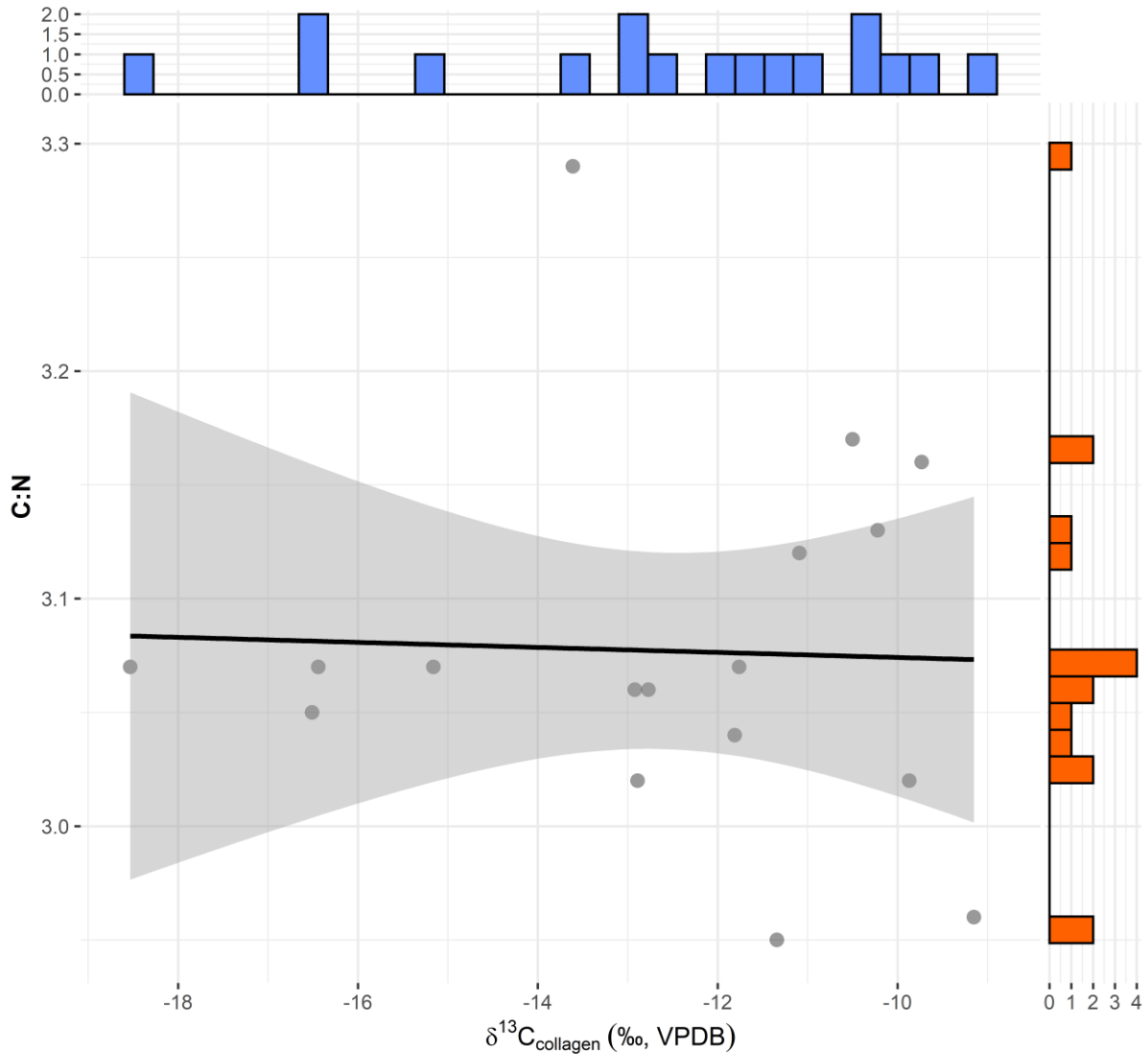


X = non-significant at $p < 0.05$ (Adjustment: Holm)

Supplementary Figure 1: correlogram of QC criteria for all samples (a) and then by taxa where the sample size is sufficient (b and c).

Relationship between C:N and $\delta^{13}\text{C}$ in collagen samples

$S = 854.28$, $p = 0.86$, $\hat{\rho}_{\text{Spearman}} = -0.05$, $\text{CI}_{95\%} [-0.53, 0.46]$, $n_{\text{pairs}} = 17$



Supplementary Figure 2: plot of C:N and $\delta^{13}\text{C}$ demonstrating no significant correlation between these variables.

MANOVA results

```
> fit_known_v2 <- manova(y ~ Location*Diet) # gives results of multivariate ANOVA
```

```
> summary(fit_known_v2)
```

	Df	Pillai	approx F	num Df	den Df	Pr(>F)
Location	3	0.99159	10.4888	6	64	4.464e-08 ***
Diet	2	0.41490	4.1880	4	64	0.004486 **
Location:Diet	4	0.33443	1.6063	8	64	0.140624
Residuals	32					

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
>
```

```
> summary.aov(fit_known_v2) # breaks results down by each dependent variable
```

Response C :

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Location	3	141.656	47.219	6.8436	0.001084 **
Diet	2	3.815	1.908	0.2765	0.760243
Location:Diet	4	54.698	13.674	1.9819	0.121024
Residuals	32	220.789	6.900		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Response N :

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Location	3	164.167	54.722	24.772	1.774e-08 ***
Diet	2	45.721	22.860	10.349	0.0003418 ***
Location:Diet	4	6.875	1.719	0.778	0.5477221
Residuals	32	70.689	2.209		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1