

*The following supplements accompany the article*

# **Rescaling stable isotope data for standardized evaluations of food webs and species niches**

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**Supplement 1.** Example of how species averages are re-scaled from measured isotope values (data from, for example, Louisiana bay fish data shown in Figs. 1 and 2). Rescaled values for individual species are obtained by subtracting the community mean from measured values and multiplying by (1.0‰/community SD) for  $\delta^{13}\text{C}$  and by (1.5‰/community SD) for  $\delta^{15}\text{N}$ , with the 1.0 and 1.5‰ values representing typical average SD values for fish communities considered in this study. Confidence limits for the species are similarly re-scaled by multiplying by (1.0‰/community SD) for  $\delta^{13}\text{C}$  and by (1.5‰/community SD) for  $\delta^{15}\text{N}$ , so that in the end the significant differences and relative spacing between means present in the measured data are preserved in the re-scaled data.

Location	Species	average $\delta^{13}\text{C}$	average $\delta^{15}\text{N}$	SD $\delta^{13}\text{C}$	SD $\delta^{15}\text{N}$	count $\delta^{13}\text{C}$	count $\delta^{15}\text{N}$	SEM $\delta^{13}\text{C}$	SEM $\delta^{15}\text{N}$	95% CL $\delta^{13}\text{C}$	95% CL $\delta^{15}\text{N}$
Manilla Village	Hardhead Catfish	-20.69	13.24	0.93	0.59	17.00	17.00	0.23	0.14	0.48	0.31
Manilla Village	Atlantic Croaker	-21.45	12.54	2.02	0.79	12.00	12.00	0.58	0.23	1.28	0.50
Manilla Village	Ladyfish	-22.16	10.82	2.28	0.71	9.00	9.00	0.76	0.24	1.74	0.54
Manilla Village	Gulf Menhaden	-21.60	12.60	1.96	1.81	12.00	12.00	0.57	0.52	1.24	1.15
Manilla Village	Spot	-22.36	12.19	1.70	1.33	7.00	7.00	0.64	0.50	1.54	1.21
	community mean across species	-21.65	12.28								
	community SD across species	0.66	0.90								
		rescaled average $\Delta^{13}\text{C}$	rescaled average $\Delta^{15}\text{N}$							rescaled 95% CL $\Delta^{13}\text{C}$	rescaled 95% CL $\Delta^{15}\text{N}$
Manilla Village	Hardhead Catfish	1.47	1.61							0.73	0.51
Manilla Village	Atlantic Croaker	0.30	0.44							1.96	0.84
Manilla Village	Ladyfish	-0.77	-2.43							2.66	0.90
Manilla Village	Gulf Menhaden	0.08	0.53							1.90	1.91
Manilla Village	Spot	-1.07	-0.14							2.35	2.01
Location	Species	average $\delta^{13}\text{C}$	average $\delta^{15}\text{N}$	SD $\delta^{13}\text{C}$	SD $\delta^{15}\text{N}$	count $\delta^{13}\text{C}$	count $\delta^{15}\text{N}$	SEM $\delta^{13}\text{C}$	SEM $\delta^{15}\text{N}$	95% CL $\delta^{13}\text{C}$	95% CL $\delta^{15}\text{N}$
Grand Terre	Hardhead Catfish	-19.00	13.87	2.09	0.96	13.00	13.00	0.58	0.27	1.26	0.58
Grand Terre	Atlantic Croaker	-20.16	11.97	2.59	1.67	11.00	11.00	0.78	0.50	1.73	1.11
Grand Terre	Ladyfish	-19.66	11.50	2.26	1.95	13.00	13.00	0.63	0.54	1.36	1.18
Grand Terre	Gulf Menhaden	-20.49	10.46	1.54	1.54	6.00	6.00	0.63	0.63	1.56	1.55
Grand Terre	Spot	-20.01	12.57	1.69	1.69	11.00	11.00	0.51	0.51	1.13	1.13
	community mean across species	-19.86	12.07								
	community SD across species	0.57	1.26								
		rescaled average $\Delta^{13}\text{C}$	rescaled average $\Delta^{15}\text{N}$							rescaled 95% CL $\Delta^{13}\text{C}$	rescaled 95% CL $\Delta^{15}\text{N}$
Grand Terre	Hardhead Catfish	1.53	2.13							2.22	0.69
Grand Terre	Atlantic Croaker	-0.52	-0.13							3.05	1.32
Grand Terre	Ladyfish	0.35	-0.68							2.40	1.40
Grand Terre	Gulf Menhaden	-1.10	-1.91							2.74	1.84
Grand Terre	Spot	-0.26	0.59							1.99	1.35

**Supplement 2.** Example of how to produce rescaled data for individuals in a series of steps that culminate in values that will give the same results as use of species averages that are illustrated above in Supplement 1. Data are for Louisiana bay fish from Manilla Village used in Figs. 1 and 2. The steps illustrate first calculations for C isotope data then N isotope data where rescaling of standard deviations involves multiplying by ‰/community SD (for  $\delta^{13}\text{C}$ ) or 1.5‰/community SD (for  $\delta^{15}\text{N}$ ), with the 1.0 and 1.5‰ values representing typical average SD values for fish communities considered in this study. Equations are given, followed by a spreadsheet example using these equations to produce rescaled individual data.

Note that some of the following steps for rescaling individual data are not very intuitive (especially step 4 below). These steps adjust the spread or dispersion of the data to bring it in line with the uniform scale set by community-level standard deviation. These adjustments in dispersion are adjustments that affect the errors such as SD and 95%CL. Separate steps (6 and 7 below) rescale the mean values to account for baseline differences in food webs being compared.

#### Carbon Isotope Rescaling Steps:

Step 1. The rescaling involves first using means to obtain the rescaled standard deviation ( $\text{SD}_{\text{RESCALED}}$ ) for a particular species (species A) in a community. If the SD for individuals in species A is  $\text{SD}_{\text{INDIVIDUALS IN SPECIES A}}$  and the SD for the means of species across a community is  $\text{SD}_{\text{SPECIES AVERAGES}}$ , then  $\text{SD}_{\text{RESCALED}}$  for the species is:

$$\text{SD}_{\text{RESCALED}} = \text{SD}_{\text{INDIVIDUALS IN SPECIES A}} / \text{SD}_{\text{SPECIES MEANS}}.$$

Step 2. Next the simple difference is calculated for individuals vs. the species mean:

$$\Delta = \text{individual value in species A} - \text{mean value for individuals in species A}.$$

Step 3. The  $\Delta$  values are squared and summed across the N observations as  $(\sum_{1 \text{ to } N} \Delta^2)$ .

Step 4. An appropriate multiplier (X) for the observed individual  $\Delta$  values is derived from the  $SD_{\text{RESCALED}}$  values. That is, if  $SD_{\text{RESCALED}} = \sqrt{\{[\sum_{1 \text{ to } N} (X * \Delta)^2] / (N-1)\}}$ ,

$$X = \sqrt{\{[(SD_{\text{RESCALED IN SPECIES A}})^2 * (N-1)] / [\sum_{1 \text{ to } N} (\Delta)^2]\}}.$$

Step 5. The individual value as deviation from species mean is then  $= \Delta * X$ .

Step 6. A semi-final step is to calculate the rescaled mean value for the species

$$\text{Mean Species A}_{\text{RESCALED}} =$$

$$(\text{Mean for Species A} - \text{Mean across community species}) / (\text{SD across community species}).$$

Step 7. The final rescaled value for an individual in Species A is obtained by adding the right sides of the last two equations:

$$\text{Individual}_{\text{RESCALED}} = \Delta * X + (\text{Mean for Species A} - \text{Mean across community species}) / (\text{SD across community species}).$$

Nitrogen Isotope Rescaling Steps.

Steps are the same as above for Carbon Isotopes, but a value of 1.5‰ rather than an implicit 1‰ is inserted in steps 1, 6 and 7, and a different value for X is calculated in step 4:

$$\text{Step 1. } SD_{\text{RESCALED IN SPECIES A FOR N ISOTOPES}} = 1.5 * SD_{\text{INDIVIDUALS IN SPECIES A}} / SD_{\text{SPECIES MEANS}}.$$

$$\text{Step 4. } X_{\text{FOR N ISOTOPES}} = \sqrt{\{[(SD_{\text{RESCALED IN SPECIES A FOR N ISOTOPES}})^2 * (N-1)] / [\sum_{1 \text{ to } N} (\Delta)^2]\}}.$$

$$\text{Step 6. Mean Species A}_{\text{RESCALED FOR N}} = 1.5 * (\text{Mean for Species A} - \text{Mean across community species}) / (\text{SD across community species}).$$

$$\text{Step 7. Individual}_{\text{RESCALED FOR N ISOTOPES}} = \Delta * X_{\text{FOR N ISOTOPES}} + 1.5 * (\text{Mean for Species A} - \text{Mean across community species}) / (\text{SD across community species}).$$

1. Species Averages for Manilla Village fish community:

	species mean $\delta^{13}\text{C}$	species mean $\delta^{15}\text{N}$
Hardhead Catfish	-20.69	13.24
Atlantic Croaker	-21.45	12.54
Ladyfish	-22.16	10.82
Gulf Menhaden	-21.60	12.60
Spot	-22.36	12.19
community mean across species	-21.65	12.28
community SD across species	0.66	0.90

2. Rescaling C Isotope Data for Individual Hardhead Catfish from Manilla Village:

Catfish #:	$\delta^{13}\text{C}$	step 1 $\text{SD}_{\text{RESCALED}}$	step 2	step 3	step 4 X	step 5	step 6 rescaled catfish species mean	step 7 <b>final rescaled individual value</b> $\Delta^{13}\text{C}, \text{‰}$
1	-20.75	1.42	-0.06	0.00	1.53	-0.10	1.47	<b>1.37</b>
2	-20.66		0.03	0.00		0.05		<b>1.51</b>
3	-19.89		0.80	0.64		1.22		<b>2.69</b>
4	-20.09		0.61	0.37		0.92		<b>2.39</b>
5	-19.44		1.25	1.56		1.90		<b>3.37</b>
6	-21.26		-0.57	0.33		-0.87		<b>0.59</b>
7	-21.80		-1.11	1.24		-1.70		<b>-0.23</b>
8	-19.96		0.73	0.53		1.11		<b>2.57</b>
9	-20.24		0.45	0.20		0.69		<b>2.15</b>
10	-22.66		-1.97	3.89		-3.01		<b>-1.54</b>
11	-19.72		0.98	0.95		1.49		<b>2.95</b>
12	-20.43		0.26	0.07		0.40		<b>1.87</b>
13	-21.19		-0.50	0.25		-0.76		<b>0.71</b>
14	-20.73		-0.04	0.00		-0.07		<b>1.40</b>
15	-21.78		-1.08	1.18		-1.65		<b>-0.19</b>
16	-21.72		-1.03	1.06		-1.57		<b>-0.11</b>
17	-19.41		1.28	1.64		1.95		<b>3.42</b>
mean	-20.69			13.90			mean	1.47
sd	0.93			sum of squares			sd	1.42
count	17						count	17
sem	0.23						sem	0.34
95% cl	0.48						95% cl	0.73

3. Rescaling N Isotope Data for Individual Hardhead Catfish from Manilla Village:

Catfish #:	$\delta^{15}\text{N}$	step 1 $SD_{\text{RESCALED}}$	step 2	step 3	step 4 X	step 5	step 6 rescaled catfish species mean	step 7 <b>final rescaled individual value</b> $\Delta^{15}\text{N}, \text{‰}$
1.00	13.94	0.99	0.70	0.49	1.67	1.17	1.61	<b>2.77</b>
2.00	13.48		0.24	0.06		0.40		<b>2.01</b>
3.00	13.65		0.40	0.16		0.67		<b>2.28</b>
4.00	13.67		0.42	0.18		0.71		<b>2.31</b>
5.00	13.67		0.42	0.18		0.71		<b>2.31</b>
6.00	13.94		0.70	0.49		1.17		<b>2.78</b>
7.00	12.29		-0.96	0.91		-1.59		<b>0.01</b>
8.00	12.84		-0.40	0.16		-0.66		<b>0.95</b>
9.00	13.70		0.45	0.21		0.76		<b>2.36</b>
10.00	12.00		-1.24	1.53		-2.06		<b>-0.46</b>
11.00	13.33		0.09	0.01		0.14		<b>1.75</b>
12.00	13.22		-0.03	0.00		-0.04		<b>1.56</b>
13.00	13.07		-0.17	0.03		-0.29		<b>1.32</b>
14.00	12.95		-0.29	0.09		-0.49		<b>1.12</b>
15.00	12.39		-0.85	0.73		-1.42		<b>0.19</b>
16.00	13.11		-0.14	0.02		-0.23		<b>1.38</b>
17.00	13.88		0.64	0.41		1.07		<b>2.68</b>
mean	13.24			5.66			mean	1.61
sd	0.59			sum of squares			sd	0.99
count	17						count	17
sem	0.14						sem	0.24
95% cl	0.31						95% cl	0.51

**Supplement 3.** Calculating P values and significant differences when isotope data is available for individual fish within species.

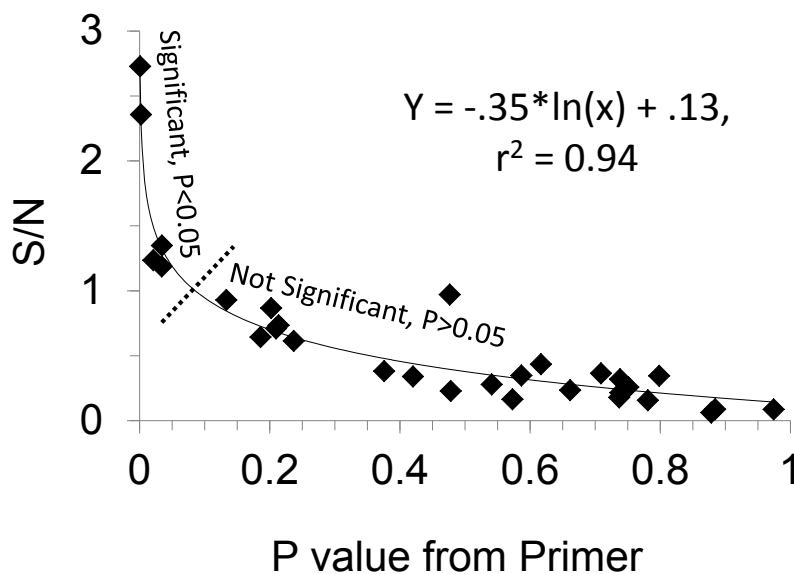
The multivariate statistical program, Permanova, can be used to calculate P values and significant differences between groups using bivariate-isotope data (e.g., Gerard and Muhling 2010, Newman et al. 2010). For the fish examples of this manuscript, we followed the Permanova approach of Newman et al. (2010) when C and N isotope data was available for individual fish within species. Here we give details about the Permanova steps involved for our Method 1 (same species) and Method 2 (between species) comparisons.

- 1) We consider the rescaling steps shown given above in Supplement 2 to be the equivalent of the normalization step used in Permanova (Newman et al. 2010), and we used the steps outlined in Supplement 2 to produce rescaled ( $\Delta^{13}\text{C}$ ,  $\Delta^{15}\text{N}$ ) values for individuals.
- 2) For Method 1, this rescaled data could then be directly used in the Permanova tests, i.e., rescaled values for individuals in species 1 in food web 1 can be compared directly to analogous individual values for species 1 in food web 2.
- 3) For Method 2, we first followed the Method 2 steps in the main text with the rescaled individual data, i.e., we considered two species in food web 1 and subtracted (x,y) values of one species from (x,y) values of the other to create a “tip” value for a vector connecting the two species. For example, if an individual in species 1 in food web 1 has a rescaled isotope value of (1,1) and an individual in species 2 in the same food web has a rescaled isotope values of (2,2), then the vector ”tip” connecting these points has a (1,1) value when recalculated to species 1 as (0,0). Using this approach, vector “tip” values for food web 1 were calculated for all possible connecting lines between individuals in species 1 and species 2. This set of vector “tip” values was then compared with the corresponding set of “tip” values calculated for food web 2, with this comparison done with Permanova that produced P values for the Method 2 comparisons.

**Supplement 4.** Comparison of two approaches for evaluating statistical significance with food web data and species data. The two approaches are 1) S/N ratios calculated with means and 2) P values calculated in Primer with individual-level data.

We used data from four sites in Moreton Bay (GI, PI 1, PI 3, and PI 7) to make pairwise species comparisons. Five species were collected at each site. Measured individual data were rescaled according to Supplement 2 and evaluated for species-level differences using a Euclidean distance similarity matrix in PERMANOVA +, and add-on to Primer, a statistical program for the analysis of multivariate data that yields P (significance) values. The same individual data were also used to calculate rescaled means and errors as in Supplement 1, and S/N ratios were calculated for the pairwise comparisons.

Comparisons between the two methods showed that S/N ratios >1 had significance values of  $P < 0.05$ . We used this result generally with the S/N ratios to infer significant ( $P < 0.05$ ) differences between species and food webs when S/N was 1 or greater.





## LITERATURE CITED

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