Supplementary Materials for

Distinguishing zooplankton fecal pellets as a component of the biological pump using compound-specific isotope analysis of amino acids

Shannon C. Doherty[1], Amy E. Maas[2], Deborah K. Steinberg[3], Brian N. Popp[4], Hilary G. Close[1*]

[1]Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL
[2]Bermuda Institute of Ocean Sciences, St. Georges GE01, Bermuda
[3]Virginia Institute of Marine Science, William & Mary, Gloucester Point, Virginia
[4]Department of Earth Sciences, University of Hawaii at Mānoa, Honolulu, Hawaii

*Corresponding author: hclose@rsmas.miami.edu

Supplementary table 1. Cruise and Collection details

Cruise	Date	Deployment Location Lat Long	Time of net deployment (local)	Net type	Mesh size (um)	Maximum depth (m)	Total tow time (min)	Number of animals	Incubation time	Filter size of FP
RR 1813 (EXPORTS)	September 2 & 3, 2018	50.58116 144.74885	22:02 -- 23:28 August (2)	1 m ring net	3x 500, 1600	100	4 x 14	Salps: 19 aggregates 2 solitary V. propinqua: nd (many)	overnight	GF/F (0.7 μm)
AE1819	July 3 & 4, 2018	31' 4.014 64' 10.75	23:50 (July 3)	Reeve	153	90	54	52 solitary	1.5 hr	GF/F (0.7 μm)
AE1910	May 22, 2019	32 33.387 64 33.609	01:14	Reeve	153	200	65	0.25–1mm size fraction 1–2.5mm size fraction	11 hr	6 μm
		32 32.259 64 33.821	02:24	Reeve	153	200	73	>1 mm size fraction	11 hr	6 μm
LMG 1801 (PAL LTER)	January 22, 2018	68 03.506 69 14.758	06:48:00	2x2m frame Metro net	700	120	27	8-12	9 hr	GF/F (0.7 μm)

Supplementary Table 2. Full $\delta^{15}N$ values of 14 AA for all samples. Error is the standard deviation of replicate runs; single run uncertainty displayed in figures was estimated using the average standard deviation for all runs of an individual amino acid (listed in final row).

		$\delta^{15}N$ (‰)												
Sample	# replicate injections	Ala	Gly	Thr	Ser	Val	Leu	Ile	Pro	Asp	Glu	Phe	Tyr	Lys
E. superba	2	13.9 ± 0.6	-0.4 ± 0.6	-10.6 ± 0.1	-1.1 ± 1.1	10.2 ± 3.4	11.9 ± 0.8	8.7 ± 0.7	10.1 ± 0.6	11.8 ± 0.7	15.2 ± 1.1	2.6 ± 1.3	4.3 ± 1.2	-2.6 ± 1.3
E. superba FP	1	10.6 ± 1.0	7.4 ± 1.0	-4.4 ± 1.0	-0.4 ± 1.0	5.8 ± 1.0	3.6 ± 1.0	nd	4.4 ± 1.0	5.4 ± 1.0	7.8 ± 1.0	1.6 ± 1.0	2.3 ± 1.0	-1.7 ± 1.0
NE Pacific *S. aspera*	3	13.2 ± 0.4	0.0 ± 0.4	-9.6 ± 0.5	-3.2 ± 1.0	10.0 ± 0.3	5.1 ± 0.1	7.9 ± 0.7	7.7 ± 0.1	7.8 ± 0.4	11.5 ± 0.4	0.2 ± 0.1	3.0 ± 0.1	-0.7 ± 0.4
NE Pacific *S. aspera* FP	3	4.0 ± 0.2	0.5 ± 0.2	-4.1 ± 0.5	-3.5 ± 0.1	6.0 ± 0.7	1.4 ± 0.5	2.1 ± 0.7	2.7 ± 0.1	-3.0 ± 0.3	5.5 ± 0.2	2.7 ± 0.7	nd -	-1.7 ± 0.2
Sargasso Sea mixed 0.25-1 mm	2	14.2 ± 0.1	4.5 ± 1.5	-10.5 ± 1.3	3.3 ± 1.3	11.1 ± 1.3	10.6 ± 0.4	10.5 ± 0.2	9.8 ± 1.0	10.4 ± 0.6	13.8 ± 1.5	1.7 ± 0.5	3.9 ± 1.0	0.3 1.5
Sargasso Sea mixed 0.25-1 mm FP	1	10.3 ± 1.0	2.3 ± 1.0	-7.6 ± 1.0	6.5 ± 1.0	1.0 ± 1.0	9.0 ± 1.0	nd	9.9 ± 1.0	7.5 ± 1.0	13.7 ± 1.0	0.4 ± 1.0	2.7 ± 1.0	nd
Sargasso Sea mixed 1-2.5 mm	3	16.0 ± 0.1	3.1 ± 0.0	-11.3 ± 0.3	1.3 ± 0.2	11.5 ± 0.2	10.7 ± 0.1	11.4 ± 0.3	9.1 ± 0.1	11.7 ± 0.1	15.7 ± 0.2	1.4 ± 0.1	3.6 ± 0.3	0.6 ± 0.2
Sargasso Sea mixed 1-2.5 mm FP	1	9.3 ± 1.0	3.7 ± 1.0	-10.6 ± 1.0	1.4 ± 1.0	12.7 ± 1.0	11.3 ± 1.0	-1.3 ± 1.0	9.7 ± 1.0	12.3 ± 1.0	18.3 ± 1.0	1.5 ± 1.0	2.9 ± 1.0	nd
Sargasso Sea mixed >1 mm	1	14.8 ± 1.0	3.8 ± 1.0	-13.0 ± 1.0	0.6 ± 1.0	10.2 ± 1.0	10.2 ± 1.0	9.3 ± 1.0	8.6 ± 1.0	10.8 ± 1.0	18.7 ± 1.0	2.8 ± 1.0	1.9 ± 1.0	-1.4 ± 1.0
Sargasso Sea mixed >1 mm FP	2	12.7 ± 0.1	2.9 ± 0.0	-14.5 ± 0.3	1.2 ± 0.2	11.0 ± 0.2	10.1 ± 0.1	8.4 ± 0.3	7.4 ± 0.1	9.5 ± 0.1	13.0 ± 0.2	-0.2 ± 0.1	2.0 ± 0.3	-2.4 ± 0.2
Sargasso Sea *Salpa sp.*	3	11.2 ± 0.4	0.3 ± 0.8	-10.2 ± 0.2	-2.6 ± 1.1	9.0 ± 0.9	4.7 ± 0.4	6.3 ± 0.2	5.9 ± 0.1	7.1 ± 0.2	7.8 ± 0.6	-1.1 ± 0.3	1.5 ± 0.8	-1.4 ± 0.5
Sargasso Sea *Salpa sp.* FP	1	6.9 ± 1.0	4.8 ± 1.0	-7.2 ± 1.0	2.6 ± 1.0	6.3 ± 1.0	4.0 ± 1.0	1.9 ± 1.0	nd	4.4 ± 1.0	9.6 ± 1.0	3.3 ± 1.0	2.1 ± 1.0	nd
V. propinqua	3	16.6 ± 0.2	0.2 ± 0.1	-14.5 ± 0.7	-1.1 ± 0.1	10.6 ± 0.0	10.4 ± 0.2	11.1 ± 0.3	8.9 ± 0.2	10.4 ± 0.2	16.1 ± 0.3	1.3 ± 0.6	3.4 ± 0.6	-1.0 ± 0.3
V. propinqua FP	1	12.3 ± 1.0	0.5 ± 1.0	-11.5 ± 1.0	-1.5 ± 1.0	8.2 ± 1.0	7.4 ± 1.0	7.6 ± 1.0	8.0 ± 1.0	8.7 ± 1.0	12.2 ± 1.0	0.3 ± 1.0	-0.6 ± 1.0	-0.2 ± 1.0

Supplementary Table 3. Results (p-values) of ANOVA and Tukey HSD tests for pairs of endmembers. Green boxes highlight p-values <0.05.

	$\delta^{15}N_{Thr-Phe}$	$\delta^{15}N_{Ala-Phe}$	$\delta^{15}N_{Glx-Phe}$	$\delta^{15}N_{Asp-Phe}$	$\delta^{15}N_{Val-Phe}$	$\delta^{15}N_{Pro-Phe}$	$\delta^{15}N_{Gly-Phe}$	$\delta^{15}N_{Leu-Phe}$	$\delta^{15}N_{Ser-Phe}$	ΣV
ANOVA	4.04E-13	<2e-16	<2e-16	3.67E-16	1.90E-13	6.74E-14	1.34E-04	<2e-16	7.25E-08	1.71E-01
MD-FP	2.31E-02	5.34E-01	4.49E-02	9.66E-01	7.90E-01	9.97E-01	9.52E-01	4.13E-01	6.99E-01	8.74E-01
Phyto-FP	0.00E+00	2.07E-04	5.00E-07	6.02E-02	1.18E-02	4.33E-01	6.41E-01	6.20E-06	2.25E-01	2.06E-01
Zoop-FP	6.97E-01	2.00E-07	1.97E-03	1.64E-05	1.83E-03	9.10E-06	1.89E-01	2.81E-04	6.42E-02	8.74E-01
Phyto-MD	5.16E-02	1.53E-05	2.00E-01	3.88E-01	2.98E-03	4.65E-01	9.79E-01	4.19E-02	9.77E-01	8.50E-01
Zoop-MD	4.19E-02	1.35E-02	2.00E-07	1.45E-04	2.85E-01	1.60E-03	1.30E-01	9.30E-06	8.62E-03	9.88E-01
Zoop-Phyto	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	2.78E-04	0.00E+00	3.00E-07	1.96E-01

Supplementary Table 4. Phe-normalized $\delta^{15}N$ values of Thr and Ala used in Figure 6, with metadata.

End-member	Source	Collection location	Species, sample type, time collected and/or depth	$\delta^{15}N_{Thr\text{-}Phe}$ (‰)	$\delta^{15}N_{Ala\text{-}Phe}$ (‰)
Microbially-degraded organic matter	Yamaguchi & McCarthy (2018)	North Pacific Subtropical Gyre	UPOM 670 m 500kD	-6.1	8.6
			UPOM 670 m 0.1 µm	-5.5	8.4
			HMW DOM 21 m	-2.0	10.2
			HMW DOM 915 m	-1.3	13.9
Zooplankton biomass	Hannides et al. (2020)	Station ALOHA	25 m, 0.2 mm, day	-7.2	12.7
			125 m, 0.2 mm, day	-9.3	14.0
			25 m, 1 mm, night	-8.7	16.5
			125 m, 1 mm, night	-10.9	18.1
	Hannides et al. (2009)		*Pleuromamma xiphias*	-11	17.9
			Pleuromamma xiphias	-8.6	19
			Pleuromamma xiphias	-7.9	20.1
			Pleuromamma xiphias	-10	18.7
			Neocalanus robustior	-7.4	14.6
			Neocalanus robustior	-6.8	14.1
			Neocalanus robustior	-7.6	14
			Oithona spp.	-9.8	14.4
			Oithona spp.	-8.2	15.8
			Oithona spp.	-12.1	12.9
			Oithona spp.	-9.4	15.1
			euphausiids (8 mm)	-13.6	16.5
			euphausiids (8 mm)	-8	14.6
			euphausiids (8 mm)	-10	14.3
			euphausiids (8 mm)	-9.4	17.3
			1-2 mm sieved	-11.3	15.7
			1-2 mm sieved	-10	15.5
			1-2 mm sieved	-8.6	16.8
	Hannides et al. (2013)		75m, 0.2 mm, day	-7.03	11.55
			75m, 0.5 mm, day	-7.24	14.33
			75m , 1 mm, day	-8.41	13.91
			75m, 2 mm, day	-11.10	16.03
			75m, 0.2 mm, night	-9.02	10.25
			75, 0.5 mm, night	-8.95	13.25
			75, 1 mm, night	-9.10	14.85

End-member	Source	Collection location	Species, sample type, time collected and/or depth	$\delta^{15}N_{Thr\text{-}Phe}$ (‰)	$\delta^{15}N_{Ala\text{-}Phe}$ (‰)
Zooplankton biomass (continued)	McClelland & Montoya (2003)	Tropical North Atlantic	Station 32, 100m	-2.8	11.4
			Station 30, 100m	-0.9	10.9
			Station 26, 100 m	-4.4	11.2
			Station 21, 100 m	-7.2	11.2
			Station 18, 100 m	-4.6	10.7
	Romero-Romero et al. (2020)	Equatorial Pacific	25m, night	-5.4	18.8
			75m, night	-10.3	17.5
			125m, night	-10.4	19.3
			175m, night	-4.5	19.6
			25m, night	3.5	16.8
			75m, night	-2.4	11.1
			175m, night	-14.8	19.8
			25m, day	-11.8	19.3
			75m, day	-4.7	16.5
			175m, day	-13.6	18.7
			25m, day	-6.8	14.2
			75m, day	-4.5	13.9
			125m, day	-3.4	19.9
			175m, day	-7.7	19.4
	This study	Southern Ocean	*E superba*	-13.1	11.3
		NE Pacific	*V. propinqua*	-15.8	15.3
			Salpa aspera	-9.9	12.9
		Sargasso Sea	*Salpa sp.*	-9.1	12.3
			Mixed 0.25-1 mm	-12.2	12.5
			Mixed 1-2.5 mm	-12.7	14.0
			Mixed >1 mm	-15.4	12.1

End-member	Source	Collection location	Species, sample type, time collected and/or depth	$\delta^{15}N_{Thr\text{-}Phe}$ (‰)	$\delta^{15}N_{Ala\text{-}Phe}$ (‰)
Fecal pellets	This study	Southern Ocean	*E superba*	-5.9	9.1
		NE Pacific	*V. propinqua*	-11.2	11.5
			Salpa aspera	-6.8	1.3
		Sargasso Sea	*Salpa sp.*	-10.5	3.6
			Mixed 0.25-1 mm	-8.0	9.9
			Mixed 1-2.5 mm	-12.1	7.8
			Mixed >1 mm	-16.7	10.7
Phytoplankton	McCarthy et al. (2013)	Culture	*Synechococcus sp.*	1.0	3.7
			Synechococcus sp.	-0.2	6.42
			Prochlorococcus marinus	1.4	2.88
			Trichodesmium erythaeum	-0.2	-2.05
			Thalassiosira psuedonana	-1.3	-0.49
			Amphidinium carterea	6.3	1.89
			Psuedo-nitzchia multiseries	-4.5	-3.51
			Skeletonema marinoi	1.7	-2.26
			Synechococcus sp.	2.1	-2.15
			Cyanothece sp.	4.4	3.57
			Cyanothece sp.	5.3	7.66
	McClelland & Montoya (2003)		*Trichodesmium erythaeum*	3.0	0.4