



*Supplement of*

## **Microbial strong organic-ligand production is tightly coupled to iron in hydrothermal plumes**

**Colleen L. Hoffman et al.**

*Correspondence to:* Colleen L. Hoffman (clhoffma@gmail.com) and Patrick J. Monreal (pmonreal@uw.edu)

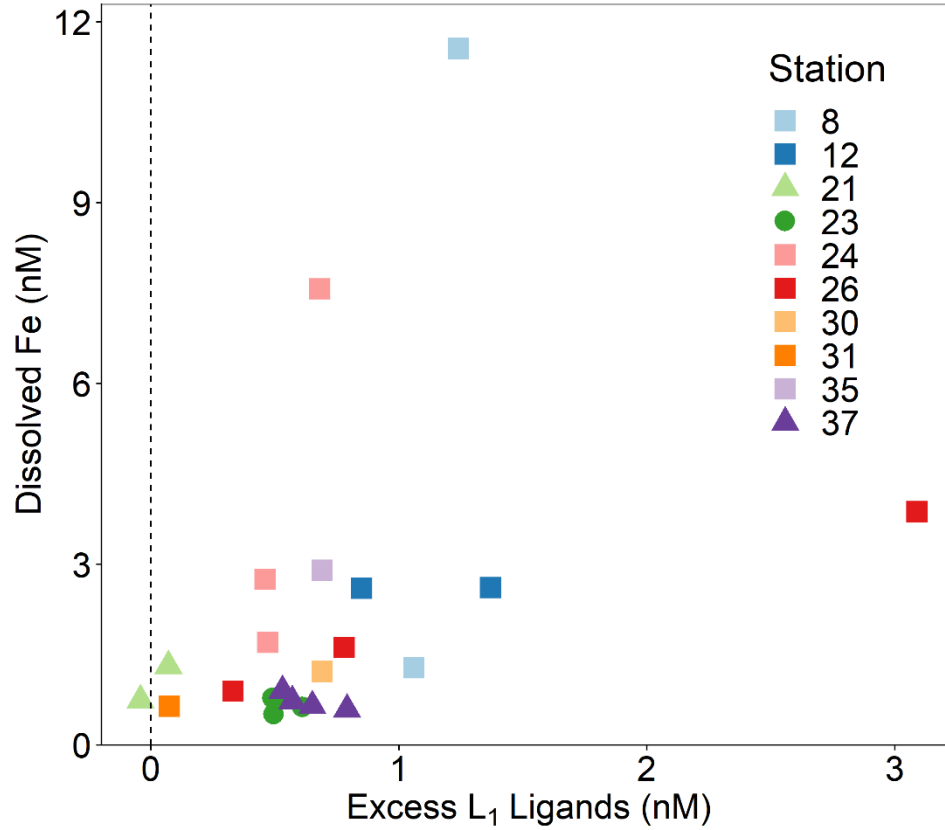
The copyright of individual parts of the supplement might differ from the article licence.

## **S1. Supporting Information Text**

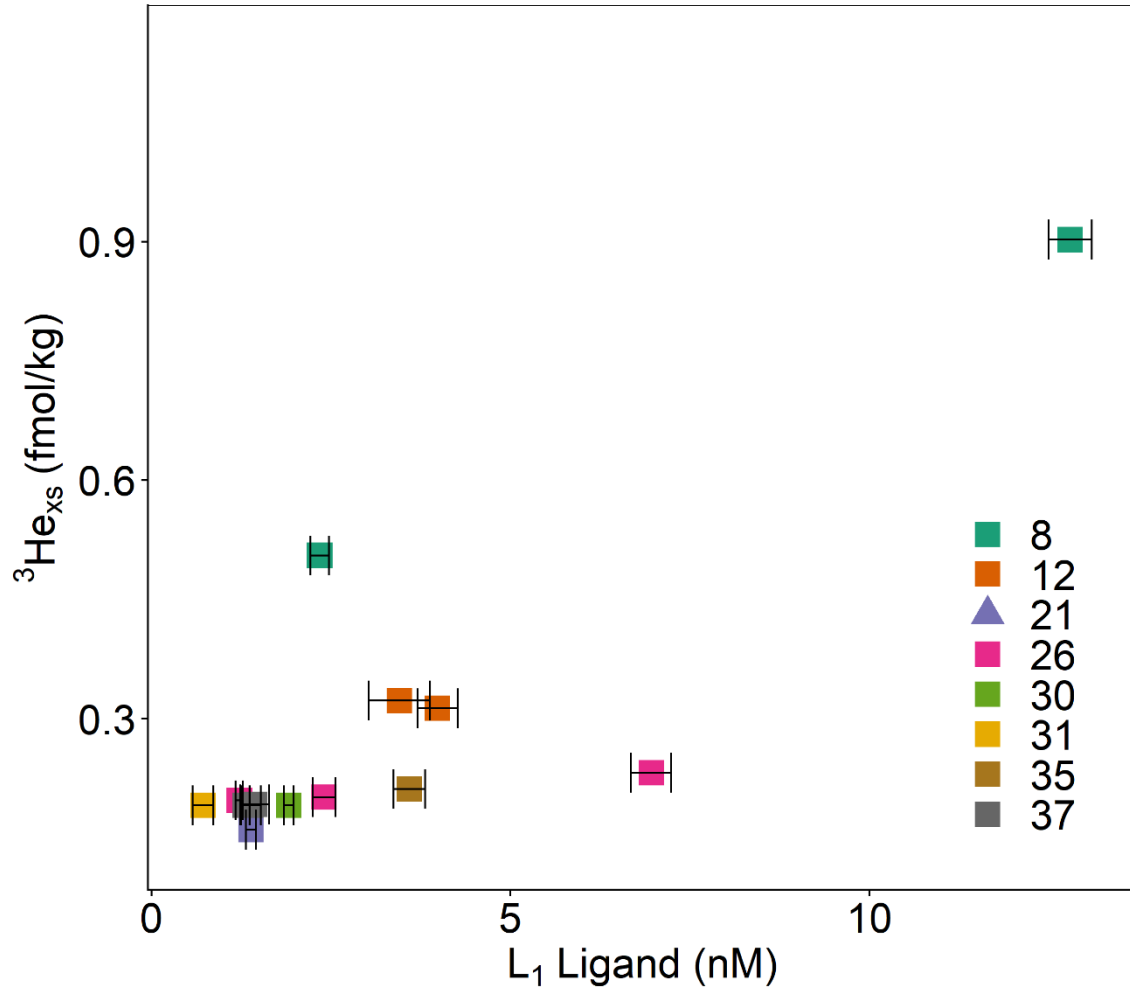
### **S1.1 Additional Methods**

**Competitive Ligand Exchange-Adsorptive Cathodic Stripping Voltammetry.** Two sets of Fe additions were used when conducting forward titrations to ensure full titration of samples. Dissolved Fe in the 40 samples process via forward titration ranged from 0.4-11.67 nM. For Lost City samples and one sample from Close E of TAG with a dissolved Fe concentration of 0.41 nM, dissolved Fe additions were 0, 0, 0.1, 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 7.5, and 10 nM. For the other 33 samples where a forward titration was conducted (**Table S1**), dissolved Fe additions were 0, 0, 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 7.5, 10, and 15 nM. Each sample was then equilibrated for at least 12 hours before analysis on the *BASi*.

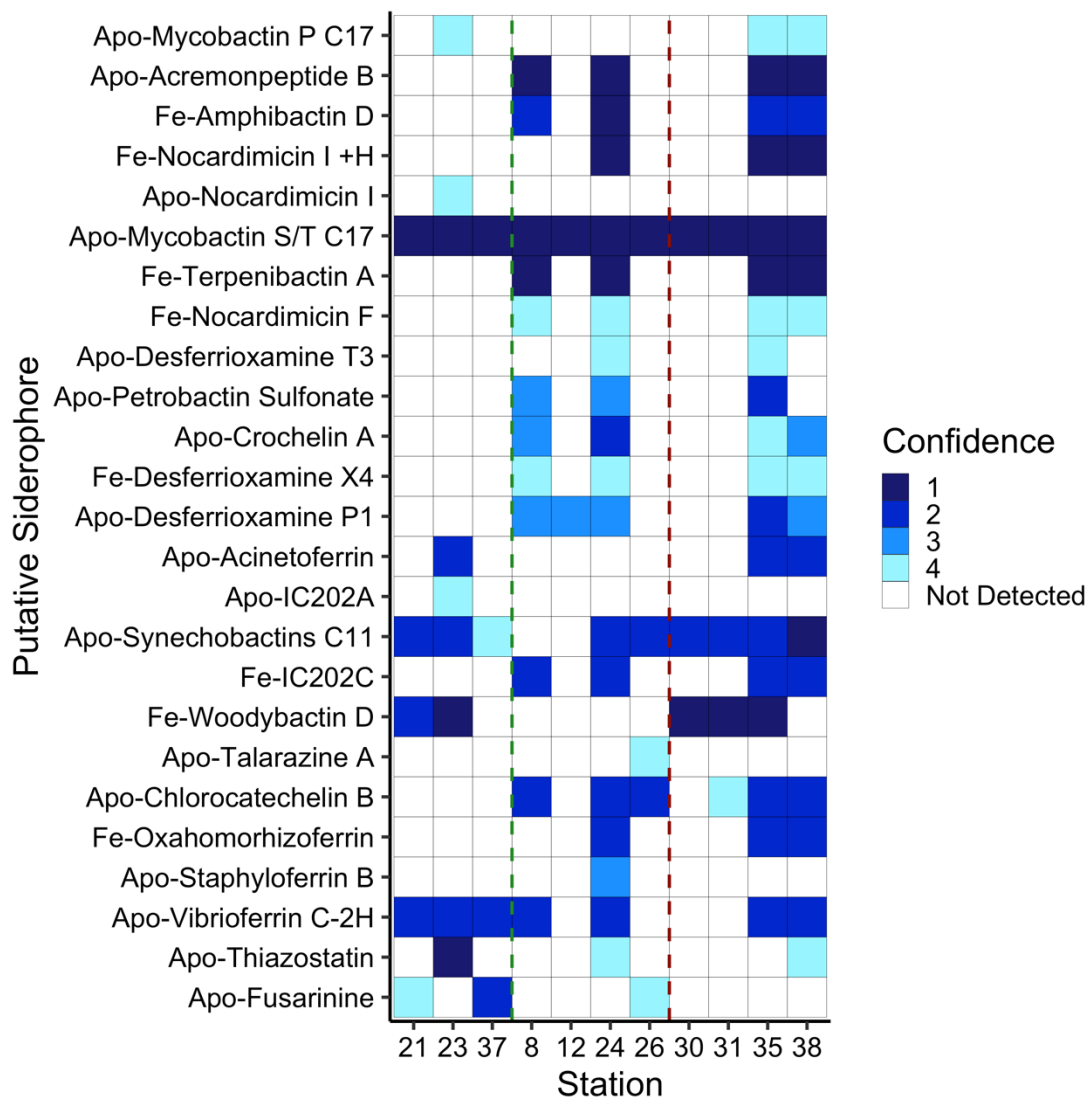
## S2. Supporting Information Figures



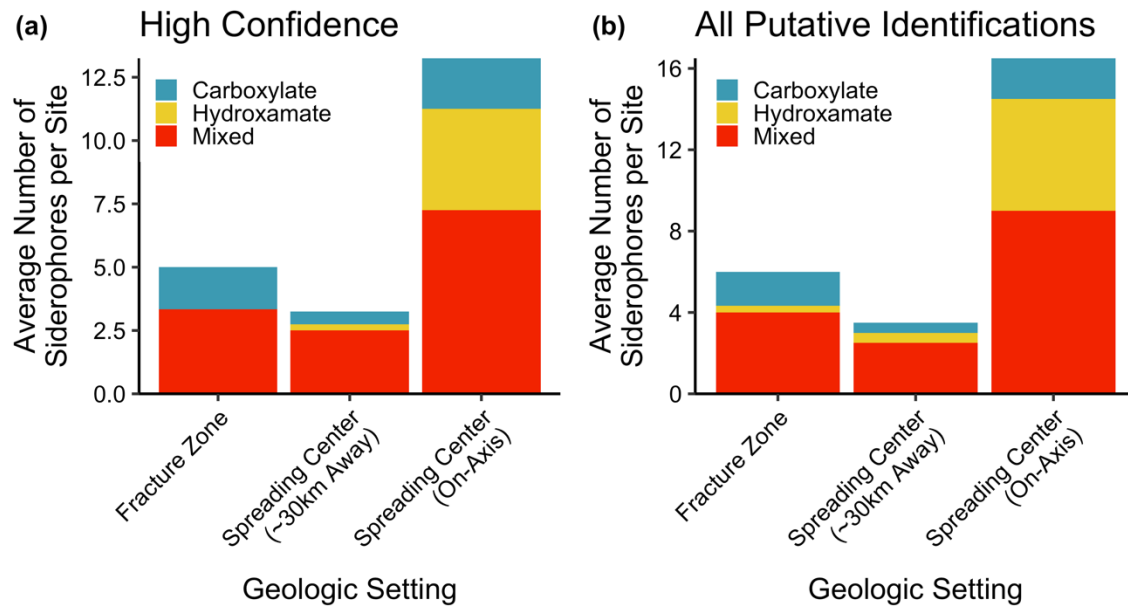
**Fig. S1. Excess L<sub>1</sub> along the Mid-Atlantic Ridge.** Excess L<sub>1</sub> ligands along the MAR. Hayes Fracture Zone (Station 21) was the only vent field to have one sample to not contain excess L<sub>1</sub> ligands. The dashed line is the zero line on the y-axis. Points with positive values correspond to samples with excess ligand present. Points with negative values correspond to samples with no excess ligand present. No L<sub>1</sub> or excess L<sub>1</sub> ligands were present at St. 38 Rainbow. Square symbols refer to spreading centers, triangles refer to fracture zones, and circles refer to alkaline vents.



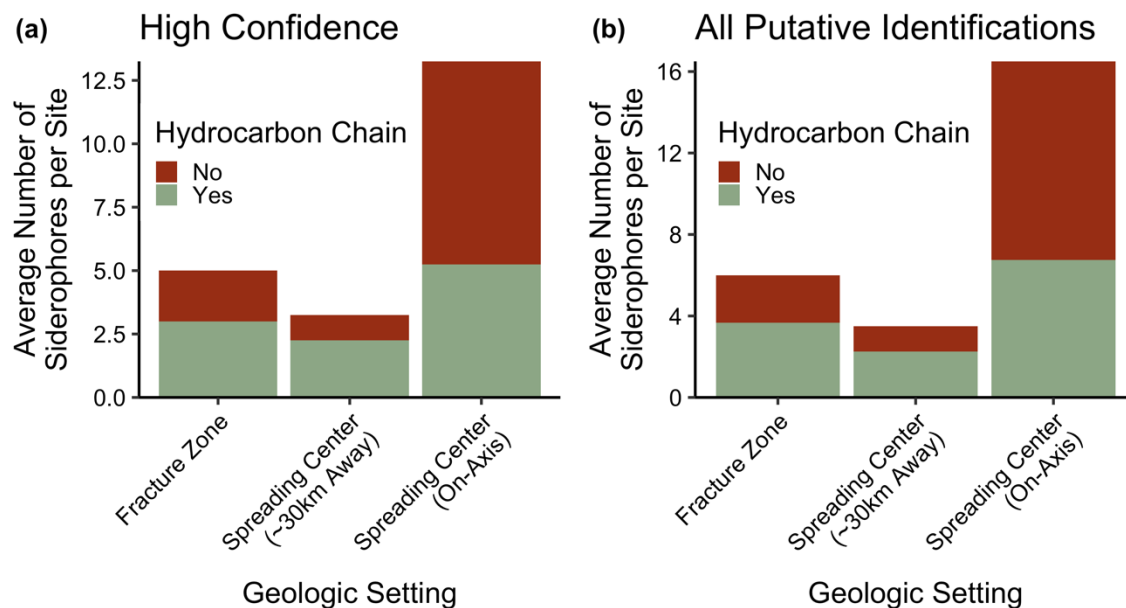
**Fig. S2. L<sub>1</sub> ligands versus <sup>3</sup>He<sub>xs</sub> along the Mid-Atlantic Ridge.** L<sub>1</sub> ligands did not correlate with the <sup>3</sup>He<sub>xs</sub> across the different plumes along the Mid-Atlantic Ridge. Expected <sup>3</sup>He<sub>xs</sub> values were derived from the conservative relationship between dMn/<sup>3</sup>He<sub>xs</sub> in hydrothermal plumes (Lough et al., 2022). No <sup>3</sup>He<sub>xs</sub> was derived for St. 23 Lost City, St. 24 Broken Spur and one sample at St. 21 Hayes Fracture Zone (**Table S2**). Square symbols refer to spreading centers and triangles refer to fracture zones.



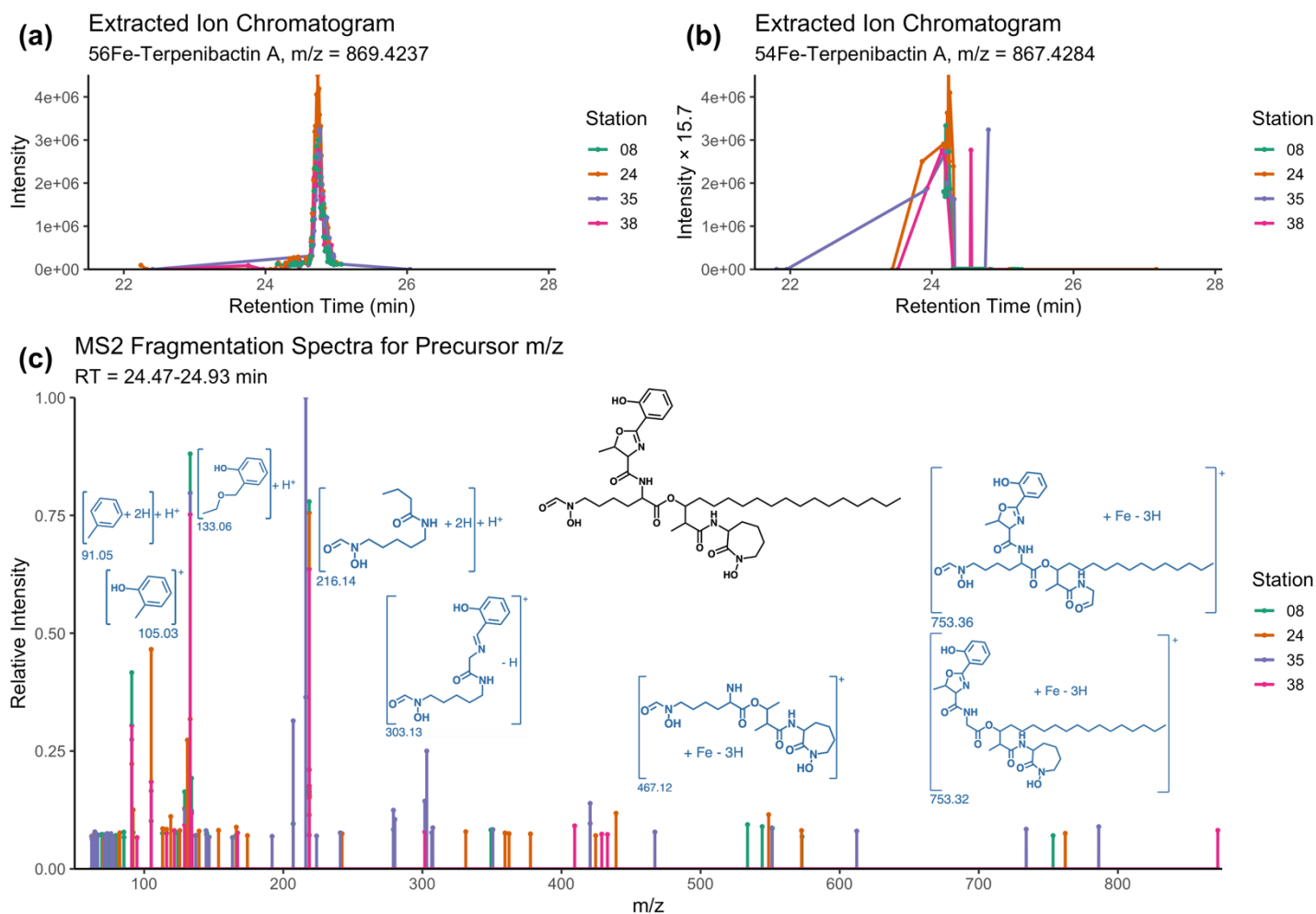
**Fig. S3. All putative siderophore identifications made in hydrothermal plumes along the MAR.** Heat map of siderophore detections at each site, similar to Fig. 2a of main text, but all siderophores and all confidence levels are visualized. The blue gradients indicates the confidence level of the identification, with level 1 representing the highest confidence level. In the text, confidence levels 1 and 2 are considered high confidence, while data from those only identified with confidence levels 3 and 4 not used in figures or statistics. Whites boxes indicate no detection at that particular site. The y-axis is ordered from top to bottom in terms of descending mass of the apo (without Fe) form. The vertical green lines separate fracture/diffuse dominant sites from off-axis sites, while the vertical red lines separate off-axis from on-axis sites. More information about the putative compounds are listed in Table S5 and definitions of the confidence levels are found in the methods of the main text and are outlined in Table S4.



**Fig. S4. Distribution of types of siderophores.** Bar graph of types of putative siderophores identified with (a) high confidence (i.e. at least confidence level 1 and 2 at a site) and (b) all confidence levels, separated by the type of site. Sites above spreading centers averaged more putative siderophores than both those above fracture zone or lower temperature sites and those ~30km from the spreading center. Mixed-type siderophores dominated identification, with hydroxamate siderophores also significant above spreading centers.

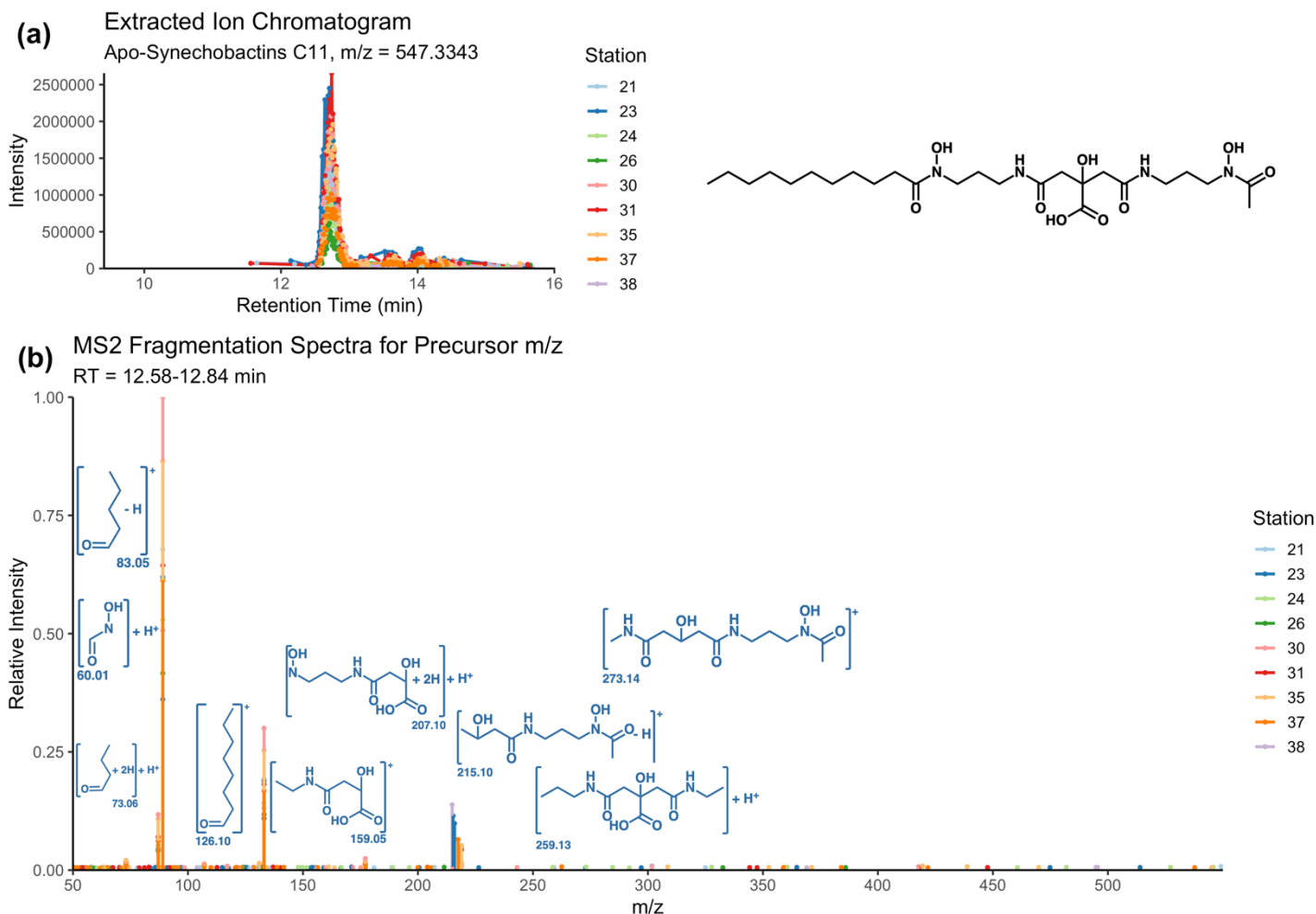


**Fig. S5. Distribution of amphiphilic siderophores.** Bar graph of types of putative siderophores identified with (a) high confidence (i.e. at least confidence level 1 and 2 at a site) and (b) all confidence levels, separated by the type of site. A “Yes” indicates that the structure of the siderophore contains a terminal hydrocarbon chain of 7+ carbon atoms. In total, over half (57%) of putative siderophores identified in this study contained a hydrocarbon chain.

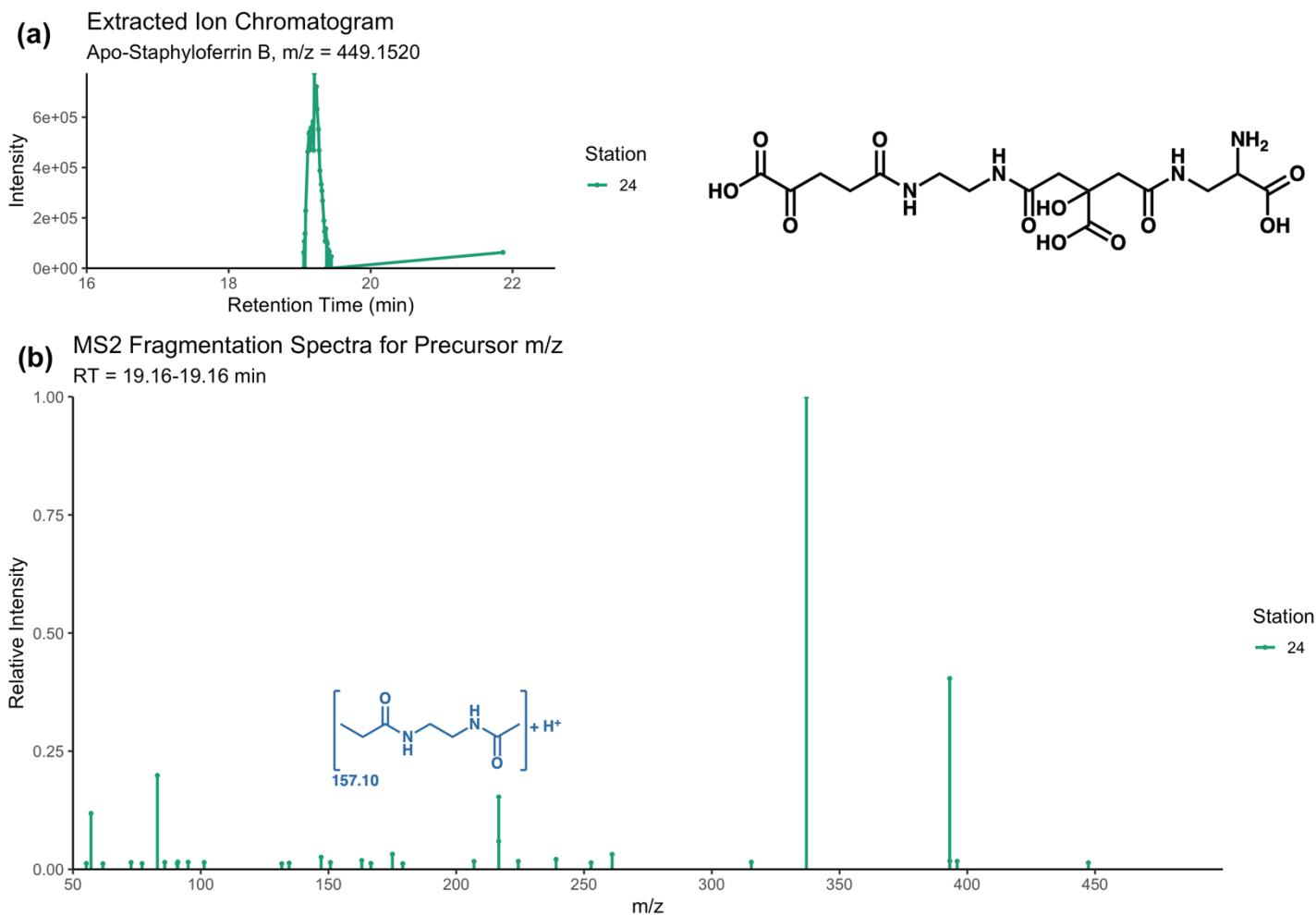


**Fig. S6. Confidence Level 1 example MS<sup>1</sup> and MS<sup>2</sup> spectra.** Example MS<sup>1</sup> (a-b) and MS<sup>2</sup> (c) spectra of putative siderophore Fe-Terpenibactin A is presented as an example of putative siderophores identified at the highest confidence level. The peak in the extracted ion chromatogram (EIC) corresponding to the  $^{56}\text{Fe}$ -bound mass is presented in (a), while the EIC corresponding to the less-abundant  $^{54}\text{Fe}$ -bound mass is presented in (b), scaled by a factor of 15.7 (crustal abundance of  $^{56}\text{Fe}/^{54}\text{Fe}$ ). Little is known about the isotopic fractionation associated with Fe-siderophore binding, but the fact that the  $^{54}\text{Fe}$ -Terpenibactin A signal scales to a similar intensity to that of  $^{56}\text{Fe}$ -Terpenibactin A provides strong evidence that the candidate compound is indeed bound to Fe. This strategy has been used in past siderophore identification workflows (Boiteau et al., 2016). Following analysis of the EICs, MS<sup>2</sup> fragments were extracted from the same  $m/z$  and retention time window and compared against *in silico* spectra for the candidate structure generated by MetFrag. For this particular compound, too many matching fragments were identified to display, but select fragments are presented in (c). The well-defined MS<sup>1</sup> peaks, consistent  $^{54}\text{Fe}$  and  $^{56}\text{Fe}$  EICs, and high-intensity fragments matching *in silico* fragmentation yields a detection with the highest confidence level. While this figure has spectra from multiple sites, confidence levels were typically assigned at each site independent of fragments at other sites.

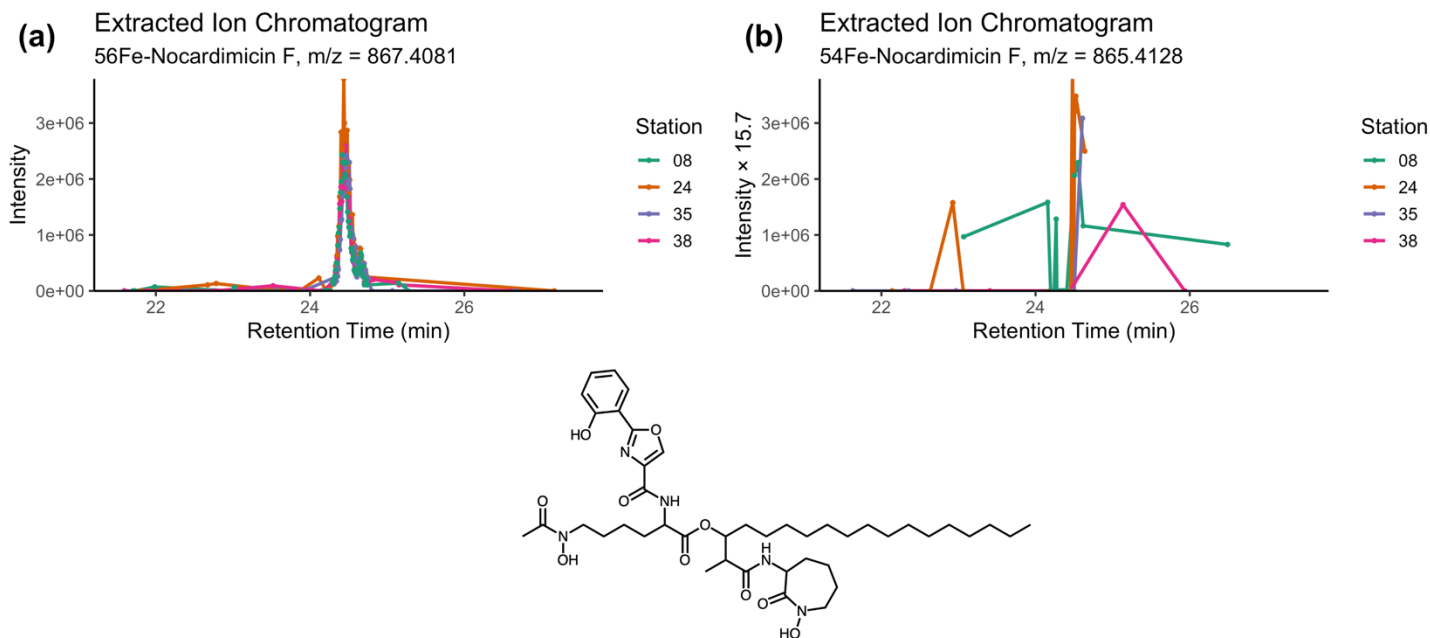




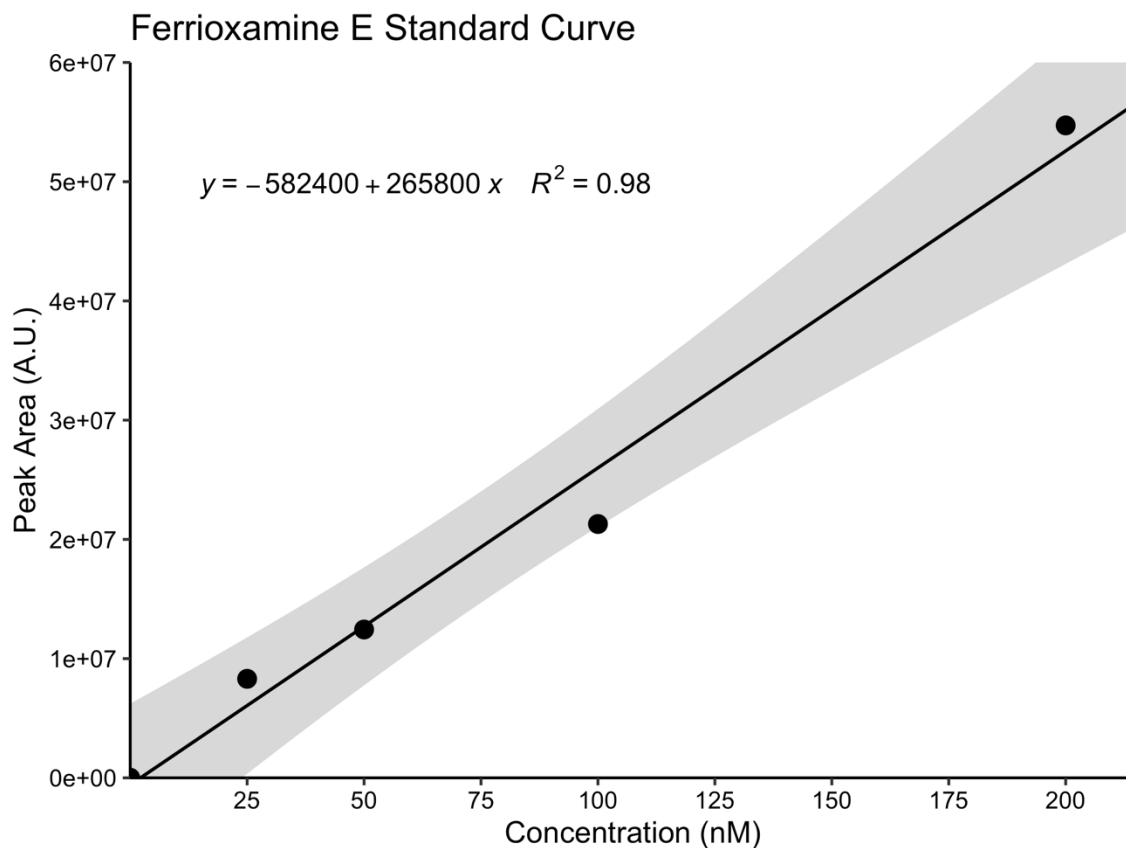
**Fig. S7. Confidence Level 2 example MS<sup>1</sup> and MS<sup>2</sup> spectra.** In a similar format to Fig. S6, example MS<sup>1</sup> (a) and MS<sup>2</sup> (b) spectra of putative siderophore Apo-Synechobactins C<sub>11</sub> is presented as an example of putative siderophores identified at confidence level 2. The peak in the extracted ion chromatogram (EIC) corresponding to the apo mass is presented in (a). Since this candidate structure is the apo form and not the  $m/z$  bound to Fe, the <sup>54</sup>Fe isotopologue EIC is not plotted. Following analysis of the EIC, MS<sup>2</sup> fragments were extracted from the same  $m/z$  and retention time window and compared against *in silico* spectra for the candidate structure generated by MetFrag. Fragments found in MS<sup>2</sup> spectra that were also predicted by MetFrag are shown in (b). Many of these fragment masses were low-intensity in the MS<sup>2</sup> data, except for the fragment  $m/z$  215.10 detected at St. 38, which was one of the most intense fragments in that sample. The well-defined MS<sup>1</sup> peaks and siderophore-like fragments matching *in silico* fragmentation — albeit at lower relative intensities — yields a detection at confidence level 2 for most sites. The detection at St. 38, however, was assigned a confidence level 1 due to the fragment with  $m/z = 215.10$ .



**Fig. S8. Confidence Level 3 example MS<sup>1</sup> and MS<sup>2</sup> spectra.** In a similar format to **Fig. S6**, example MS<sup>1</sup> (a) and MS<sup>2</sup> (b) spectra of putative siderophore Apo-Staphyloferrin B is presented as an example of putative siderophores identified at confidence level 3. The peak in the extracted ion chromatogram (EIC) corresponding to the apo mass is presented in (a). Since this candidate structure is the apo form and not the  $m/z$  bound to Fe, the <sup>54</sup>Fe isotopologue EIC is not plotted. Following analysis of the EIC, MS<sup>2</sup> fragments were extracted from the same  $m/z$  and retention time window and compared against *in silico* spectra for the candidate structure generated by MetFrag. Only one fragment was found in MS<sup>2</sup> spectra that was also predicted by MetFrag (b). While there was a well-defined MS<sup>1</sup> peaks that corresponded to the exact mass of Staphyloferrin B, only one fragment matched *in silico* fragmentation, yielding a detection at confidence level 3 at St. 24. These low-confidence detections are not included in most of the figures and relationships presented in the main text (unless detections were made at higher confidence at other sites and retention times align) but are presented in the supplement to aid future work.



**Fig. S9. Confidence Level 4 example MS<sup>1</sup> spectra.** In a similar format to **Fig. S6**, example MS<sup>1</sup> (a-b) spectra of putative siderophore Fe-Nocardimicin F is presented as an example of putative siderophores identified at the lowest confidence level, 4. The peak in the extracted ion chromatogram (EIC) corresponding the <sup>56</sup>Fe-bound mass is presented in (a), while the EIC corresponding to the less-abundant <sup>54</sup>Fe-bound mass is presented in (b), scaled by a factor of 15.7 (crustal abundance of <sup>56</sup>Fe/<sup>54</sup>Fe). Little is known about the isotopic fractionation associated with Fe-siderophore binding, but the fact that the <sup>54</sup>Fe-Nocardimicin F signal scales to a similar intensity to that of <sup>56</sup>Fe-Nocardimicin F provides strong evidence that the candidate compound is indeed bound to Fe. That said, no MS<sup>2</sup> spectra was available for this candidate *m/z*, yielding a confidence level of 4. These low-confidence detections are not included in most of the figures and relationships presented in the main text (unless detections were made at higher confidence at other sites and retention times align) but are presented in the supplement to aid future work. Interestingly, Nocardimicin F and Terpenibactin A are very structurally similar and the MS<sup>1</sup> peaks in our data corresponding to their masses were of similar magnitude. The *m/z* of Nocardimicin F is within the 7.5 ppm tolerance of the *m/z* Terpenibactin A minus two hydrogen atoms, so it is possible that this compound is related to the putative Terpenibactin A compound through one degree of unsaturation in the hydrocarbon chain, which is common in marine siderophores (Gauglitz and Butler, 2013). Our data, however, is unable to resolve this level of structural detail.



**Fig. S10. Ferrioxamine E Standard Curve.** A 5-point standard curve with known concentrations of siderophore ferrioxamine E at 0 nM, 25 nM, 50nM, 100 nM, and 200nM was used for quantification of putative siderophores. Commercial standards are not available for most siderophores, and different compounds have distinct ionization efficiencies in ESI-MS. Thus, the siderophore concentrations reported here are estimates of siderophore concentrations in these environments based on the structure and ionization efficiency of siderophore ferrioxamine E. The standard error of the slope of the curve was 20726 a.u./nM, yielding a limit of detection in the eluent of 0.257 nM. Eluent concentrations of 0.257 nM equate to sample concentrations of 0.07-0.21 pM depending on sample-to-eluent volume ratio at each site (**Table S1**). All putative detections below this limit were ultimately discarded. Additionally, 1 mM of cyanocobalamin was added as an internal standard to each sample aliquot to address any changes in sensitivity during LC-ESI-MS runs. Shaded area in the figure represents the 95% confidence interval of the linear regression.



**Fig. S11. Relative abundance of prokaryotic taxa.** Bar height indicates the proportion of 16S rRNA genes recovered in each sample, separated by depth and site location. Colors correspond to taxonomy. Only the 10 most abundant families in each sample are depicted, which is not always 100% of proportion of 16S rRNA genes recovered. Asterisks denote families containing genera hypothesized to produce siderophores.

### S3. Supporting Information Tables

**Table S1. FRidge GA13 sample processing information**

Location	Abbr.	Station #	Total # of Samples	# of CSV samples	16S rRNA	Volume of seawater on SPE column (L)*	Volume of Eluent ( $\mu$ L)*
Lucky Strike <sup>†</sup>	LS	7	-	-	yes	-	-
Lucky Strike <sup>†</sup>	LS	8	6	3	-	1.50	416.1
200 km E of Rainbow	-	11	-	-	yes	-	-
33 km E of Rainbow	CER	12	5	2	-	0.95	500
Rainbow	R	38	3	1	-	0.65	542.4
10 km S of Rainbow	-	17	-	-	yes	-	-
Hayes Fracture Zone	HFZ	21	6	3	-	1.05	500
Lost City	LC	23	8	6	-	1.10	500
Broken Spur	BS	24	8	4 (2)	-	1.20	401.2
29 km N of TAG	CNT	26	7	5	-	1.00	500
30 km W of TAG	CWT	30	6	3	-	1.05	500
30 km E of TAG	CET	31	7	5	-	1.00	500
TAG	TAG	35	10	2 (8)	-	1.40	434.2
Low Temp Slope	LTS	37	8	6	-	1.10	500

For CSV, 150 mL and 100 mL of water was needed to run forward titrations and reverse titrations (in parentheses), respectively.

\*For LC-MS, water samples were pooled and filtered from each vent site – see methods section for more details. Both volume of seawater concentrated, and final eluent volume are reported above.

- = not applicable/not sampled

<sup>†</sup> = Lucky Strike Station 7 and 8 were sampled at the same latitude and longitude (**Fig. 1a**)

**Table S2. Forward Titration Ligand concentrations and binding strengths from samples taken along the MAR.**

Vent Name	Station	GT #	Depth m	[dFe] nM	<sup>3</sup> He <sub>ss</sub> <sup>†</sup> fmol	[L <sub>1</sub> ] nM	[L <sub>2</sub> ] nM	[L <sub>3</sub> ] nM	[L <sub>4</sub> ] nM	Log K <sub>1</sub>	Log K <sub>2</sub>	Log K <sub>3</sub>	Log α <sub>1</sub>	Log α <sub>2</sub>	Log α <sub>3</sub>	Flag L <sub>1</sub>	Flag L <sub>2</sub>	Flag L <sub>3</sub>
BS	24	1251	2950	0.94	<i>n.a.</i>		1.62 ± 0.2		1.62 ± 0.2		11.922 ± 0.387			2.75				3
BS	24	1252	2887	2.75	<i>n.a.</i>	3.21 ± 0.06		37.1 ± 3.2	40.31 ± 3.26	13.42 ± 0.114		9.778 ± 0.044	4.08		2.31	4		2
BS	24	1253	2841	7.57	<i>n.a.</i>	8.25 ± 0.47			8.25 ± 0.47	12.131 ± 0.364			2.96			3		
BS	24	1260	2500	1.7	<i>n.a.</i>	2.17 ± 0.28		58.5 ± 26.3	60.67 ± 26.58	12.434 ± 0.466		8.745 ± 0.219	3.11		1.5	4		2
CER	12	628	2200	2.6	0.323	3.45 ± 0.43		10.4 ± 4.0	13.85 ± 4.43	12.091 ± 0.363		9.811 ± 0.221	3.02		1.7	3		2
CER	12	632	2000	2.61	0.313	3.98 ± 0.28			3.98 ± 0.28	12.132 ± 0.273			3.27			4		
CET	31	1610	3700	0.49	0.191			4.93±0.89	4.93±0.89			10.509±0.158		2.16				1
CET	31	1612	3350	0.41	0.191		2.39 ± 0.31	69.4 ± 30.4	71.79 ± 30.71		11.469 ± 0.172	8.75 ± 0.219		2.77	1.59		3	2
CET	31	1613	3150	0.47	0.191		3.65 ± 0.365		3.65 ± 0.365		11.054 ± 0.138			2.56			1	
CET	31	1615	2800	0.64	0.191	0.714 ± 0.143		79.7 ± 24.9	80.414 ± 25.043	12.601 ± 0.662		8.837 ± 0.155	2.47		1.74	1		2
CET	31	1616	2600	0.8	0.192		2.25±0.27		2.25±0.27		11.504±0.201			2.67			3	
CNT	26	1346	3801	1.93	0.205		5.57 ± 0.45	13.4 ± 5.3	18.97 ± 5.75		11.748 ± 0.117	9.677 ± 0.242		3.31	1.74		4	2
CNT	26	1348	3400	3.87	0.232	6.96 ± 0.28			6.96 ± 0.28	12.06 ± 0.096			3.55			4		
CNT	26	1349	3200	2.86	0.23			5.64 ± 0.99	5.64 ± 0.99			10.583 ± 0.238		2.02				1
CNT	26	1351	2800	1.62	0.202	2.4 ± 0.16			2.4 ± 0.16	12.018 ± 0.21			2.91			3		
CNT	26	1352	2400	0.89	0.198	1.22 ± 0.05		2.05 ± 0.22	3.27 ± 0.27	12.778 ± 0.415		10.663 ± 0.075	3.3		1.73	4		1
CWT	30	1539	2800	0.87	0.193		1.6 ± 0.2		1.6 ± 0.2		11.636 ± 0.291			2.52			1	
CWT	30	1540	2700	1.22	0.191	1.91 ± 0.07		25.3 ± 6.4	27.21 ± 6.47	12.547 ± 0.22		9.026 ± 0.158	3.39		1.41	4		2
CWT	30	1542	2500	0.82	0.192		2.77 ± 0.37		2.77 ± 0.37		11.228 ± 0.168			2.52			1	
HFZ	21	1129	4452	0.74	<i>n.a.</i>	0.697 ± 0.139		34.9 ± 6.6	35.597 ± 6.739	12.419 ± 0.528		9.515 ± 0.109		2.05				2
HFZ	21	1136	2850	1.31	0.161	1.38 ± 0.07		10.2 ± 3.2	11.58 ± 3.27	13.189 ± 0.396		9.592 ± 0.168	3.03		1.54	3		2
HFZ	21	1138	2500	1.084	0.163		1.85 ± 0.08		1.85 ± 0.08		11.998 ± 0.108			2.88			3	
LC	23	1203	757	0.63	<i>n.a.</i>	1.24 ± 0.16		9.05 ± 3.06	10.29 ± 3.22	12.23 ± 0.336		9.676 ± 0.194	3.02		1.6	3		2

LC	23	1204	753	0.81	<i>n.a.</i>		6.19 ± 0.34	6.19 ± 0.34	11.164 ± 0.067		2.89			3			
LC	23	1205	747	0.73	<i>n.a.</i>	1.238 ± 0.062		8.086 ± 1.33	9.324 ± 1.392	12.02 ± 0.114		9.573 ± 0.081	2.73	1.44	3	2	
LC	23	1207	727	0.51	<i>n.a.</i>	1.005 ± 0.075		2.244 ± 0.269	3.249 ± 0.344	12.115 ± 0.151		10.645 ± 0.09	2.81	1.88	3	1	
LC	23	1208	700	0.78	<i>n.a.</i>	1.27 ± 0.08		15 ± 3.5	16.27 ± 3.58	12.189 ± 0.171		9.319 ± 0.14	2.88	1.47	3	2	
LC	23	1209	685	0.58	<i>n.a.</i>		2.527 ± 0.215		2.527 ± 0.215		11.346 ± 0.091		2.64			3	
LTS	37	1970	2400	0.9	0.192	1.43 ± 0.2		24.2 ± 11.2	25.63 ± 11.4	12.151 ± 0.365		9.163 ± 0.229	2.88	1.53	3	2	
LTS	37	1972	2200	0.87	0.192		1.78 ± 0.19	7.08 ± 2.04	8.86 ± 2.23		11.531 ± 0.173	9.624 ± 0.168	2.49	1.42		1	2
LTS	37	1973	2100	0.73	0.191	1.3 ± 0.07			1.3 ± 0.07	12.191 ± 0.244			2.95		3		
LTS	37	1975	1900	0.71	0.191		1.47 ± 0.24		1.47 ± 0.24		11.789 ± 0.295		2.67			3	
LTS	37	1976	1800	0.65	0.191	1.3 ± 0.06			1.3 ± 0.06	12.048 ± 0.151			2.86		3		
LTS	37	1977	1700	0.59	0.191	1.38 ± 0.14			1.38 ± 0.14	12.179 ± 0.365			3.07		3		
LS	8	409	1725	11.56	0.902	12.8 ± 0.3			12.8 ± 0.3	13.096 ± 0.458			3.54			4	
LS	8	415	1620	11.67	0.84		12.6 ± 0.3		12.6 ± 0.3		11.924 ± 0.143		2.9			3	
LS	8	418	1550	1.28	0.505	2.34 ± 0.13			2.34 ± 0.13	12.519 ± 0.72			4.19			4	
R	38	1009	2285	25.87	0.446			27.3 ± 1.3	27.3 ± 1.3			10.967 ± 0.192		3.4		4	
TAG	35	1802	3600	2.9	0.212	3.59 ± 0.22			3.59 ± 0.22	12.022 ± 0.601			2.86			3	
TAG	35	1821	1821	1.34	0.192		2.26 ± 0.35		2.26 ± 0.35		11.689 ± 0.497		2.65			3	

L<sub>1</sub>, L<sub>2</sub>, and L<sub>3</sub> are defined in the literature (Gledhill and Buck, 2012).

<sup>†</sup> Expected <sup>3</sup>He<sub>con</sub> values were derived from the conservative relationship between dMn/<sup>3</sup>He<sub>con</sub> in hydrothermal plumes (Lough et al., 2022).

*n.a.* = <sup>3</sup>He<sub>con</sub> data not available

Log  $\alpha$  are reported for each ligand calculation

A quality flag was the given to each ligand value

1 - within analytical window and data has low error

2 - data within analytical window but has high error (> 1)

3 - edge of analytical window (within 0.5)

4 - outside of analytical window (>0.5)



**Table S3. Reverse Titration Ligand concentrations and binding strengths from samples taken along the MAR.**

Vent Name	Station	GT #	Depth m	[dFe] nM	<sup>3</sup> He <sub>xs</sub> <sup>†</sup> fmol	[L <sub>2</sub> ] nM	[L <sub>3</sub> ] nM	[L <sub>τ</sub> ] nM	Log K <sub>2</sub>	Log K <sub>3</sub>	Flag
BS	24	1255	2833	21.31	<i>n.a.</i>	4.24±0.355		4.24±0.355	11.7±0.3		1
BS	24	1258	2809	21.06	<i>n.a.</i>	6.31±0.664		6.31±0.664	11.82±0.417		3
TAG	35	1806	3453	19.01	0.284		9.19±0.538	9.19±0.538		10.66±0.166	1
TAG	35	1807	3429	26.99	0.326		8.27±0.539	8.27±0.539		10.68±0.178	1
TAG	35	1811	3335	30.68	1.122		20.1±2.66	20.1±2.66		10.22±0.361	2
TAG	35	1812	3322	64.36	0.788		6.14±1.64	6.14±1.64		10.58±0.677	2
TAG	35	1813	3313	34.22	0.777						
TAG	35	1817	3336	90.25	0.849		37.7±5.93	37.7±5.93		10.04±0.438	2
TAG	35	1818	3195	84.53	0.431	9.55±1.59		9.55±1.59	11.58±0.654		2
TAG	35	1819	3100	55.71	0.201						

L<sub>1</sub>, L<sub>2</sub>, and L<sub>3</sub> are defined in the literature (Gledhill and Buck, 2012).

The following values used to calculate the analytical window,  $\alpha_{Fe} = 10^{9.80}$  and  $\beta_{FeNN3} = 5.12 \times 10^{26}$ , were previously reported in the literature for titrations buffered to ~pH8 (Hawkes et al., 2013b, a).

This gives us an analytical window (log  $\alpha_{FeNN3}$ ) between 7.80 - 13.52 for each of our titration curves

<sup>†</sup> Expected <sup>3</sup>He<sub>xs</sub> values were derived from the conservative relationship between dMn/<sup>3</sup>He<sub>xs</sub> in hydrothermal plumes (Lough et al., 2022).

*n.a.* = <sup>3</sup>He<sub>xs</sub> data not available

A quality flag was the given to each ligand value

1 - within analytical window and data has low error

2 - data within analytical window but has high error (> 1)

3 - edge of analytical window (within 0.5)

4 - outside of analytical window (>0.5)

**Table S4. Average Strong binding ligands and Siderophore concentrations along the MAR**

Location	Station	Avg L <sub>1</sub> ligand (nM)	Avg Siderophore Concentration (pM)
Lucky Strike	8	7.57±7.40	0.438±0.042
33 km E of Rainbow	12	3.72±0.37	0.269±0.080
Hayes Fracture Zone	21	1.04±0.48	0.548±0.073
Lost City	23	1.24±0.15	0.579±0.069
Broken Spur	24	4.54±3.25	0.450±0.051
29 km N of TAG	26	3.53±3.03	0.242±0.076
30 km W of TAG	30	1.91	0.969±0.073
30 km E of TAG	31	0.71	0.548±0.076
TAG	35	3.59	0.440±0.047
Low Temp Slope	37	1.35±0.06	0.282±0.069
Rainbow	38	<i>n.a.</i>	0.863±0.128

Standard deviations are reported for average L<sub>1</sub> concentrations, while, for average siderophore concentrations, the error is reported as 1.96\*SE of the desferrioxamine E standard curve multiplied by the concentration factor of each sample.

*n.a.* = no L<sub>1</sub> ligands were found in the sample(s) that were run using CSV.

**Table S5. Brief description of confidence levels**

<b>Confidence Level</b>	<b>MS<sup>1</sup></b>	<b>MS<sup>2</sup></b>	<b>Fragmentation Pattern</b>
1*	✓	✓	Multiple fragments in the MS <sup>2</sup> data matched those predicted by MetFrag and at least one was in the top three most intense fragments
2*	✓	✓	Multiple lower-intensity fragments in the MS <sup>2</sup> data matched those predicted by MetFrag
3	✓	✓	Little to no fragments found in MS <sup>2</sup> data match those predicted by MetFrag
4	✓	-	-

\*Confidence levels were designed such that putative detections made with either confidence level 1 or 2 contained siderophore-like fragments in MS<sup>2</sup> data, regardless of definitive identification. That is, we have reasonable confidence that the putative siderophore at least contains siderophore moieties.

**Table S6. Putative siderophores detected with LC-ESI-MS**

Vent Name	Station	Geology	Putative Siderophore	Form	m/z	Confidence Level	Type	Hydrocarbon Chain?*	Retention Time (min)	Raw Peak Area	Peak Area × Conc. Factor	DFOE-calibrated Conc. in SW (pM)	Conc. in SW with 40% Efficiency (pM)**	Conc. in SW with 10% Efficiency (pM)***
LS	8	Spreading Center	Terpenibactin A	Fe	869.424	1	Mixed	Yes	24.8	377950	104.843	0.394	0.986	3.944
LS	8	Spreading Center	Acremonpeptide B	Apo	848.452	1	Hydroxamate	No	24.8	476264	132.115	0.497	1.243	4.970
LS	8	Spreading Center	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	180723	50.133	0.189	0.471	1.886
CER	12	Spreading Center	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	151918	79.957	0.301	0.752	3.008
HFZ	21	Fracture Zone	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	507896	241.855	0.910	2.275	9.098
LC	23	Fracture Zone	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	707028	321.376	1.209	3.023	12.090
LC	23	Fracture Zone	Woodybactin D	Fe	544.172	1	Carboxylate	Yes	16.9	103258	46.936	0.177	0.441	1.766
LC	23	Fracture Zone	Thiazostatin	Apo	339.084	1	Mixed	No	19	87227	39.649	0.149	0.373	1.492
BS	24	Spreading Center	Amphibactin D	Fe	885.415	1	Hydroxamate	Yes	24.8	353010	118.023	0.444	1.110	4.440
BS	24	Spreading Center	Nocardimicin I +H	Fe	884.447	1	Mixed	Yes	24.5	159387	53.289	0.200	0.501	2.005
BS	24	Spreading Center	Terpenibactin A	Fe	869.424	1	Mixed	Yes	24.8	577343	193.025	0.726	1.815	7.262
BS	24	Spreading Center	Acremonpeptide B	Apo	848.452	1	Hydroxamate	No	24.8	790407	264.259	0.994	2.485	9.941
BS	24	Spreading Center	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	296850	99.247	0.373	0.933	3.734
CNT	26	Spreading Center	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	213887	106.943	0.402	1.006	4.023
CWT	30	Spreading Center	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	509863	242.792	0.913	2.283	9.134

CWT	30	Spreading Center	Woodybactin D	Fe	544.172	1	Carboxylate	Yes	16.9	770593	366.949	1.380	3.451	13.804
CET	31	Spreading Center	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	556283	278.142	1.046	2.616	10.464
CET	31	Spreading Center	Woodybactin D	Fe	544.172	1	Carboxylate	Yes	16.9	90528	45.264	0.170	0.426	1.703
TAG	35	Spreading Center	Nocardimicin I +H	Fe	884.447	1	Mixed	Yes	24.5	101851	31.588	0.119	0.297	1.188
TAG	35	Spreading Center	Terpenibactin A	Fe	869.424	1	Mixed	Yes	24.8	414993	128.707	0.484	1.210	4.842
TAG	35	Spreading Center	Acremonpeptide B	Apo	848.452	1	Hydroxamate	No	24.8	508219	157.621	0.593	1.482	5.930
TAG	35	Spreading Center	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	491344	152.387	0.573	1.433	5.733
TAG	35	Spreading Center	Woodybactin D	Fe	544.172	1	Carboxylate	Yes	16.9	81066	25.142	0.095	0.236	0.946
LTS	37	Fracture Zone	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	219752	99.887	0.376	0.939	3.758
R2	38	Spreading Center	Nocardimicin I +H	Fe	884.447	1	Mixed	Yes	24.5	89956	75.065	0.282	0.706	2.824
R2	38	Spreading Center	Terpenibactin A	Fe	869.424	1	Mixed	Yes	24.8	358436	299.101	1.125	2.813	11.252
R2	38	Spreading Center	Acremonpeptide B	Apo	848.452	1	Hydroxamate	No	24.8	472652	394.410	1.484	3.709	14.838
R2	38	Spreading Center	Mycobactin S/T C17	Apo	828.512	1	Mixed	Yes	15	426981	356.299	1.340	3.351	13.404
R2	38	Spreading Center	Synechobactins C11	Apo	547.334	1	Mixed	Yes	12.7	261667	218.351	0.821	2.054	8.214
LS	8	Spreading Center	Amphibactin D	Fe	885.415	2	Hydroxamate	Yes	24.7	229587	63.688	0.240	0.599	2.396
LS	8	Spreading Center	IC202C	Fe	570.246	2	Hydroxamate	No	23.5	363338	100.790	0.379	0.948	3.792
LS	8	Spreading Center	Chlorocatechelin B	Apo	461.144	2	Mixed	No	18.9	428902	118.977	0.448	1.119	4.476
LS	8	Spreading Center	Vibrioferrin C-2H	Apo	447.125	2	Carboxylate	No	14.7	581310	161.255	0.607	1.517	6.066

HFZ	21	Fracture Zone	Synechobactins C11	Apo	547.334	2	Mixed	Yes	12.7	282675	134.607	0.506	1.266	5.064
HFZ	21	Fracture Zone	Woodybactin D	Fe	544.172	2	Carboxylate	Yes	16.9	147103	70.049	0.264	0.659	2.635
HFZ	21	Fracture Zone	Vibrioferrin C-2H	Apo	447.125	2	Carboxylate	No	14.7	422356	201.122	0.757	1.892	7.566
LC	23	Fracture Zone	Acinetoferrin	Apo	585.350	2	Mixed	Yes	18.6	101336	46.062	0.173	0.433	1.733
LC	23	Fracture Zone	Synechobactins C11	Apo	547.334	2	Mixed	Yes	12.7	429185	195.084	0.734	1.835	7.339
LC	23	Fracture Zone	Vibrioferrin C-2H	Apo	447.125	2	Carboxylate	No	14.7	602509	273.868	1.030	2.576	10.303
BS	24	Spreading Center	Crochelin A	Apo	696.353	2	Mixed	No	26	377773	126.302	0.475	1.188	4.751
BS	24	Spreading Center	IC202C	Fe	570.246	2	Hydroxamate	No	23.5	534621	178.742	0.672	1.681	6.724
BS	24	Spreading Center	Synechobactins C11	Apo	547.334	2	Mixed	Yes	12.7	164152	54.882	0.206	0.516	2.065
BS	24	Spreading Center	Oxahomorphizoferrin	Fe	506.047	2	Carboxylate	No	23.4	290782	97.218	0.366	0.914	3.657
BS	24	Spreading Center	Chlorocatechelin B	Apo	461.144	2	Mixed	No	18.9	75889	25.372	0.095	0.239	0.954
BS	24	Spreading Center	Vibrioferrin C-2H	Apo	447.125	2	Carboxylate	No	14.7	465962	155.787	0.586	1.465	5.861
CNT	26	Spreading Center	Synechobactins C11	Apo	547.334	2	Mixed	Yes	12.7	88291	44.145	0.166	0.415	1.661
CNT	26	Spreading Center	Chlorocatechelin B	Apo	461.144	2	Mixed	No	18.9	101696	50.848	0.191	0.478	1.913
CWT	30	Spreading Center	Synechobactins C11	Apo	547.334	2	Mixed	Yes	12.7	341539	162.638	0.612	1.530	6.118
CET	31	Spreading Center	Synechobactins C11	Apo	547.334	2	Mixed	Yes	12.7	420695	210.347	0.791	1.978	7.913
TAG	35	Spreading Center	Amphibactin D	Fe	885.415	2	Hydroxamate	Yes	24.6	257644	79.907	0.301	0.752	3.006
TAG	35	Spreading Center	Petrobactin Sulfonate	Apo	771.287	2	Mixed	No	24.2	1046933	324.699	1.222	3.054	12.215

TAG	35	Spreading Center	Desferrioxamine P1	Apo	616.331	2	Hydroxamate	No	26.5	791716	245.545	0.924	2.309	9.237
TAG	35	Spreading Center	Acinetoferrin	Apo	585.350	2	Mixed	Yes	18.6	159590	49.496	0.186	0.466	1.862
TAG	35	Spreading Center	IC202C	Fe	570.246	2	Hydroxamate	No	23.5	424015	131.505	0.495	1.237	4.947
TAG	35	Spreading Center	Synechobactins C11	Apo	547.334	2	Mixed	Yes	12.8	374766	116.231	0.437	1.093	4.373
TAG	35	Spreading Center	Oxahomorphozoferrin	Fe	506.047	2	Carboxylate	No	23.4	381526	118.328	0.445	1.113	4.451
TAG	35	Spreading Center	Chlorocatechelin B	Apo	461.144	2	Mixed	No	18.9	206280	63.976	0.241	0.602	2.407
TAG	35	Spreading Center	Vibrioferrin C-2H	Apo	447.125	2	Carboxylate	No	14.7	343044	106.393	0.400	1.001	4.002
LTS	37	Fracture Zone	Vibrioferrin C-2H	Apo	447.125	2	Carboxylate	No	14.7	107803	49.001	0.184	0.461	1.843
LTS	37	Fracture Zone	Fusarinine	Apo	261.145	2	Mixed	No	16.6	145829	66.286	0.249	0.623	2.494
R2	38	Spreading Center	Amphibactin D	Fe	885.415	2	Hydroxamate	Yes	24.7	232026	193.616	0.728	1.821	7.284
R2	38	Spreading Center	Acinetoferrin	Apo	585.350	2	Mixed	Yes	18.6	117191	97.791	0.368	0.920	3.679
R2	38	Spreading Center	IC202C	Fe	570.246	2	Hydroxamate	No	23.5	381777	318.578	1.198	2.996	11.985
R2	38	Spreading Center	Oxahomorphozoferrin	Fe	506.047	2	Carboxylate	No	23.4	142918	119.259	0.449	1.122	4.486
R2	38	Spreading Center	Chlorocatechelin B	Apo	461.144	2	Mixed	No	18.9	103894	86.696	0.326	0.815	3.261
R2	38	Spreading Center	Vibrioferrin C-2H	Apo	447.125	2	Carboxylate	No	14.7	210102	175.322	0.660	1.649	6.596
LS	8	Spreading Center	Petrobactin Sulfonate	Apo	771.287	3	Mixed	No	24.1	480146	133.193	0.501	1.253	5.011
LS	8	Spreading Center	Crochelin A	Apo	696.353	3	Mixed	No	26	282119	78.260	0.294	0.736	2.944
LS	8	Spreading Center	Desferrioxamine P1	Apo	616.331	3	Hydroxamate	No	26.5	797065	221.106	0.832	2.079	8.318

CER	12	Spreading Center	Desferrioxamine P1	Apo	616.331	3	Hydroxamate	No	26.5	119545	62.918	0.237	0.592	2.367
BS	24	Spreading Center	Petrobactin Sulfonate	Apo	771.287	3	Mixed	No	24.1	154561	51.675	0.194	0.486	1.944
BS	24	Spreading Center	Desferrioxamine P1	Apo	616.331	3	Hydroxamate	No	26.5	632217	211.371	0.795	1.988	7.952
BS	24	Spreading Center	Staphyloferrin B	Apo	449.152	3	Mixed	No	19.3	132154	44.183	0.166	0.416	1.662
R2	38	Spreading Center	Crochelin A	Apo	696.353	3	Mixed	No	26	206343	172.185	0.648	1.619	6.478
R2	38	Spreading Center	Desferrioxamine P1	Apo	616.331	3	Hydroxamate	No	26.5	768105	640.954	2.411	6.028	24.112
LS	8	Spreading Center	Nocardimicin F	Fe	867.408	4	Mixed	Yes	24.5	393157	109.062	0.410	1.026	4.103
LS	8	Spreading Center	Desferrioxamine X4	Fe	682.299	4	Hydroxamate	No	28.2	126870	35.194	0.132	0.331	1.324
HFZ	21	Fracture Zone	Fusarinine	Apo	261.145	4	Mixed	No	16.5	169109	80.528	0.303	0.757	3.029
LC	23	Fracture Zone	Mycobactin P C17	Apo	870.559	4	Mixed	Yes	21.4	72348	32.885	0.124	0.309	1.237
LC	23	Fracture Zone	Nocardimicin I	Apo	830.528	4	Mixed	Yes	15	80033	36.379	0.137	0.342	1.369
LC	23	Fracture Zone	IC202A	Apo	573.398	4	Hydroxamate	No	18.9	185180	84.173	0.317	0.792	3.167
BS	24	Spreading Center	Nocardimicin F	Fe	867.408	4	Mixed	Yes	24.5	523154	174.908	0.658	1.645	6.580
BS	24	Spreading Center	Desferrioxamine T3	Apo	773.441	4	Hydroxamate	No	24.9	125850	42.076	0.158	0.396	1.583
BS	24	Spreading Center	Desferrioxamine X4	Fe	682.299	4	Hydroxamate	No	28.2	130558	43.650	0.164	0.411	1.642
BS	24	Spreading Center	Thiazostatin	Apo	339.084	4	Mixed	No	19	136590	45.667	0.172	0.429	1.718
CNT	26	Spreading Center	Talarazine A	Apo	469.266	4	Hydroxamate	No	22	76585	38.293	0.144	0.360	1.441
CNT	26	Spreading Center	Fusarinine	Apo	261.145	4	Mixed	No	16.5	110730	55.365	0.208	0.521	2.083



CET	31	Spreading Center	Chlorocatechelin B	Apo	461.144	4	Mixed	No	18.9	97801	48.901	0.184	0.460	1.840
TAG	35	Spreading Center	Mycobactin P C17	Apo	870.559	4	Mixed	Yes	21.4	118442	36.734	0.138	0.345	1.382
TAG	35	Spreading Center	Nocardimicin F	Fe	867.408	4	Mixed	Yes	24.5	466429	144.660	0.544	1.361	5.442
TAG	35	Spreading Center	Desferrioxamine T3	Apo	773.441	4	Hydroxamate	No	24.9	192467	59.692	0.225	0.561	2.246
TAG	35	Spreading Center	Crochelin A	Apo	696.353	4	Mixed	No	26	78347	24.299	0.091	0.229	0.914
TAG	35	Spreading Center	Desferrioxamine X4	Fe	682.299	4	Hydroxamate	No	29	132006	40.941	0.154	0.385	1.540
LTS	37	Fracture Zone	Synechobactins C11	Apo	547.334	4	Mixed	Yes	12.7	185568	84.349	0.317	0.793	3.173
R2	38	Spreading Center	Mycobactin P C17	Apo	870.559	4	Mixed	Yes	21.4	70466	58.801	0.221	0.553	2.212
R2	38	Spreading Center	Nocardimicin F	Fe	867.408	4	Mixed	Yes	24.5	398350	332.408	1.251	3.126	12.505
R2	38	Spreading Center	Desferrioxamine X4	Fe	682.299	4	Hydroxamate	No	28.2	81880	68.326	0.257	0.643	2.570
R2	38	Spreading Center	Thiazostatin	Apo	339.084	4	Mixed	No	19	78507	65.511	0.246	0.616	2.465

\*A 'Yes' under 'Hydrocarbon Chain' indicates that the putative structure contains a hydrocarbon chain of 7 or more carbons

\*\*40% efficiency is based on the efficiency of ENV columns for recovering siderophore desferrioxamine E in solid-phase extractions from seawater (Waska et al., 2015)

\*\*\*10% efficiency is based on the typical recovery of the bulk Fe-binding organic pool with ENV columns, which comes from comparing the concentration of [Fe-L]-SPE from LC-ICP-MS with that of [Fe-L] from cathodic-stripping voltammetry (Bundy et al., 2016)

**Table S7. Genera detected in this study along with corresponding siderophores production ability**

<b>Taxonomy</b>	<b>Relative Abundance</b>	<b>Location</b>	<b>Filter Size</b>	<b>Depth (m)</b>	<b>Putative Siderophores</b>
Bacteria ; Nitrospinota ; Nitrospina ; Nitrospinales ; Nitrospinaceae ; Nitrospina	0.095	200 km E of Rainbow	0.2 µm	600	STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacterales ; Pseudoalteromonadaceae ; Pseudoalteromonas	0.001	200 km E of Rainbow	0.2 µm	600	DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , DEFERRIOXAMIN B , DEFERRIOXAMINE , UNKNOWN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacterales ; Colwelliaceae ; Colwellia	0.001	200 km E of Rainbow	0.2 µm	600	UNKNOWN
Bacteria ; Nitrospinota ; Nitrospina ; Nitrospinales ; Nitrospinaceae ; Nitrospina	0.094	200 km E of Rainbow	0.2 µm	1600	STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacterales ; Pseudoalteromonadaceae ; Pseudoalteromonas	0.004	200 km E of Rainbow	0.2 µm	1600	DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , DEFERRIOXAMIN B , DEFERRIOXAMINE , UNKNOWN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Marinobacteraceae ; Marinobacter	0.001	200 km E of Rainbow	0.2 µm	1600	PUTREBACTIN / AVAROFERRIN , XANTHOFERRIN , UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacterales ; Moritellaceae ; Moritella	0.0003	200 km E of Rainbow	0.2 µm	1600	COLICIN V , UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Coxiellales ; Coxiellaceae ; Coxiella	0.0002	200 km E of Rainbow	0.2 µm	1600	UNKNOWN

Bacteria ; Nitrospinota ; Nitrospina ; Nitrospinales ; Nitrospinaceae ; Nitrospina	0.035	200 km E of Rainbow	0.2 µm	2250	STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Pseudoalteromonadaceae ; Pseudoalteromonas	0.009	200 km E of Rainbow	0.2 µm	2250	DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , DEFERRIOXAMIN B , DEFERRIOXAMINE , UNKNOWN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Pseudomonadaceae ; Pseudomonas	0.001	200 km E of Rainbow	0.2 µm	2250	XANTHOFERRIN , UNKNOWN , SYRINGAFACTIN , DEFERRIOXAMINE E , ARTHROFACTIN A , PUTREBACTIN / AVAROFERRIN , VIBRIOFERRIN , CICHOFACTIN A / CICHOFACTIN B , PUTISOLVIN , SYRINGOMYCIN , NUNAPEPTIN / NUNAMYCIN , COELICHELIN , DEFERRIOXAMIN B / DEFERRIOXAMINE E , CORPEPTIN A / CORPEPTIN B , ORFAMIDE A / ORFAMIDE C , ACINETO FERRIN , DEFERRIOXAMIN B , CAROTENOID , PYOVERDIN , GACAMIDE A , BANANAMIDE 1 / BANANAMIDE 2 / BANANAMIDE 3
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Colwelliaceae ; Colwellia	0.001	200 km E of Rainbow	0.2 µm	2250	UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Marinobacteraceae ; Marinobacter	0.001	200 km E of Rainbow	0.2 µm	2250	PUTREBACTIN / AVAROFERRIN , XANTHOFERRIN , UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Alteromonadaceae ; Alteromonas	0.0001	200 km E of Rainbow	0.2 µm	2250	UNKNOWN
Bacteria ; Nitrospinota ; Nitrospina ; Nitrospinales ; Nitrospinaceae ; Nitrospina	0.095	200 km E of Rainbow	0.2 µm	600	STAPHYLOBACTIN

Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacterales ; Pseudoalteromonadaceae ; Pseudoalteromonas	0.001	200 km E of Rainbow	0.2 µm	600	DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , DEFERRIOXAMIN B , DEFERRIOXAMINE , UNKNOWN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacterales ; Colwelliaceae ; Colwellia	0.001	200 km E of Rainbow	0.2 µm	600	UNKNOWN
Bacteria ; Nitrospinota ; Nitrospina ; Nitrospinales ; Nitrospinaceae ; Nitrospina	0.094	200 km E of Rainbow	0.2 µm	1600	STAPHYLOBACTIN
Bacteria ; Nitrospinota ; Nitrospina ; Nitrospinales ; Nitrospinaceae ; Nitrospina	0.009	Lucky Strike	0.2 µm	1670	STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacterales ; Pseudoalteromonadaceae ; Pseudoalteromonas	0.007	Lucky Strike	0.2 µm	1670	DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , DEFERRIOXAMIN B , DEFERRIOXAMINE , UNKNOWN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Halomonadaceae ; Halomonas	0.003	Lucky Strike	0.2 µm	1670	STAPHYLOBACTIN , UNKNOWN , DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , AEROBACTIN , XANTHOFERRIN , VIBRIOFERRIN , BISUCABERIN B
Bacteria ; Proteobacteria ; Alphaproteobacteria ; Sphingomonadales ; Sphingomonadaceae ; Sphingobium	0.003	Lucky Strike	0.2 µm	1670	UNKNOWN , XANTHOFERRIN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacterales ; Vibrionaceae ; Vibrio	0.002	Lucky Strike	0.2 µm	1670	VIBRIOFERRIN , PUTREBACTIN / AVAROFERRIN , AEROBACTIN , AMPHI- ENTEROBACTIN 1 / AMPHI- ENTEROBACTIN 2 / AMPHI- ENTEROBACTIN 3 / AMPHI- ENTEROBACTIN 4 , UNKNOWN

Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Marinobacteraceae ; Marinobacter	0.002	Lucky Strike	0.2 µm	1670	PUTREBACTIN / AVAROFERRIN , XANTHOFERRIN , UNKNOWN
Bacteria ; Proteobacteria ; Alphaproteobacteria ; Rhodospirillales ; Thalassospiraceae ; Thalassospira	0.001	Lucky Strike	0.2 µm	1670	UNKNOWN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Shewanellaceae ; Shewanella	0.001	Lucky Strike	0.2 µm	1670	DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , XANTHOFERRIN , UNKNOWN , VIBRIOFERRIN , STAPHYLOBACTIN
Bacteria ; Bacteroidota ; Bacteroidia ; Flavobacteriales ; Flavobacteriaceae ; Tenacibaculum	0.0003	Lucky Strike	0.2 µm	1670	BISUCABERIN B , UNKNOWN
<hr/>					
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Pseudoalteromonadaceae ; Pseudoalteromonas	0.024	Lucky Strike	3 µm	1670	DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , DEFERRIOXAMIN B , DEFERRIOXAMINE , UNKNOWN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Shewanellaceae ; Shewanella	0.017	Lucky Strike	3 µm	1670	DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , XANTHOFERRIN , UNKNOWN , VIBRIOFERRIN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Moritellaceae ; Moritella	0.015	Lucky Strike	3 µm	1670	COLICIN V , UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Alteromonadaceae ; Alteromonas	0.013	Lucky Strike	3 µm	1670	UNKNOWN
Bacteria ; Proteobacteria ; Alphaproteobacteria ; Sphingomonadales ;	0.011	Lucky Strike	3 µm	1670	UNKNOWN , XANTHOFERRIN , STAPHYLOBACTIN

Sphingomonadaceae ; Sphingobium						VIBRIOFERRIN , PUTREBACTIN / AVAROFERRIN , AEROBACTIN , AMPHI- ENTEROBACTIN 1 / AMPHI- ENTEROBACTIN 2 / AMPHI- ENTEROBACTIN 3 / AMPHI- ENTEROBACTIN 4 , UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Vibrionaceae ; Vibrio	0.005	Lucky Strike	3 µm	1670		
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Marinobacteraceae ; Marinobacter	0.005	Lucky Strike	3 µm	1670		PUTREBACTIN / AVAROFERRIN , XANTHOFERRIN , UNKNOWN
Bacteria ; Bacteroidota ; Bacteroidia ; Flavobacteriales ; Flavobacteriaceae ; Flavobacterium	0.004	Lucky Strike	3 µm	1670		BISUCABERIN B , PUTREBACTIN / AVAROFERRIN , UNKNOWN , DESFERRIOXAMINE E
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Colwelliaceae ; Colwellia	0.004	Lucky Strike	3 µm	1670		UNKNOWN
Bacteria ; Nitrospina ; Nitrospina ; Nitrospinales ; Nitrospinaceae ; Nitrospina	0.004	Lucky Strike	3 µm	1670		STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Halomonadaceae ; Halomonas	0.004	Lucky Strike	3 µm	1670		STAPHYLOBACTIN , UNKNOWN , DESFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , AEROBACTIN , XANTHOFERRIN , VIBRIOFERRIN , BISUCABERIN B
Bacteria ; Proteobacteria ; Alphaproteobacteria ; Rhodobacteriales ; Rhodobacteraceae ; Tateyamaria	0.003	Lucky Strike	3 µm	1670		UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Coxiellales ; Coxiellaceae ; Coxiella	0.002	Lucky Strike	3 µm	1670		UNKNOWN

Bacteria ; Proteobacteria ; Alphaproteobacteria ; Rhodobacterales ; Rhodobacteraceae ; Paracoccus	0.002	Lucky Strike	3 µm	1670	UNKNOWN , CHEJUNOLIDE A / CHEJUNOLIDE B
Bacteria ; Actinobacteriota ; Actinobacteria ; Corynebacteriales ; Nocardiaceae ; Rhodococcus	0.002	Lucky Strike	3 µm	1670	UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Sphingobacteriaceae ; Sinobacterium	0.001	Lucky Strike	3 µm	1670	PUTREBACTIN / AVAROFERRIN
Bacteria ; Proteobacteria ; Alphaproteobacteria ; Rhodospirillales ; Thalassospiraceae ; Thalassospira	0.001	Lucky Strike	3 µm	1670	UNKNOWN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Vibrionaceae ; Photobacterium	0.001	Lucky Strike	3 µm	1670	UNKNOWN , DESFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , AEROBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Psychromonadaceae ; Psychromonas	0.0004	Lucky Strike	3 µm	1670	UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Halomonadaceae ; Salinicola	0.0004	Lucky Strike	3 µm	1670	PUTREBACTIN / AVAROFERRIN , AEROBACTIN , DESFERRIOXAMINE E
Bacteria ; Spirochaetota ; Spirochaetia ; Spirochaetales ; Spirochaetaceae ; Treponema	0.0003	Lucky Strike	3 µm	1670	UNKNOWN
Bacteria ; Actinobacteriota ; Actinobacteria ; Propionibacteriales ; Nocardioideae ; Nocardioideae	0.0002	Lucky Strike	3 µm	1670	DESFERRIOXAMINE B , DESFERRIOXAMINE , DESFERRIOXAMINE E

---

Bacteria ; Nitrospinota ; Nitrospina ; Nitrospinales ; Nitrospinaceae ; Nitrospina	0.024	10 km S of Rainbow	0.2 µm	2000	STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Pseudoalteromonadaceae ; Pseudoalteromonas	0.006	10 km S of Rainbow	0.2 µm	2000	DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , DEFERRIOXAMIN B , DEFERRIOXAMINE , UNKNOWN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Alphaproteobacteria ; Sphingomonadales ; Sphingomonadaceae ; Sphingobium	0.004	10 km S of Rainbow	0.2 µm	2000	UNKNOWN , XANTHOFERRIN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Halomonadaceae ; Halomonas	0.002	10 km S of Rainbow	0.2 µm	2000	STAPHYLOBACTIN , UNKNOWN , DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , AEROBACTIN , XANTHOFERRIN , VIBRIOFERRIN , BISUCABERIN B
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Alteromonadaceae ; Alteromonas	0.001	10 km S of Rainbow	0.2 µm	2000	UNKNOWN
Bacteria ; Bacteroidota ; Bacteroidia ; Flavobacteriales ; Flavobacteriaceae ; Flavobacterium	0.001	10 km S of Rainbow	0.2 µm	2000	BISUCABERIN B , PUTREBACTIN / AVAROFERRIN , UNKNOWN , DEFERRIOXAMINE E
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Colwelliaceae ; Colwellia	0.0003	10 km S of Rainbow	0.2 µm	2000	UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Alteromonadaceae ; Alteromonas	0.115	10 km S of Rainbow	3 µm	2000	UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ;	0.019	10 km S of Rainbow	3 µm	2000	DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , DEFERRIOXAMIN B ,



Pseudoalteromonadaceae ; Pseudoalteromonas					DEFERRIOXAMINE , UNKNOWN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Alphaproteobacteria ; Sphingomonadales ; Sphingomonadaceae ; Sphingobium	0.015	10 km S of Rainbow	3 µm	2000	UNKNOWN , XANTHOFERRIN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Halomonadaceae ; Halomonas	0.013	10 km S of Rainbow	3 µm	2000	STAPHYLOBACTIN , UNKNOWN , DEFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , AEROBACTIN , XANTHOFERRIN , VIBRIOFERRIN , BISUCABERIN B
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacterales ; Moritellaceae ; Moritella	0.009	10 km S of Rainbow	3 µm	2000	COLICIN V , UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacterales ; Vibrionaceae ; Vibrio	0.007	10 km S of Rainbow	3 µm	2000	VIBRIOFERRIN , PUTREBACTIN / AVAROFERRIN , AEROBACTIN , AMPHI- ENTEROBACTIN 1 / AMPHI- ENTEROBACTIN 2 / AMPHI- ENTEROBACTIN 3 / AMPHI- ENTEROBACTIN 4 , UNKNOWN
Bacteria ; Nitrospinota ; Nitrospina ; Nitrospinales ; Nitrospinaceae ; Nitrospina	0.007	10 km S of Rainbow	3 µm	2000	STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Pseudomonadaceae ; Pseudomonas	0.004	10 km S of Rainbow	3 µm	2000	XANTHOFERRIN , UNKNOWN , SYRINGAFECTIN , DEFERRIOXAMINE E , ARTHROFACTIN A , PUTREBACTIN / AVAROFERRIN , VIBRIOFERRIN , CICHOFACTIN A / CICHOFACTIN B , PUTISOLVIN , SYRINGOMYCIN , NUNAPEPTIN / NUNAMYCIN , COELICHELIN , DEFERRIOXAMIN B / DEFERRIOXAMINE E , CORPEPTIN A / CORPEPTIN B , ORFAMIDE A / ORFAMIDE C , ACINETOFERRIN , DEFERRIOXAMIN B , CAROTENOID , PYOVERDIN ,

Bacteria ; Bacteroidota ; Bacteroidia ; Flavobacteriales ; Flavobacteriaceae ; Flavobacterium	0.004	10 km S of Rainbow	3 µm	2000	GACAMIDE A , BANANAMIDE 1 / BANANAMIDE 2 / BANANAMIDE 3 BISUCABERIN B , PUTREBACTIN / AVAROFERRIN , UNKNOWN , DESFERRIOXAMINE E
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Marinobacteraceae ; Marinobacter	0.003	10 km S of Rainbow	3 µm	2000	PUTREBACTIN / AVAROFERRIN , XANTHOFERRIN , UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Vibrionaceae ; Photobacterium	0.003	10 km S of Rainbow	3 µm	2000	UNKNOWN , DESFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , AEROBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Coxiellales ; Coxiellaceae ; Coxiella	0.002	10 km S of Rainbow	3 µm	2000	UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Colwelliaceae ; Colwellia	0.002	10 km S of Rainbow	3 µm	2000	UNKNOWN
Bacteria ; Proteobacteria ; Alphaproteobacteria ; Sphingomonadales ; Sphingomonadaceae ; Sphingomonas	0.002	10 km S of Rainbow	3 µm	2000	STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Halomonadaceae ; Salinicola	0.001	10 km S of Rainbow	3 µm	2000	PUTREBACTIN / AVAROFERRIN , AEROBACTIN , DESFERRIOXAMINE E
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Shewanellaceae ; Shewanella	0.001	10 km S of Rainbow	3 µm	2000	DESFERRIOXAMINE E , PUTREBACTIN / AVAROFERRIN , XANTHOFERRIN , UNKNOWN , VIBRIOFERRIN , STAPHYLOBACTIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ;	0.001	10 km S of Rainbow	3 µm	2000	DESFERRIOXAMINE E

Pseudomonadales ; Saccharospirillaceae ; Oleibacter Bacteria ; Proteobacteria ; Gammaproteobacteria ; Francisellales ; Francisellaceae ; Fangia	0.001	10 km S of Rainbow	3 µm	2000	UNKNOWN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Spongiiibacteraceae ; Sinobacterium	0.0001	10 km S of Rainbow	3 µm	2000	PUTREBACTIN / AVAROFERRIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Enterobacteriales ; Psychromonadaceae ; Psychromonas	0.0005	10 km S of Rainbow	3 µm	2000	UNKNOWN
Bacteria ; Proteobacteria ; Alphaproteobacteria ; Rhodobacteriales ; Rhodobacteraceae ; Paracoccus	0.0005	10 km S of Rainbow	3 µm	2000	UNKNOWN , CHEJUNOLIDE A / CHEJUNOLIDE B
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Burkholderiales ; Oxalobacteraceae ; Massilia	0.0004	10 km S of Rainbow	3 µm	2000	CUPRIACHELIN , UNKNOWN , XANTHOFERRIN
Bacteria ; Proteobacteria ; Gammaproteobacteria ; Pseudomonadales ; Moraxellaceae ; Psychrobacter	0.0002	10 km S of Rainbow	3 µm	2000	VIBRIOFERRIN , ACINETOFERRIN

Solid lines separate the three different stations. Dashed lines separate 0.2 µm and 3 µm filter sizes investigated in this study.

St. 7 = Lucky Strike

St. 11 = 200 km E of Rainbow

St. 17 = 10 km S of Rainbow

See **Supplementary Table 1** for additional information.

## SI References

Boiteau, R. M., Mende, D. R., Hawco, N. J., McIlvin, M. R., Fitzsimmons, J. N., Saito, M. A., Sedwick, P. N., DeLong, E. F., and Repeta, D. J.: Siderophore-based microbial adaptations to iron scarcity across the eastern Pacific Ocean, *Proc. Natl. Acad. Sci.*, 113, 14237–14242, <https://doi.org/10.1073/pnas.1608594113>, 2016.

Bundy, R. M., Jiang, M., Carter, M., and Barbeau, K. A.: Iron-Binding Ligands in the Southern California Current System: Mechanistic Studies, *Front. Mar. Sci.*, 3, 1–17, <https://doi.org/10.3389/fmars.2016.00027>, 2016.

Gauglitz, J. M. and Butler, A.: Amino acid variability in the peptide composition of a suite of amphiphilic peptide siderophores from an open ocean *Vibrio* species, *J. Biol. Inorg. Chem.*, 18, 489–497, <https://doi.org/10.1007/s00775-013-0995-3>.Amino, 2013.

Gledhill, M. and Buck, K. N.: The organic complexation of iron in the marine environment: A review, *Front. Microbiol.*, 3, 1–17, <https://doi.org/10.3389/fmicb.2012.00069>, 2012.

Hawkes, J. A., Gledhill, M., Connelly, D. P., and Achterberg, E. P.: Characterisation of iron binding ligands in seawater by reverse titration, *Anal. Chim. Acta*, 766, 53–60, <https://doi.org/10.1016/j.aca.2012.12.048>, 2013a.

Hawkes, J. A., Connelly, D. P., Gledhill, M., and Achterberg, E. P.: The stabilisation and transportation of dissolved iron from high temperature hydrothermal vent systems, *Earth Planet. Sci. Lett.*, 375, 280–290, <https://doi.org/10.1016/j.epsl.2013.05.047>, 2013b.

Lough, A. J. M., Tagliabue, A., Demasy, C., Resing, J. A., Mellett, T., Wyatt, N. J., and Lohan, M. C.: The impact of hydrothermal vent geochemistry on the addition of iron to the deep ocean, *Biogeosciences Discuss.*, [preprint], 1–23, <https://doi.org/10.5194/bg-2022-73>, 2022.

Waska, H., Koschinsky, A., Ruiz Chanco, M. J., and Dittmar, T.: Investigating the potential of solid-phase extraction and Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) for the isolation and identification of dissolved metal-organic complexes from natural waters, *Mar. Chem.*, 173, 78–92, <https://doi.org/10.1016/j.marchem.2014.10.001>, 2015.