***Supplementary Material*:**

**Extracellular reef metabolites across the protected Jardines de la Reina, Cuba reef system**

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Supplementary Table 1. Locations, depths, and physicochemical measurements of the water column across Jardines de la Reina (JR) coral reefs and the off reef (OR) sites.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Date | Site | Type | Latitude | Longitude | Depth types (m) | CTD  depth (m) | Temp.1  (°C) | pH | Salinity  (PSU) | DO2 (mg/L) |
| 11/5/17 | JR4 | Reef | 21° 03.825 N | 79° 25.635 W | Surface | 0.38 | 28.62 | 8.16 | 36.91 | 8.13 |
|  | Reef depth | 10.84 | 28.65 | 8.25 | 36.94 | 6.48 |
| 11/6/17 | JR5 | Reef | 20° 57.924 N | 79° 12.273 W | Surface | 0.40 | 27.75 | 8.17 | 37.54 | 6.76 |
|  | Reef depth | 14.71 | 28.63 | 8.26 | 36.96 | 6.38 |
| 11/7/17 | JR6 | Reef | 20° 50.634 N | 79° 01.299 W | Surface | 0.38 | 28.29 | 8.15 | 37.39 | 6.53 |
|  | Reef depth | 7.46 | 28.73 | 8.27 | 37.05 | 6.51 |
| 11/8/17 | JR13 | Reef | 20° 34.747 N | 78° 26.563 W | Surface | 0.35 | 28.69 | 8.27 | 37.04 | 6.35 |
|  | Reef depth | 10.04 | 28.64 | 8.28 | 37.05 | 6.23 |
| 11/9/17 | JR14 | Reef | 20° 30.390 N | 78° 22.864 W | Surface | 0.53 | 28.78 | 8.25 | 37.06 | 6.45 |
|  | Reef depth | 11.12 | 28.11 | 8.29 | 37.19 | 6.37 |
| 11/9/17 | OR1 | Off reef | 20° 30.680 N | 78° 24.798 W | Surface | - | - | - | - | - |
| 11/10/17 | JR12 | Reef | 20° 37.446 N | 78° 35.326 W | Surface | 0.39 | 28.84 | 8.20 | 37.07 | 6.54 |
|  | Reef depth | 12.05 | 28.65 | 8.23 | 37.09 | 5.93 |
| 11/11/17 | JR11 | Reef | 20° 40.802 N | 78° 45.275 W | Surface | 0.50 | 28.68 | 8.22 | 37.04 | 6.34 |
|  | Reef depth | 13.84 | 28.58 | 8.26 | 37.10 | 5.44 |
| 11/11/17 | OR2 | Off reef | 20° 44.017 N | 78° 52.835 W | Surface | - | - | - | - | - |
| 11/12/17 | JR10 | Reef | 20° 46.500 N | 78° 55.134 W | Surface | 0.35 | 28.49 | 8.29 | 36.98 | 6.45 |
|  | Reef depth | 9.59 | 28.54 | 8.29 | 36.98 | 5.93 |
| 11/20/17 | JR2 | Reef | 21° 18.200 N | 79° 35.464 W | Surface | 0.38 | 28.13 | 8.29 | 36.87 | 6.72 |
|  | Reef depth | 10.98 | 28.16 | 8.31 | 36.94 | 6.53 |

1 = Temperature

2 = Dissolved oxygen

Supplementary Table 2. Detection and concentration ranges (when applicable) of targeted metabolites in off reef, reef surface, and reef depth seawater.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Metabolites | Ionization mode | Sample type | % of samples | Concentration range  (nM or pM) |
| 2,3 – Dihydroxypropane – 1– sulfonate | Negative | Off reef | 100 | 9.40 – 10.6 nM |
|  |  | Reef surface | 100 | 3.45 – 15.3 nM |
|  |  | Reef depth | 100 | 4.34 – 17.2 nM |
| 4 – Aminobenzoic acid | Positive | Off reef | 100 | 26.3 – 50.6 pM |
|  |  | Reef surface | 100 | 18.9 – 63.5 pM |
|  |  | Reef depth | 100 | 20.1– 67.7 pM |
| 4 – Hydroxybenzoic acid | Negative | Off reef | 100 | 70.7 – 91.2 pM |
|  |  | Reef surface | 100 | 47.7 – 140 pM |
|  |  | Reef depth | 100 | 55.7 – 122 pM |
| 5ʹ – Methylthioadenosine (MTA) | Positive | Off reef | 75 | 0 – 1.38 pM |
|  |  | Reef surface | 100 | 1.08 – 5.01 pM |
|  |  | Reef depth | 100 | 1.33 – 4.58 pM |
| b – Nicotinamide adenine dinucleotide hydrate (NADH) | Positive | Off reef | 50 | 0 – 5.80 pM |
| Reef surface | 67 | 0 – 5.66 pM |
| Reef depth | 56 | 0 – 3.16 pM |
| Adenosine 5ʹ – monophosphate | Positive | Off reef | 75 | 0 – 0.772 nM |
| Reef surface | 0 | 0 – 1.63 nM |
| Reef depth | 50 | 0 – 3.94 nM |
| Adenosine | Positive | Off reef | 100 | 22.3 – 30.9 pM |
|  |  | Reef surface | 100 | 23.9 – 58.4 pM |
|  |  | Reef depth | 100 | 28.6 – 70.3 pM |
| Caffeine | Positive | Off reef | 100 | 18.8 – 80.6 pM |
|  |  | Reef surface | 100 | 13.9 – 194 pM |
|  |  | Reef depth | 100 | 14.5 – 30.7 pM |
| Chitobiose | Positive | Off reef | 75 | 0 – 1.73 nM |
|  |  | Reef surface | 84 | 0 – 3.18 nM |
|  |  | Reef depth | 78 | 0 – 3.37 nM |
| Chitotriose | Positive | Off reef | 75 | 0 – 133 pM |
|  |  | Reef surface | 23 | 0 – 144 pM |
|  |  | Reef depth | 23 | 0 – 140 pM |
| Citric acid | Negative | Off reef | 100 | 1.761 – 3.22 nM |
|  |  | Reef surface | 100 | 0 – 9.40 nM |
|  |  | Reef depth | 100 | 1.54 – 6.47 nM |
| Desthiobiotin | Positive | Off reef | 100 | 13.2 – 16.2 pM |
| Reef surface | 89 | 0 – 16.8 pM |
| Reef depth | 73 | 0 – 18.7 pM |
| Glutathione (oxidized) | Positive | Off reef | 100 | 251 – 568 pM |
|  |  | Reef surface | 100 | 142 – 498 pM |
|  |  | Reef depth | 100 | 154 – 989 pM |
| Guanosine | Positive | Off reef | 0 | - |
|  |  | Reef surface | 84 | 0 – 29.3 pM |
|  |  | Reef depth | 84 | 0 – 39.0 pM |
| Inosine | Negative | Off reef | 100 | 4.95 – 5.94 pM |
|  |  | Reef surface | 100 | 4.61 – 14.2 pM |
|  |  | Reef depth | 100 | 3.44 – 13.0 pM |
| Kynurenine | Positive | Off reef | 100 | 3.22 – 6.94 pM |
|  |  | Reef surface | 84 | 0 – 12.8 pM |
|  |  | Reef depth | 78 | 0 – 6.96 pM |
| Leucine | Positive | Off reef | 75 | 0 – 0.626 nM |
|  |  | Reef surface | 95 | 0 – 2.01 nM |
|  |  | Reef depth | 95 | 0 – 1.27 nM |
| Malic acid | Negative | Off reef | 100 | 17.7 – 24.7 nM |
|  |  | Reef surface | 100 | 11.5 – 27.8 nM |
|  |  | Reef depth | 100 | 9.54 – 27.3 nM |
| *N* – acetylmuramic acid | Positive | Off reef | 100 | 302 – 424 pM |
| Reef surface | 89 | 0 – 532 pM |
| Reef depth | 84 | 0 – 630 pM |
| Pantothenic acid | negative | Off reef | 0 | - |
| Reef surface | 78 | 0 – 6.55 pM |
| Reef depth | 89 | 0 – 4.72 pM |
| Phenylalanine | Positive | Off reef | 100 | 49.9 – 69.6 pM |
|  |  | Reef surface | 100 | 30.7 – 395 pM |
|  |  | Reef depth | 100 | 38.5– 133 pM |
| Pyridoxine | Positive | Off reef | 100 | 17.6 – 23.3 pM |
|  |  | Reef surface | 84 | 0 – 24.4 pM |
|  |  | Reef depth | 67 | 0 – 33.9 pM |
| Riboflavin | Positive | Off reef | 75 | 0 – 0.85 pM |
|  |  | Reef surface | 100 | 1.06 – 3.55 pM |
|  |  | Reef depth | 100 | 1.78 – 7.13 pM |
| S – 5ʹadenosyl – L – homocysteine | Positive | Off reef | 100 | 0.811 – 1.93 pM |
|  | Reef surface | 95 | 0 – 2.79 pM |
|  | Reef depth | 100 | 0 – 2.45 pM |
| Syringic acid | Negative | Off reef | 50 | 0 – 14.01 pM |
|  |  | Reef surface | 39 | 0 – 30.4 pM |
|  |  | Reef depth | 39 | 0 – 28.4 pM |
| Taurocholic acid | Negative | Off reef | 100 | 1.68 – 5.34 pM |
|  |  | Reef surface | 100 | 5.88 – 33.9 pM |
|  |  | Reef depth | 100 | 10.3 – 84.5 pM |
| Thymidine | Negative | Off reef | 25 | 0 – 11.7 pM |
|  |  | Reef surface | 73 | 0 – 18.2 pM |
|  |  | Reef depth | 56 | 0 – 16.7 pM |
| Tryptamine | Positive | Off reef | 50 | 0 – 21.4 pM |
|  |  | Reef surface | 50 | 0 – 20.2 pM |
|  |  | Reef depth | 50 | 0 – 19.9 pM |
| Tryptophan | Positive | Off reef | 75 | 0 – 5.76 pM |
|  |  | Reef surface | 67 | 0 – 16.9 pM |
|  |  | Reef depth | 89 | 0 – 5.75 pM |
| Tyrosine | Positive | Off reef | 100 | 0.481 – 0.659 nM |
|  |  | Reef surface | 100 | 0.479 – 1.76 nM |
|  |  | Reef depth | 100 | 0.445 – 1.16 nM |
| Xanthine | Positive | Off reef | 0 | - |
|  | Reef surface | 73 | 0 – 0.655 nM |
|  | Reef depth | 67 | 0 – 1.06 nM |
| Xanthosine | Negative | Off reef | 50 | 0 – 7.28 pM |
|  | Reef surface | 62 | 0 – 11.1 pM |
|  | Reef depth | 28 | 0 – 6.33 pM |

Supplementary Table 3. List of targeted compounds that were detected, but not quantified.

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| Metabolites |
| 4-Amino-5-aminomethyl-2-methylpyrimidine (AmMP) |
| γ-aminobutyric acid (GABA) |
| Acetyltaurine |
| Adenine |
| Alpha – ketoglutaric acid |
| Arginine |
| Choline |
| Ciliatine |
| Citrulline |
| Dihydroxyacetone phosphate |
| Ectoine |
| Fosfomycin |
| Glucose 6-phosphate |
| Glutamic acid |
| Guanine |
| Proline |
| Sn-glycerol 3-phosphate |
| Succinic acid |
| Threonine/homoserine |
| Uridine 5-monophosphate |
| Valine |



Supplementary Figure 1. Non-metric multidimensional scaling analysis conducted on the square-root transformed Bray-Curtis untargeted feature dissimilarities. To make this plot, feature peak intensities were not normalized by volume or total organic carbon concentrations because of the inclusion of the pooled samples, for which volume or total organic carbon measurement is not possible. Symbol color represents general sample type (Reef, Pool, or Off reef).



Supplementary Figure 2. Significant (FDR-adjusted p-value < 0.05) positive correlations (Pearson’s r > 0.70) between untargeted metabolite features and environmental parameters.



Supplementary Figure 3. Significant (FDR-adjusted p-value < 0.05) negative correlations (Pearson’s r < -0.70) between untargeted metabolite features and environmental parameters.



Supplementary Figure 4. Significant (FDR-adjusted p-value < 0.05) correlations between targeted metabolite features and environmental parameters. Above each scatter plot (A – W), the Pearson’s R and FDR adjusted p-value are displayed for that correlation. Symbol color represents the region where reef samples were collected (central, eastern, western) and the off reef sample type.