

SUPPLEMENTARY INFORMATION

Impact of ocean acidification on the metabolome of the brown macroalgae *Lobophora rosacea* from New Caledonia

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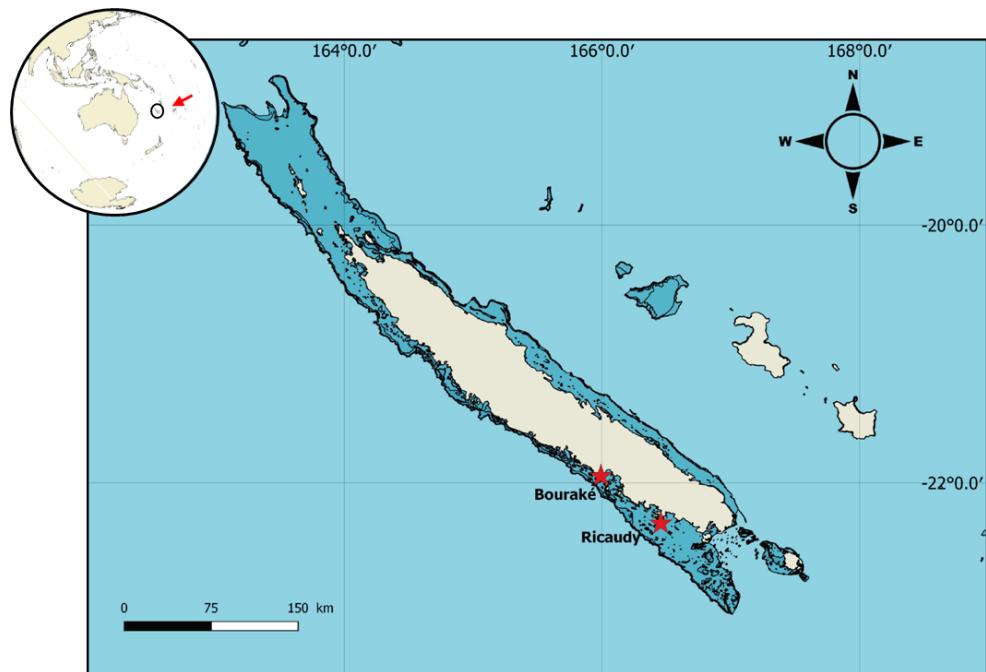


Figure S1. Map of the collected sites (Bouraké and Ricaudy) in the southwest lagoon of Nouméa, New Caledonia.



Figure S2. Picture of *Lobophora rosacea* at Ricaudy site. The alga is growing attached to the bedrock by a basal mound of hairs niched beneath *Acropora spp.* branches.

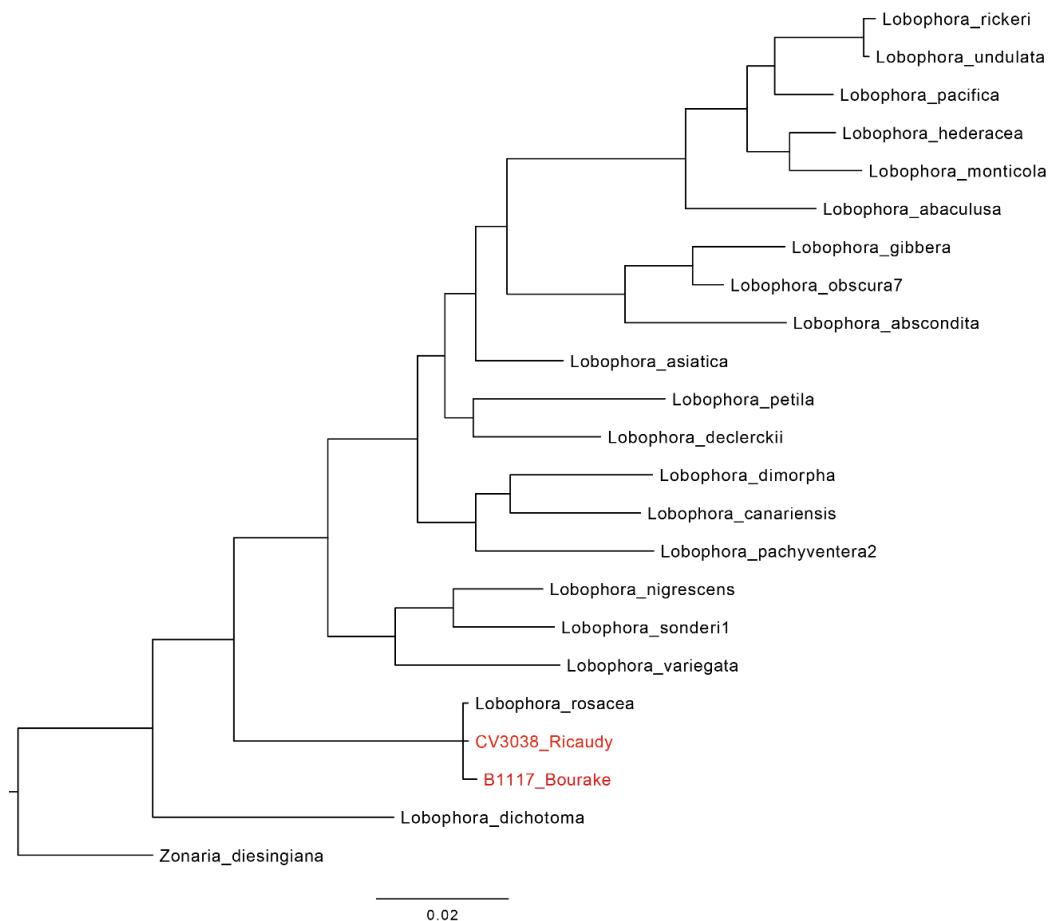


Figure S3. Phylogenetic tree reconstruction of 20 *Lobophora* species using Bayesian inference (BI) implemented in MrBayes v3.2, using the chloroplast marker psbA. Voucher numbers of *L. rosacea* collected at Ricaudy (control pH) and Bouraké (low pH) are indicated.

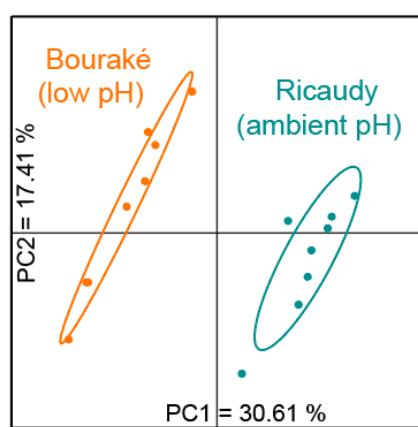


Figure S4. Principal component analysis (PCA) score plots of the methanol extracts of *Lobophora rosacea* metabolome from control (Ricaudy) and low pH (Bouraké).

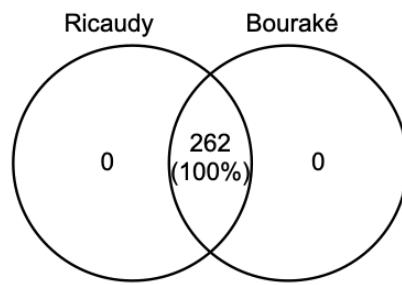


Figure S5. Venn diagram test of *Lobophora rosacea* MeOH extracts from algae sampled at Ricaudy (control pH) vs Bouraké (low pH).

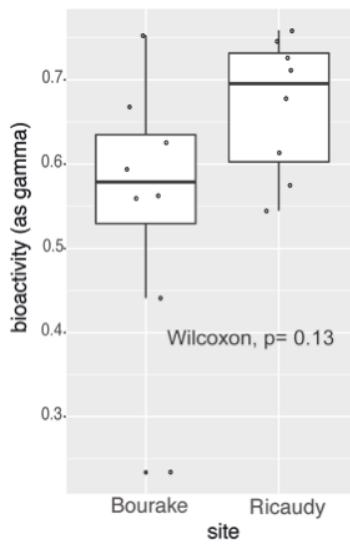


Figure S6. Macroalgal bioactivities (as gamma units) boxplots in MeOH extracts according to pH condition in the New Caledonian lagoon: Bouraké (low pH) vs Ricaudy (control pH) ($n = 8$, Wilcoxon rank sum test, $p = 0.13$).

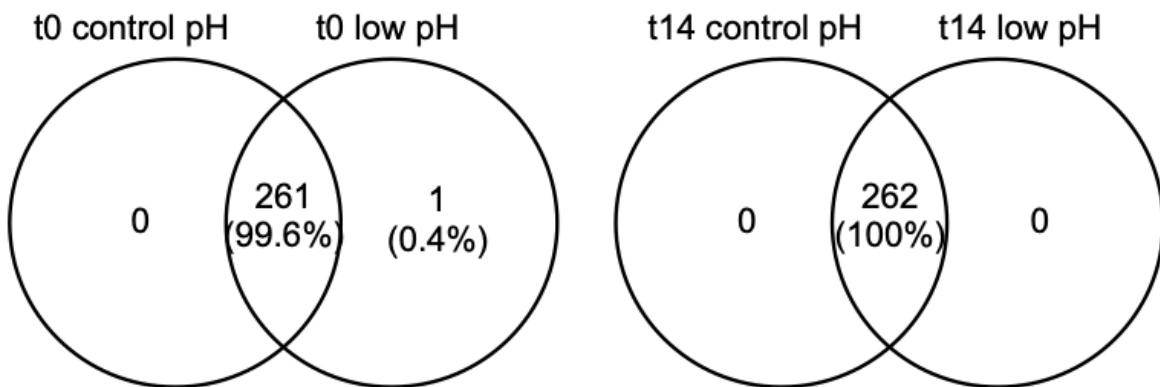


Figure S7. Venn diagrams on *Lobophora rosacea* MeOH extracts at t0 and t14 (algae exposed to control vs low pH conditions) during the *ex situ* experiment.

Table S1. Seawater temperature and pH measured in control and low pH tanks from t0 to t14 for aquaria experiment. Carbonate chemistry parameters were calculated with CO2Sys (Lewis & Wallace, 1998) using mean total alkalinity (TA), salinity (35), temperature (T) and pH.

| days | T (°C) | TA ($\mu\text{mol} \cdot \text{kg}^{-1}$) | pH | pCO ₂ (μatm) | HCO ₃ ⁻ ($\mu\text{mol} \cdot \text{kg}^{-1}$) | CO ₃ ²⁻ ($\mu\text{mol} \cdot \text{kg}^{-1}$) | CO ₂ ($\mu\text{mol} \cdot \text{kg}^{-1}$) | ΩCa | ΩAr |
|---|------------|--|-------------|---|---|---|---|-------------|-------------|
| <i>Control pH tanks</i> | | | | | | | | | |
| 0 (t0) | 24.0 ± 0.1 | 2328 ± 4 | 8.14 ± 0.02 | 311 ± 17 | 1723 ± 22 | 246 ± 8 | 9 ± 1 | 5.92 ± 0.19 | 3.89 ± 0.13 |
| 1 | 23.9 ± 0.1 | 2337 ± 6 | 8.15 ± 0.01 | 304 ± 11 | 1721 ± 16 | 251 ± 4 | 9 | 6.03 ± 0.1 | 3.96 ± 0.06 |
| 2 | 23.8 ± 0.1 | 2337 ± 6 | 8.16 ± 0.01 | 289 ± 10 | 1704 ± 16 | 258 ± 5 | 8 | 6.19 ± 0.11 | 4.07 ± 0.07 |
| 3 | 23.7 ± 0.1 | 2337 ± 6 | 8.13 ± 0.01 | 316 ± 10 | 1739 ± 14 | 244 ± 4 | 9 | 5.85 ± 0.1 | 3.84 ± 0.07 |
| 4 | 23.7 ± 0.1 | 2345 ± 8 | 8.15 ± 0.02 | 299 ± 19 | 1724 ± 25 | 253 ± 11 | 9 ± 1 | 6.09 ± 0.26 | 4.00 ± 0.17 |
| 5 | 23.6 ± 0.1 | 2351 ± 5 | 8.13 ± 0.03 | 317 ± 27 | 1749 ± 30 | 246 ± 13 | 9 ± 1 | 5.90 ± 0.31 | 3.88 ± 0.20 |
| 6 | 23.8 ± 0.1 | 2349 ± 7 | 8.11 ± 0.02 | 343 ± 17 | 1773 ± 21 | 235 ± 8 | 10 ± 1 | 5.65 ± 0.18 | 3.71 ± 0.12 |
| 7 | 24.0 ± 0.3 | 2347 ± 9 | 8.11 ± 0.04 | 338 ± 43 | 1763 ± 47 | 238 ± 19 | 10 ± 1 | 5.73 ± 0.46 | 3.76 ± 0.31 |
| 8 | 24.3 ± 0.4 | 2342 ± 2 | 8.14 ± 0.07 | 314 ± 71 | 1723 ± 81 | 253 ± 34 | 9 ± 2 | 6.07 ± 0.81 | 4.00 ± 0.54 |
| 9 | 23.6 ± 0.1 | 2342 ± 2 | 8.09 ± 0.01 | 353 ± 8 | 1782 ± 7 | 228 ± 3 | 10 | 5.49 ± 0.07 | 3.60 ± 0.05 |
| 10 | 24.3 ± 0.2 | 2337 ± 7 | 8.18 ± 0.04 | 273 ± 31 | 1675 ± 50 | 270 ± 17 | 8 ± 1 | 6.49 ± 0.41 | 4.27 ± 0.27 |
| 11 | 23.6 | 2341 ± 4 | 8.09 ± 0.01 | 359 ± 12 | 1787 ± 9 | 226 ± 5 | 11 | 5.43 ± 0.12 | 3.56 ± 0.08 |
| 12 | 23.5 ± 0.1 | 2345 ± 9 | 8.10 ± 0.01 | 345 ± 11 | 1777 ± 8 | 232 ± 6 | 10 | 5.57 ± 0.14 | 3.66 ± 0.09 |
| 13 (t14) | 24.1 ± 0.1 | 2345 ± 9 | 8.10 ± 0.01 | 346 ± 7 | 1770 ± 10 | 235 ± 2 | 10 | 5.64 ± 0.06 | 3.71 ± 0.04 |
| <i>Acidified condition: tanks pH 7.65</i> | | | | | | | | | |
| 0 (t0) | 24.0 ± 0.1 | 2333 ± 1 | 7.66 ± 0.04 | 1135 ± 126 | 2089 ± 24 | 100 ± 9 | 33 ± 4 | 2.40 ± 0.22 | 1.58 ± 0.15 |
| 1 | 24.1 ± 0.1 | 2335 ± 4 | 7.66 ± 0.03 | 1118 ± 71 | 2088 ± 10 | 101 ± 5 | 32 ± 2 | 2.43 ± 0.13 | 1.60 ± 0.08 |
| 2 | 24.0 ± 0.1 | 2335 ± 4 | 7.66 ± 0.03 | 1131 ± 69 | 2091 ± 9 | 100 ± 5 | 33 ± 2 | 2.40 ± 0.12 | 1.58 ± 0.08 |
| 3 | 24.0 ± 0.2 | 2335 ± 4 | 7.63 ± 0.02 | 1217 ± 63 | 2105 ± 9 | 94 ± 5 | 35 ± 2 | 2.26 ± 0.12 | 1.48 ± 0.08 |
| 4 | 23.6 ± 0.1 | 2337 ± 9 | 7.62 ± 0.03 | 1248 ± 97 | 2114 ± 9 | 91 ± 7 | 37 ± 3 | 2.19 ± 0.16 | 1.44 ± 0.11 |
| 5 | 23.9 ± 0.1 | 2348 ± 12 | 7.63 ± 0.03 | 1214 ± 105 | 2116 ± 19 | 95 ± 7 | 35 ± 3 | 2.28 ± 0.17 | 1.50 ± 0.11 |
| 6 | 23.9 ± 0.1 | 2340 ± 10 | 7.65 ± 0.04 | 1176 ± 106 | 2103 ± 15 | 97 ± 8 | 34 ± 3 | 2.34 ± 0.2 | 1.53 ± 0.13 |
| 7 | 24.5 ± 0.3 | 2333 ± 10 | 7.67 ± 0.03 | 1105 ± 83 | 2079 ± 11 | 104 ± 8 | 32 ± 3 | 2.50 ± 0.2 | 1.64 ± 0.13 |
| 8 | 24.6 ± 0.1 | 2335 ± 9 | 7.63 ± 0.06 | 1225 ± 175 | 2100 ± 19 | 96 ± 12 | 35 ± 5 | 2.32 ± 0.28 | 1.53 ± 0.18 |
| 9 | 23.8 ± 0.1 | 2335 ± 9 | 7.71 ± 0.06 | 993 ± 138 | 2064 ± 30 | 111 ± 13 | 29 ± 4 | 2.67 ± 0.31 | 1.75 ± 0.20 |

| | | | | | | | | | |
|----------|----------------|---------------|-----------------|---------------|---------------|-------------|------------|-----------------|-----------------|
| 10 | 24.1 ± 0.3 | 2337 ± 12 | 7.68 ± 0.01 | 1081 ± 27 | 2083 ± 13 | 104 ± 1 | 31 ± 1 | 2.50 ± 0.02 | 1.65 ± 0.01 |
| 11 | 23.8 ± 0.1 | 2342 ± 12 | 7.64 ± 0.02 | 1182 ± 49 | 2107 ± 10 | 96 ± 4 | 35 ± 1 | 2.31 ± 0.09 | 1.52 ± 0.06 |
| 12 | 23.6 ± 0.1 | 2347 ± 13 | 7.66 ± 0.03 | 1134 ± 87 | 2104 ± 4 | 99 ± 7 | 33 ± 3 | 2.39 ± 0.17 | 1.57 ± 0.11 |
| 13 (t14) | 24.2 ± 0.1 | 2347 ± 13 | 7.65 ± 0.03 | 1163 ± 91 | 2104 ± 25 | 99 ± 6 | 34 ± 3 | 2.39 ± 0.13 | 1.57 ± 0.09 |

Seawater carbonate chemistry

Sixty mL of seawater were collected twice a week from each aquarium, filtered at $0.45 \mu\text{m}$ (GF/F Whatman) and stored in the dark at 4°C to avoid biological alteration. The pH was measured at 0.1 mL increments of 0.01 N HCl at 25°C using a Metrohm titration system (848 Titrino Plus). For each sample, three 20 mL sub-samples were analyzed. Total alkalinity (AT) was calculated from the Gran function applied to pH variations from 4.2 to 3.0 as mEq L^{-1} from the slope of the HCl volume *versus* pH curve. Data were corrected by titrations of AT standards provided by A.G. Dickson. For each experimental treatment, the parameters of the carbonate system [$p\text{CO}_2$, CO_3^{2-} , HCO_3^{3-} , and saturation state of aragonite (Ω_{arag})] were calculated from pH_T , mean AT , temperature and mean salinity (35) using the free-access CO2SYS package (Pierrot and Wallace 2006).

Table S2. *Lobophora rosacea* ions responsible for the discrimination between low pH (Bouraké) and control pH (Ricaudy) conditions. RT = retention time (s). The mSigma (mS) value is a measure for the goodness of fit between experimental mass and isotopic pattern with theoretical ones: lower is the mS, better is the annotation

| name | m/z | RT | err ppm | mS | ion | ion formula |
|-----------------------------|-----------|-----|---------|-------|-----------------------------------|--|
| <i>low pH - Bouraké</i> | | | | | | |
| M284T395 | 284.1857 | 395 | 3.4 | 8.4 | [M+H] ⁺ | C ₁₅ H ₂₆ NO ₄ |
| M305T395 | 305.1148 | 395 | -4.5 | 57.5 | [M+H] ⁺ | C ₁₅ H ₁₇ N ₂ O ₅ |
| M344T543 | 344.3157 | 543 | -2.9 | 17.6 | [M+NH ₄] ⁺ | C ₂₀ H ₄₂ NO ₃ |
| M378T646 | 378.3576 | 646 | - | - | - | - |
| M496T514 | 496.2906 | 514 | 2.8 | 20.5 | [M+H] ⁺ | C ₂₆ H ₄₂ NO ₈ |
| M505T476 | 505.2539 | 476 | - | - | - | - |
| M536T527 | 536.3797 | 527 | 1 | 6.8 | [M+H] ⁺ | C ₂₇ H ₅₄ NO ₉ |
| M555T641 | 555.466 | 641 | - | - | - | - |
| M563T492 | 563.3186 | 492 | - | - | - | - |
| M579T479 | 579.2812 | 479 | - | - | - | - |
| M589T504 | 589.24459 | 504 | - | - | - | - |
| M598T638 | 598.4889 | 638 | - | - | - | - |
| M599T475 | 599.3179 | 475 | - | - | - | - |
| M600T488 | 600.34155 | 488 | - | - | - | - |
| M605T566 | 605.2397 | 566 | -0.3 | 55.2 | [M+H] ⁺ | C ₃₅ H ₃₃ N ₄ O ₆ |
| M644T635 | 643.5192 | 635 | - | - | - | - |
| <i>control pH - Ricaudy</i> | | | | | | |
| M119T599 | 119.0855 | 599 | - | - | - | - |
| M299T504 | 299.2367 | 504 | -2.7 | 28.5 | [M+H] ⁺ | C ₂₁ H ₃₁ O |
| M315T455 | 315.2319 | 455 | -0.1 | 24.6 | [M+H] ⁺ | C ₂₁ H ₃₁ O ₂ |
| M316T583 | 316.2634 | 583 | 1.5 | 7.6 | [M+NH ₄] ⁺ | C ₂₁ H ₃₄ NO |
| M317T456 | 317.2474 | 456 | 0.2 | 5.9 | [M+H] ⁺ | C ₂₁ H ₃₃ O ₂ |
| M318T600 | 318.2786 | 600 | -0.9 | 13.8 | [M+NH ₄] ⁺ | C ₂₁ H ₃₆ NO |
| M334T515 | 334.274 | 515 | 0 | 6.3 | [M+NH ₄] ⁺ | C ₂₁ H ₃₆ NO ₂ (lobophorenol B) |
| M335T506 | 335.1971 | 506 | 6.1 | 37.5 | [M+Na] ⁺ | C ₂₁ H ₂₈ NaO ₂ |
| M336T524 | 336.2896 | 524 | 0.4 | 14 | [M+NH ₄] ⁺ | C ₂₁ H ₃₈ NO ₂ (lobophorenol C) |
| M340T566 | 340.2634 | 566 | -0.8 | 31.9 | [M+NH ₄] ⁺ | C ₂₃ H ₃₄ NO |
| M343T551 | 343.2606 | 551 | 6.9 | 60.1 | [M+H] ⁺ | C ₂₃ H ₃₅ O ₂ |
| M345T499 | 345.2422 | 499 | -1 | 35.5 | [M+H] ⁺ | C ₂₂ H ₃₃ O ₃ |
| M345T495 | 345.2436 | 495 | -0.6 | 11.5 | [M+H] ⁺ | C ₂₂ H ₃₃ O ₃ |
| M353T457 | 353.2086 | 457 | 0.1 | 56 | [M+Na] ⁺ | C ₂₁ H ₃₀ NaO ₃ |
| M355T463 | 355.2242 | 463 | 2.2 | 171.7 | [M+Na] ⁺ | C ₂₁ H ₃₂ NaO ₃ |
| M361T478 | 361.2373 | 478 | -1.7 | 59.8 | [M+H] ⁺ | C ₂₂ H ₃₃ O ₄ |
| M361T450_2 | 361.2557 | 450 | -8.2 | 20.6 | [M+H] ⁺ | C ₂₆ H ₃₃ O |
| M363T470 | 363.253 | 470 | 2.2 | 21.1 | [M+H] ⁺ | C ₂₂ H ₃₅ O ₄ |
| M368T484 | 368.2347 | 484 | -4.4 | 249.1 | [M+H] ⁺ | C ₂₂ H ₃₀ N ₃ O ₂ |
| M373T485 | 373.1904 | 485 | - | - | - | - |
| M373T456 | 373.2142 | 456 | - | - | - | - |
| M409T437 | 409.2116 | 437 | 3 | 133.8 | [M+H] ⁺ | C ₂₄ H ₂₉ N ₂ O ₄ |

| | | | | | | |
|----------|----------|-----|-----|------|-----------------------------------|---|
| M413T449 | 413.183 | 449 | - | - | - | - |
| M438T478 | 438.285 | 478 | 0.1 | 16 | [M+NH ₄] ⁺ | C ₂₄ H ₄₀ NO ₆ |
| M459T477 | 459.2119 | 477 | 0.1 | 16 | [M+H] ⁺ | C ₂₉ H ₃₁ O ₅ |
| M496T464 | 496.3632 | 464 | 1.6 | 44.9 | [M+H] ⁺ | C ₂₈ H ₅₀ NO ₆ |
| M516T368 | 516.3169 | 368 | - | - | - | - |
| M520T458 | 520.3635 | 458 | 2.2 | 37.2 | [M+H] ⁺ | C ₃₀ H ₅₀ NO ₆ |
| M526T491 | 526.293 | 491 | 2.4 | 81.9 | [M+H] ⁺ | C ₃₄ H ₄₀ NO ₄ |
| M528T490 | 528.3543 | 490 | - | - | - | - |
| M536T404 | 536.3714 | 404 | - | - | - | - |
| M550T593 | 550.4315 | 593 | - | - | - | - |
| M578T788 | 578.4769 | 788 | - | - | - | - |
| M596T496 | 596.3308 | 496 | - | - | - | - |
| M602T506 | 602.4615 | 506 | - | - | - | - |
| M625T557 | 625.2655 | 557 | 0.7 | 62.7 | [M+H] ⁺ | C ₃₅ H ₃₇ N ₄ O ₇ |
| M815T705 | 814.5459 | 705 | - | - | - | - |

Table S3. *Lobophora rosacea* ions responsible for the discrimination between low pH and control pH conditions, at the beginning (t0) and the end (t14) of the aquaria experiment. RT = retention time (s). The mSigma (mS) value is a measure for the goodness of fit between experimental mass and isotopic pattern with theoretical ones: lower is the mS, better is the annotation

| name | m/z | RT | err ppm | mS | ion | ion formula |
|--------------------------------|----------|-----|---------|-------|-----------------------------------|---|
| <i>t0 samples - low pH</i> | | | | | | |
| M401T324 | 401.262 | 324 | - | - | - | - |
| M430T455 | 430.2023 | 455 | -1.7 | 53.5 | [M+H] ⁺ | C ₂₇ H ₂₈ NO ₄ |
| M432T461 | 432.2178 | 461 | | | | |
| M467T329 | 467.3016 | 329 | - | - | - | - |
| M511T332 | 511.3277 | 332 | - | - | - | - |
| <i>t0 samples - control pH</i> | | | | | | |
| M318T600 | 318.2786 | 600 | -0.9 | 13.8 | [M+NH ₄] ⁺ | C ₂₁ H ₃₆ NO |
| M331T516 | 331.2089 | 516 | - | - | - | - |
| M336T524 | 336.2896 | 524 | 0.4 | 14 | [M+NH ₄] ⁺ | C ₂₁ H ₃₈ NO ₂ (lobophorenol C) |
| M345T499 | 345.2422 | 499 | -1 | 35.5 | [M+H] ⁺ | C ₂₂ H ₃₃ O ₃ |
| M363T470 | 363.253 | 470 | 2.2 | 21.1 | [M+H] ⁺ | C ₂₂ H ₃₅ O ₄ |
| M438T478 | 438.285 | 478 | 0.1 | 16 | [M+NH ₄] ⁺ | C ₂₄ H ₄₀ NO ₆ |
| M459T477 | 459.2119 | 477 | 0.1 | 16 | [M+H] ⁺ | C ₂₉ H ₃₁ O ₅ |
| <i>t14 samples - low pH</i> | | | | | | |
| M288T454 | 288.2533 | 454 | 0.2 | n.a | [M+NH ₄] ⁺ | C ₁₆ H ₃₄ NO ₃ |
| M316T496 | 316.2846 | 496 | -0.2 | 40.6 | [M+NH ₄] ⁺ | C ₁₈ H ₃₈ NO ₃ |
| M344T543 | 344.3157 | 543 | -2.9 | 17.6 | [M+NH ₄] ⁺ | C ₂₀ H ₄₂ NO ₃ |
| M438T574 | 438.3803 | 574 | 3.4 | 40.1 | [M+H] ⁺ | C ₂₄ H ₄₈ N ₅ O ₂ |
| M510T644 | 510.4366 | 644 | -1.6 | 125.6 | [M+H] ⁺ | C ₂₈ H ₅₆ N ₅ O ₃ |
| M527T570 | 527.435 | 570 | - | - | - | - |
| M555T641 | 555.466 | 641 | - | - | - | - |
| M598T638 | 598.4889 | 638 | - | - | - | - |
| M644T635 | 643.5192 | 635 | - | - | - | - |
| M659T564 | 658.5103 | 564 | - | - | - | - |