Supporting information

**Insight into unprecedented diversity of cyanopeptides in eutrophic ponds using a MS/MS networking approach**



**Figure S1**. Phytoplankton composition of studied ponds throughout the sampling season. Biomass of phytoplankton among different sampling location **A**) KL **B)** DH, **C)** KV; **D**) biomass of cyanobacterial species; **E**) cyanobacterial relative species composition; KL:Klec, DH: Dehtář, KV: Kvítkovický.

**Table S1**. Biomass of cyanobacterial species and phytoplankton classes in all three studied ponds during the sampling season. KL:Klec, DH: Dehtář, KV: Kvítkovický.

|  |  |  |  |
| --- | --- | --- | --- |
| **Cyanobacteria** | **KL** | **DH** | **KV** |
| **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** | **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** | **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** |
| *Dolichospermum circinale* | 0.23 |  | 0.44 | 8.48 |  | 1.32 |  |  | 1.25 | 2.32 |  |  |  |  |  | 1.23 | 12.79 | 0.49 |
| *Dolichospermum viguieri* | 0.14 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Dolichospermum planctonicum* |  |  |  | 9.05 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Dolichospermum spiroides* |  |  |  | 4.46 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Dolichospermum flos-aquae* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1.35 |  |
| *Dolichospermum compactum* |  |  |  |  | 1.08 |  |  |  |  |  | 1.04 |  |  |  |  |  | 2.14 |  |
| *Dolichospermum crassum* |  |  |  |  |  | 6.07 |  |  |  |  |  |  |  |  |  |  |  |  |
| *Microcystis aeruginosa* | 0.49 |  | 2.04 | 5.45 | 33.40 | 30.12 |  | 1.90 |  |  | 0.10 | 0.10 |  |  |  | 0.08 | 0.28 | 1.49 |
| *Aphanizomenon flos-aquae* | 0.31 |  | 0.53 | 3.65 | 8.06 | 3.96 |  |  | 3.32 | 5.42 | 26.63 | 8.37 |  |  |  | 1.24 | 7.23 | 0.46 |
| *Cuspidothrix issatschenkoi* | 0.88 |  |  | 1.00 | 4.82 | 1.70 |  |  | 0.50 | 0.10 | 0.95 | 2.98 |  |  | 0.13 |  |  |  |
| *Raphidiopsis raciborskii* |  |  |  |  | 3.38 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Limnothrix redekei* |  |  |  | 0.17 | 0.19 |  |  |  |  | 0.15 |  | 0.16 |  |  |  |  |  |  |
| *Planktothrix agardhii* | 0.15 |  |  |  |  |  |  |  |  | 1.36 | 0.24 | 9.49 |  |  |  |  |  |  |
| *Raphidiopsis* sp. |  |  |  |  |  | 0.19 |  |  |  |  |  |  |  |  |  |  |  |  |
| *Planktolyngbya limnetica* |  |  | 0.02 | 0.10 | 0.18 | 0.13 | 0.07 |  |  | 0.12 |  |  |  |  | 0.01 |  |  |  |
| *Anabaenopsis elenkinii* |  |  |  |  | 2.81 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Pseudanabaena* sp. |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 0.11 |  |
| *Aphanizomenon yezoense* |  |  |  |  |  |  |  |  |  | 1.17 |  |  |  |  |  |  |  |  |
| *Woronichinia naegeliana* |  |  | 2.57 | 6.09 |  |  |  |  | 0.91 | 0.91 |  |  |  |  |  |  | 1.43 |  |
| *Aphanocapsa delicatissima* | 0.04 | 0.01 | 0.61 | 0.42 | 0.96 |  | 0.03 | 0.01 |  | 0.12 |  |  | 0.10 | 0.05 | 0.10 |  |  | 0.06 |
| *Anathece minutissima* | 0.01 |  | 0.38 | 0.94 | 3.55 | 1.84 | 0.06 |  | 0.06 | 0.42 | 0.27 | 0.01 | 0.30 |  | 0.40 | 0.14 |  | 0.02 |
| *Limnococcus limneticus* |  |  | 0.00 | 12.77 | 0.70 |  |  |  | 0.13 | 0.14 | 0.26 |  | 0.32 |  | 0.39 | 0.81 |  | 0.17 |
| *Cyanodictyon imperfectum* | 0.003 | 0.001 | 0.002 |  |  |  |  | 0.001 | 0.001 | 0.001 |  |  | 0.006 |  | 0.004 |  |  |  |
| Picocyanobacteria | 0.08 | 0.01 | 0.03 | 0.05 | 0.26 | 0.04 | 0.20 | 0.32 | 0.11 | 0.39 | 0.05 | 0.14 | 1.04 | 0.26 | 0.97 | 0.06 | 0.17 | 0.04 |
| *Merismopedia punctata* | 0.08 |  | 0.55 | 0.25 | 0.69 | 0.31 | 0.07 | 0.04 |  | 0.03 | 0.04 |  | 0.83 | 0.10 | 0.01 | 0.01 |  | 0.31 |
| *Romeria elegans* |  |  |  | 0.14 | 0.22 | 0.18 | 0.01 |  |  |  |  |  |  |  |  |  | 0.02 |  |
| *Snowella litoralis* | 0.02 | 0.01 | 0.24 | 0.11 |  | 0.48 |  | 0.05 |  |  |  | 0.08 |  | 0.08 |  |  |  |  |
| *Synechococcus* sp. |  |  |  |  |  |  |  |  | 0.01 |  |  |  | 0.16 |  |  |  |  |  |
| *Synechocystis aquatilis* |  |  |  |  |  |  |  | 0.46 |  |  |  |  |  |  |  | 0.33 |  |  |
| **Total cyanobacterial biomass** | 2.43 | 0.03 | 7.40 | 53.11 | 60.30 | 46.33 | 0.43 | 2.79 | 6.28 | 12.65 | 29.57 | 21.33 | 2.77 | 0.49 | 2.01 | 3.90 | 25.51 | 3.06 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Phytoplankton classes** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Chlorophyceae | 10.80 | 13.18 | 22.37 | 45.59 | 32.99 | 20.62 | 38.00 | 43.15 | 8.69 | 9.91 | 8.26 | 7.56 | 41.81 | 62.25 | 31.14 | 7.58 | 5.14 | 12.32 |
| Cyanophyceae | 2.43 | 0.03 | 7.40 | 53.11 | 60.30 | 46.33 | 0.43 | 2.79 | 6.28 | 12.65 | 29.57 | 21.33 | 2.77 | 0.49 | 2.01 | 3.90 | 25.51 | 3.06 |
| Cryptophyceae | 0.10 | 1.70 |  | 1.17 | 0.51 | 0.87 | 6.34 | 0.13 | 1.43 | 1.33 | 2.05 | 0.99 | 1.88 | 3.56 | 1.34 | 1.39 |  | 1.16 |
| Bacillariophyceae | 0.80 | 0.92 | 7.37 | 3.40 | 6.47 | 0.04 | 0.60 | 0.16 | 0.43 | 1.40 |  | 28.71 | 0.30 | 2.34 | 14.59 | 29.14 | 14.91 | 23.27 |
| Euglenopyceae | 0.06 |  | 1.69 |  |  |  | 2.01 |  | 6.06 | 1.90 |  | 2.47 |  |  | 0.38 |  |  | 0.26 |
| Dinophyceae |  |  |  | 1.14 |  |  |  |  |  |  |  | 0.32 |  |  | 4.21 | 0.40 |  | 4.38 |
| Zygnematophyceae | 0.44 |  | 0.38 |  |  |  |  |  |  |  |  |  | 2.29 |  |  |  |  |  |
| **Total phytoplankton biomass** | 14.18 | 15.83 | 38.83 | 104.41 | 100.27 | 67.87 | 47.38 | 46.24 | 22.90 | 27.18 | 39.88 | 61.37 | 46.76 | 68.64 | 53.66 | 42.40 | 45.57 | 44.44 |
| % cyanobacteria | 17.10 | 0.22 | 19.07 | 50.87 | 60.13 | 68.27 | 0.91 | 6.04 | 27.44 | 46.52 | 74.15 | 34.76 | 5.92 | 0.72 | 3.75 | 9.19 | 55.99 | 6.88 |

**Table 2.** Detected cyanopeptides (CNPs) in all three studied ponds during the sampling season. KL:Klec, DH: Dehtář, KV: Kvítkovický.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  **CNPs** | **KL** | **DH** | **KV** |  | **Peptide****Mass** (*m/z*) | **Observed Mass** (*m/z*) | **Adduct** |  **Scans** |
| **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** | **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** | **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** | **Total**  |
| APT-908 |  |  |  |  |   |   |  |  |  |  |  |   |  |  |  |  |  |   | 3 | 908.51 | 909.52 | M+H | 252, 329, 270 |
| APT-915 |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 915.47 | 916.49 | M+H | 878 |
| APT-der |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 2 | 929.49 | 930.50 | M+H | 884, 863 |
| APT-I |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 759.45 | 760.46 | M+H | 892 |
| APT-J |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 793.44 | 794.45 | M+H | 898 |
| APT-NZ841 |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 2 | 841.44 | 842.45 | M+H | 582, 580, 408 |
| APT-T |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 2 | 865.50 | 866.51 | M+H | 342, 458, 454 |
| APT-A |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 9 | 843.42 | 844.43 | M+H | 837,846,827 |
| APT-B |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 9 | 836.45 | 837.47 | M+H | 605,604, 603 |
| APT-C |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 808.45 | 809.46 | M+H | 598 |
| APT-F |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 7 | 850.47 | 851.48 | M+H | 652, 639, 636 |
| APT-G |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 3 | 908.51 | 455.27 | M+2H | 641, 658, 674 |
| APT-H |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 2 | 922.53 | 462.27 | M+2H | 697, 664 |
| Oscillamide-Y |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 10 | 857.43 | 858.44 | M+H | 865, 861, 855 |
| [D-Asp3]MC-RR |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 3 | 1023.55 | 512.79 | M+2H | 634, 629, 641 |
| [Dha7]MC-RR |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 4 | 1023.55 | 512.78 | M+2H | 643, 630, 637 |
| [Dha7]MC-LR |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 980.53 | 981.48 | M+H | 822 |
| [DMAdda5]MC-LR |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 980.53 | 981.55 | M+H | 736 |
| MC-LR |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 12 | 994.55 | 995.56 | M+H | 810, 808, 819  |
| MC-RR |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 15 | 1037.57 | 519.79 | M+2H | 641, 667, 206 |
| MC-FR |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 1028.53 | 1029.55 | M+H | 839 |
| MC-WR |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 1067.54 | 1068.56 | M+H | 832 |
| MC-YR |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 8 | 1044.53 | 1045.54 | M+H | 781, 778, 783 |
| [Dhb7]MC-LR |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 | 1008.56 | 1009.58 | M+H | 844 |
| CPT-972 |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 2 | 972.53 | 973.54 | M+H | 700, 760 |
| MPT-MZ925 |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 925.42 | 926.43 | M+H | 856 |
| MPT-SD944 |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 944.52 | 945.53 | M+H | 706 |
| MPT-A |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 3 | 986.57 | 987.58 | M+H | 796, 772, 778 |
| MPT-B |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 2 | 958.54 | 959.55 | M+H | 696, 700 |
| MGN |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 5 | 713.40 | 714.41 | M+H | 694, 700, 703 |
| MGN-478 |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 9 | 769.46 | 770.48 | M+H | 748, 737, 365 |
| MGN-GH787 |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 2 | 787.39 | 788.40 | M+H | 696, 697 |
| MGN-SD755 |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 755.45 | 756.46 | M+H | 742 |
| Cya-B |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 9 | 753.43 | 754.44 | M+H | 717, 710, 718 |
| Nsg-BN741 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 2 | 741.43 | 742.44 | M+H |   |
| Aeruginosamide |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 9 | 560.34 | 561.35 | M+H | 719, 752, 730 |
| Kasumigamide |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 2 | 786.37 | 787.38 | M+H | 633, 645 |
| Planktocyclin |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 1 | 800.43 | 801.44 | M+H | 952 |
| RdsB |  |  |  |  |  |   |  |  |  |  |  |   |  |  |  |  |  |   | 3 | 444.24 | 445.25 | M+H | 185, 199, 156 |
| epidolastatin 12 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 1 | 968.60 | 485.29 | M+2H | 773 |
| **Total CNPs per month** | 10 | 1 | 8 | 20 | 12 | 9 | 2 | 2 | 13 | 10 | 13 | 17 | 0 | 0 | 4 | 7 | 12 | 13 | 99 |  |  |  |   |
| **Total CNPs per pond** |   |   |   |   |   | 60 |   |   |   |   |   | 57 |   |   |   |   |   | 36 |   |   |   |   |   |

Note: APTs: anabaenopeptins, MCs: microcystins, CPTs: cyanopeptolins, MGNs: microginins, and RdsB: radiosumin\_B.