Cyanobacteria and their secondary metabolites in three freshwater reservoirs in the United Kingdom

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**Figure S9. Fragmentation spectrum of Cyanopeptolin CP992** at HCD 15, 30, 45% stepped normalised collision energy. Precursor m/z, retention time (RT) and the building block string are noted at the top. The flat structure is shown with annotated building blocks and sites of fragmentation. The table specifies the m/z value and building block fragment that supports the identification of this compound.

**Figure S10. Fragmentation spectrum of Anabaenopeptilide 202A** at HCD 15, 30, 45% stepped normalised collision energy. Precursor m/z, retention time (RT) and the building block string are noted at the top. The flat structure is shown with annotated building blocks and sites of fragmentation. The table specifies the m/z value and building block fragment that supports the identification of this compound.

**Figure S11. Fragmentation spectrum of MC-FL** at HCD 15, 30, 45% stepped normalised collision energy. Precursor m/z, retention time (RT) and the building block string are noted at the top. The flat structure is shown with annotated building blocks and sites of fragmentation. The table specifies the m/z value and building block fragment that supports the identification of this compound.
Text S1. Enumeration of cells using the utermohl inverted microscope technique

For biological analyses, 1 L water samples were kept at 4-8°C in the dark after sampling. Taxonomic analysis by microscopy was performed on the day following sampling. Samples were fixed with Lugol’s Iodine (10-20 ml per liter sample). Then the samples were pressurized and transferred to a sedimentation tube. After setting, cells in the sample are directly identified and counted applying an inverted microscope.

Text S2. Enumeration of chlorophyll-a

For chlorophyll-a analysis, samples were filtered and extracted with solvent followed by spectrophotometric measurement according to a published method: Book section A8: The determination of chlorophyll ‘a’ in plant material (phytoplankton), in suspension in water (solvent extraction method), in the book: Aquatic Environments 1980 – Methods for the Examination of Waters and Associated Materials. SBN 0117516740. Briefly, after filtration step using paper filters, pigments were extracted with 14 mL methanol in a glass test tube. The solution was heated until boiling for 10 seconds. After cooling down to room temperature in the darkness, the filter paper was removed and the extract was centrifuged. The chlorophyll-a concentration was determined by spectrophotometric evaluation of the supernatant. Absorbance measurements were carried out at two wavelengths: 665 nm (the maximum absorption of chlorophyll-a), and 750 nm (compensation for ‘background turbidity’). The chlorophyll-a content (µg/L) of samples was then quantified as

\[
\frac{13.9 \cdot A \cdot v}{d \cdot V}
\]

where \(A\) is a subtraction of absorbance value obtained at 750 nm from the one obtained at 665 nm; \(v\) is the volume of the solvent in mL; \(d\) is the cell pathlength in cm; \(V\) is the volume if the initial filtered sample in liters (see A8.24b in the noted reference). The factor 13.9 is used for approximation to the reciprocal of the specific absorption coefficient at 665 nm for chlorophyll-a in methanol.
Text S3. Measurement of total ammonium, nitrate and phosphate

Total ammonium was measured according to HMSO Methods for the Examination of Waters and Associated Materials, "Ammonia in Waters 1981" (ISBN: 011 7516139). Its concentration was determined spectrophotometrically. Ammonia in the raw drinking water reacted with hypochlorite ions, which were generated from the sodium dichlorocyanurate reagent to form monochloramine. This reacted with salicylate at pH around 10.5 with presence of sodium nitroprusside formed a blue iodophenol-type compound. This was measured at 660 nm. Range of application was between 0.06 and 2 mg/L, with limit of detection (LOD) at 0.0503 mg/L.

Nitrate was measured according to HMSO Methods for the Examination of Waters and Associated Materials, "Oxidised Nitrogen in Waters, 1981" (ISBN: 011 7515930). Its concentration was determined spectrophotometrically. Nitrate ions were reduced to nitrite by hydrazine under alkaline conditions, cupric ions were used as a catalyst. Under acidic conditions, the total nitrite content of the sample was then reacted with sulphanilamide and N-1-Naphthylenediamine dihydrochloride and formed a characteristic pink dye which was read at 540 nm. Range of application was between 0.2 and 20 mg/L, with LOD at 0.1019 mg/L.

Total phosphate was measured according to HMSO Methods for the Examination of Waters and Associated Materials, "Phosphorus in Waters, Effluents and Sewage 1980" (ISBN: 011 7515825). Its concentration was determined spectrophotometrically. Phosphate ions reacted with a solution that contained molybdic acid, trivalent antimony ions and hydrogen ions for formation of a 12-Molybdophosphoric acid. This was reduced by ascorbic acid and gave a phosphomolybdenum blue complex which was measured at 660 nm. Range of application was between 0.02 and 2 mg/L, with LOD at 0.0091 mg/L.
Table S1. **Standard analytical information** including: limit of detection (LOD) and limit of quantification (LOQ) in μg/L for the reference standards and bioreagents in nanopure water and lake water from three reservoirs.

| Cyanobacterial metabolite | molecular formula | dominant precursor | nanopure LOD (µg/L) | ingibirchworth LOD (µg/L) | tophill low LOD (µg/L) | embsay LOD (µg/L) | ingibirchworth LOQ (µg/L) | tophill low LOQ (µg/L) | embsay LOQ (µg/L) |
|---------------------------|-------------------|-------------------|---------------------|--------------------------|------------------------|---------------------|--------------------------|------------------------|---------------------|
| MC-LR | C₄₉H₇₂N₁₀O₁₂ | [M+H]^+ | 0.23 | 0.71 | 1.32 | 3.99 | 2.92 | 8.84 | 0.74 | 2.26 |
| MC-RR | C₄₉H₇₂N₁₃O₁₂ | [M+2H]²⁺ | 0.31 | 0.94 | 1.40 | 4.24 | 1.45 | 4.38 | 0.58 | 1.76 |
| MC-YR | C₅₂H₇₂N₁₀O₁₃ | [M+2H]²⁺ | 0.23 | 0.71 | 1.31 | 3.98 | 0.95 | 2.80 | 1.64 | 4.97 |
| MC-LA | C₄₆H₆₇N₁₂O₁₂ | [M+H]^+ | 0.30 | 0.89 | 1.26 | 3.82 | 2.02 | 6.13 | 1.11 | 3.38 |
| MC-LF | C₅₂H₇₁N₁₂O₁₂ | [M+H]^+ | 0.27 | 0.83 | 1.66 | 5.04 | 1.44 | 4.35 | 1.74 | 5.28 |
| MC-LY | C₅₂H₇₁N₁₃O₁₃ | [M+H]^+ | 0.24 | 0.72 | 1.40 | 4.23 | 1.68 | 5.08 | 1.97 | 5.97 |
| MC-LW | C₅₄H₇₂N₈O₁₂ | [M+H]^+ | 0.27 | 0.83 | 1.76 | 5.34 | 1.37 | 4.16 | 1.43 | 4.33 |
| MC-HiIlR | C₅₀H₇₆N₁₀O₁₂ | [M+H]^+ | 0.24 | 0.71 | 0.88 | 2.66 | 1.82 | 5.50 | 0.93 | 2.83 |
| [D-Asp³]MC-LR | C₄₈H₇₂N₁₀O₁₂ | [M+H]^+ | 0.27 | 0.81 | 0.70 | 2.11 | 1.09 | 3.31 | 0.62 | 1.89 |
| MC-RR 1024 Group[^a] | C₄₈H₇₂N₁₃O₁₂ | [M+2H]²⁺ | 0.05 | 0.15 | 1.25 | 3.80 | 0.67 | 2.02 | n.a. | n.a. |
| Nodularin | C₄₁H₆₀N₈O₁₀ | [M+H]^+ | 0.25 | 0.76 | 1.06 | 3.23 | 2.09 | 6.34 | 0.47 | 1.43 |
| Anabaenopeptin A | C₄₄H₅₇N₉O₁₀ | [M+H]^+ | 0.25 | 0.75 | 11.96 | 36.26 | 1.63 | 4.94 | 1.06 | 3.21 |
| Anabaenopeptin B | C₄₁H₅₀N₁₀O₁₀ | [M+H]^+ | 0.29 | 0.88 | 4.43 | 13.43 | 1.21 | 3.67 | 0.56 | 1.70 |
| Oscillamide Y | C₄₅H₅₉N₁₀O₁₀ | [M+H]^+ | 0.27 | 0.81 | 3.31 | 10.02 | 1.76 | 5.34 | 1.80 | 5.47 |
| Cyanopeptolin A | C₄₆H₇₂N₁₀O₁₂ | [M+H]^+ | 1.01 | 3.06 | 2.18 | 6.62 | 1.66 | 5.04 | 2.35 | 7.11 |
| Aerucyclamide A | C₂₄H₃₄N₂O₂S₂ | [M+H]^+ | 0.24 | 0.74 | 2.17 | 6.58 | 0.92 | 2.78 | 3.19 | 9.66 |
| Aeruginosin 98B | C₂₉H₄₆N₁₀O₆S | [M+H]^+ | 0.35 | 1.05 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Anatoxin-a | C₁₀H₁₅NO | [M+H]^+ | 0.08 | 0.23 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Cylindrospermopsin | C₁₅H₂₁₂₅₁₉₂O₅ | [M+H]^+ | 0.02 | 0.05 | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |

n.a. = not analysed

[^a] Microcystin-RR isomeric group 1024 includes: [Dha⁺]MC-RR, [Gly⁺]D-Asp⁺Dhb⁻]MC-Rhar, [DAmda⁺]MC-RR, [D-Asp⁺]MC-RR, [D-Asp⁺,(E)-Dhb⁻]MC-RR
Table S2. Cyanopeptides detected in lake samples and the respective level of confidence in identification, as well as the reference standard or bioreagent used for quantification by class equivalent.

| cyanopeptide                   | molecular formula | dominant precursor | m/z of precursor | quantification equivalent | identification level |
|-------------------------------|-------------------|-------------------|-----------------|---------------------------|----------------------|
| MC-LR                         | C₄₀H₇₄N₁₈O₁₂      | [M+H]⁺             | 995.56          | standard available         | 1                    |
| [D-Asp³]MC-LR                 | C₄₈H₇₂N₁₈O₁₂      | [M+H]⁺             | 981.15          | standard available         | 1                    |
| MC-RR                         | C₄₀H₇₅N₁₃O₁₂      | [M+2H]²⁺           | 519.79          | standard available         | 1                    |
| MC-RR 1024 Group             | C₄₈H₇₅N₁₃O₁₂      | [M+2H]²⁺           | 512.78          | [D-Asp³,(E)-Dhb]MC-RR      | 3                    |
| MC-HiLR                       | C₅₀H₇₆N₁₀O₁₂      | [M+H]⁺             | 1009.57         | standard available         | 1                    |
| MC-VF                         | C₅₁H₆₉N₇O₁₂       | [M+H]⁺             | 972.51          | MC-LF                     | 3                    |
| MC-FL                         | C₅₂H₇₁N₇O₁₂       | [M+H]⁺             | 986.52          | MC-LF                     | 3                    |
| [D-Asp³,(E)-Dhb]MC-HtyHty     | C₅₆H₇₁N₇O₁₄      | [M+H]⁺             | 1066.51         | MC-YR                     | 3                    |
| Anabaenopeptin A              | C₄₄H₅₇N₇O₁₀       | [M+H]⁺             | 837.46          | bioreagent available      | 1                    |
| Anabaenopeptin B              | C₄₁H₅₆N₁₀O₉       | [M+H]⁺             | 844.42          | bioreagent available      | 1                    |
| Oscillamide Y                 | C₴₅H₅₉N₇O₁₀       | [M+H]⁺             | 858.44          | bioreagent available      | 1                    |
| Anabaenopeptide 202A          | C₅₁H₇₁N₉O₁₅      | [M+H]⁺             | 1050.51         | Cyanopeptolin A           | 3                    |
| Anabaenopeptin D              | C₄₄H₅₇N₇O₉       | [M+H]⁺             | 828.43          | Anabaenopeptin A          | 3                    |
| Anabaenopeptin NZ841          | C₄₅H₅₉N₇O₀       | [M+H]⁺             | 842.44          | Anabaenopeptin A          | 3                    |
| Anatoxin-a                    | C₁₀H₃₅NO           | [M+H]⁺             | 166.12          | standard available        | 1                    |
| Aeruginosin NOL1              | C₂₆H₄₆N₆O₆       | [M+H]⁺             | 533.31          | Aeruginosin 98B           | 3                    |
| Aeruginosin 850               | C₄₁H₆₆N₆O₁₃      | [M+H]⁺             | 851.48          | Aeruginosin 98B           | 3                    |
| Aeruginosin 822               | C₃₉H₅₂N₆O₁₃      | [M+H]⁺             | 823.44          | Aeruginosin 98B           | 3                    |
| Aeruginosin 98A               | C₂₆H₄₅ClN₆O₆S    | [M+H]⁺             | 689.27          | Aeruginosin 98B           | 3                    |
| Cyanopeptolin CP992           | C₄₀H₆₈N₀O₁₃      | [M+H]⁺             | 992.51          | Cyanopeptolin A           | 3                    |
| Microginin 767                | C₄₁H₆₁N₉O₉       | [M+H]⁺             | 768.45          | MC-LR                     | 3                    |
| Microginin KR604               | C₃₂H₅₂N₆O₇      | [M+H]⁺             | 605.39          | MC-LR                     | 3                    |
| Aeruginosamide                | C₃₀H₄₈N₄O₄S      | [M+H]⁺             | 561.35          | MC-LR                     | 3                    |
| Bacteriophanetetrol            | C₄₁H₇₃NO₈        | [M+H]⁺             | 708.54          | MC-LR                     | 3                    |
| Veraguamide G                 | C₃₇H₆₂N₆O₈       | [M+NH₄]⁺           | 708.49          | MC-LR                     | 3                    |
| Micropeptin LH1062             | C₅₃H₇₈N₁₀O₁₃     | [M+H]⁺             | 1063.58         | MC-LR                     | 3                    |
| Compound     | Formula       | [M+H]$^+$    | MW (Da) | MC-LR | Isomer Group |
|--------------|---------------|--------------|---------|-------|--------------|
| Nostosin B   | C$_{22}$H$_{37}$N$_5$O$_5$ | [M+H]$^+$   | 452.29  | MC-LR | 3            |
| Almiramide G | C$_{36}$H$_{64}$N$_6$O$_6$ | [M+H]$^+$   | 677.50  | MC-LR | 3            |

*Microcystin-RR isomeric group 1024 includes: [Dha$^7$]MC-RR, [Gly$_1$D-Asp$_3$Dhb$_7$]MC-Rhar, [DMAdda$_5$]MC-RR, [D-Asp$_3$]MC-RR, [D-Asp$_3$,(E)-Dhb$_7$]MC-RR
Table S3. Chlorophyll-a, total ammonium, nitrate, total phosphate, and temperature measured in Ingbirchworth reservoir samples in 2019.

| Date       | Chlorophyll-a, µg/l | Ammonium total, mg/l | Nitrate, mg/l | Phosphate total, mg/l | Temperature, °C |
|------------|---------------------|----------------------|---------------|----------------------|-----------------|
| 02.01.2019 | 12                  | 0.03                 | 4.0           | 0.03                 | 3.7             |
| 10.01.2019 | 7                   | 0.03                 | 2.6           |                      |                 |
| 18.01.2019 | 13                  | 0.026                | 4.40          | 0.03                 | 2.5             |
| 21.01.2019 | 15                  | 0.03                 | 1.7           |                      |                 |
| 29.01.2019 | 19                  | 0.03                 | 0.9           |                      |                 |
| 06.02.2019 | 28                  | 0.03                 | 0.7           |                      |                 |
| 15.02.2019 | 33                  | 0.008                | 4.29          | 0.03                 | 1.8             |
| 22.02.2019 | 23                  | 0.03                 | 3.3           |                      |                 |
| 25.02.2019 | 15                  | 0.02                 | 3.4           |                      |                 |
| 05.03.2019 | 13                  | 0.02                 | 3.8           |                      |                 |
| 13.03.2019 | 5                   | 0.03                 | 3.0           |                      |                 |
| 21.03.2019 | 19                  | 0.008                | 4.59          | 0.04                 | 4.6             |
| 29.03.2019 | 17                  | 0.03                 | 4.8           |                      |                 |
| 01.04.2019 | 11                  | 0.02                 | 5.2           |                      |                 |
| 09.04.2019 | 9                   | 0.02                 | 7.6           |                      |                 |
| 17.04.2019 | 6                   | 0.024                | 4.18          | 0.02                 | 8               |
| 25.04.2019 | 5                   | 0.03                 | 8.2           |                      |                 |
| 03.05.2019 | 6                   | 0.04                 | 9.8           |                      |                 |
| 06.05.2019 | 6                   | 0.02                 | 10.3          |                      |                 |
| 14.05.2019 | 7                   | 0.013                | 2.91          | 0.02                 | 9.6             |
| 22.05.2019 | 4                   | 0.02                 | 9.7           |                      |                 |
| 30.05.2019 | 4                   | 0.02                 | 14.1          |                      |                 |
| 07.06.2019 | 4                   | 0.01                 | 14.3          |                      |                 |
| 10.06.2019 | 4                   | 0.03                 | 13.6          |                      |                 |
| 18.06.2019 | 4                   | 0.004                | 2.44          | 0.03                 | 12.5            |
| 26.06.2019 | 6                   | 0.04                 | 12.3          |                      |                 |
| 04.07.2019 | 5                   | 0.02                 | 14.7          |                      |                 |
| 12.07.2019 | 6                   | 0.03                 | 14.3          |                      |                 |
| 15.07.2019 | 7                   | 0.047                | 1.97          | 0.03                 | 14.8            |
| 23.07.2019 | 4                   | 0.04                 | 16.9          |                      |                 |
| 31.07.2019 | 12                  | 0.08                 | 16.4          |                      |                 |
| 08.08.2019 | 19                  | 0.07                 | 16.7          |                      |                 |
| 16.08.2019 | 20                  | 0.04                 | 16.3          |                      |                 |
| 19.08.2019 | 24                  | 0.026                | 1.69          | 0.04                 | 16.3            |
| 27.08.2019 | 14                  | 0.04                 | 15.9          |                      |                 |
| 04.09.2019 | 37                  | 0.03                 | 15.5          |                      |                 |
| 12.09.2019 | 35                  | 0.03                 | 14.5          |                      |                 |
| 20.09.2019 | 21                  | 0.020                | 0.99          | 0.03                 | 14.2            |
| 23.09.2019 | 37                  | 0.03                 | 14.3          |                      |                 |
| 01.10.2019 | 17                  | 0.13                 | 13.3          |                      |                 |
| 09.10.2019 | 5                   | 0.06                 | 12.4          |                      |                 |
| 17.10.2019 | 5                   | 0.040                | 2.37          | 0.07                 | 11.1            |
| 25.10.2019 | 6                   | 0.05                 | 10.1          |                      |                 |
| 28.10.2019 | 8                   | 0.12                 | 9.5           |                      |                 |
| 05.11.2019 | 4                   | 0.07                 | 8.3           |                      |                 |
| 13.11.2019 | 4                   | 0.07                 | 7             |                      |                 |
| 21.11.2019 | 4                   | 0.040                | 3.05          | 0.06                 | 5.8             |
| 29.11.2019 | 4                   | 0.06                 | 6.1           |                      |                 |
| 02.12.2019 | 4                   | 0.06                 |               |                      |                 |
| 10.12.2019 | 4                   | 0.06                 |               |                      |                 |
Table S3. Chlorophyll-a, total ammonium, nitrate, total phosphate, and temperature measured in Tophill Low reservoir samples in 2019.

| Date       | Chlorophyll-a, µg/l | Ammonium total, mg/l | Nitrate, mg/l | Phosphate total, mg/l | Temperature, °C |
|------------|---------------------|----------------------|---------------|------------------------|-----------------|
| 08.01.2019 | 0.047               | 11.50                | 0.13          |                        |                 |
| 16.01.2019 | 0.004               | 10.37                | 0.12          |                        | 0.13            |
| 24.01.2019 | 0.005               | 10.30                | 0.07          |                        | 0.08            |
| 01.02.2019 | 0.043               | 9.62                 | 0.08          |                        | 0.07            |
| 04.02.2019 | 0.055               | 9.37                 | 0.09          |                        | 0.07            |
| 12.02.2019 | 0.014               | 8.65                 | 0.05          |                        | 0.07            |
| 20.02.2019 | 0.008               | 7.36                 | 0.10          |                        | 0.06            |
| 28.02.2019 | 0.093               | 7.95                 | 0.07          |                        | 0.06            |
| 08.03.2019 | 0.055               | 7.64                 | 0.03          |                        | 0.03            |
| 11.03.2019 | 0.004               | 6.65                 | 0.05          |                        | 0.03            |
| 19.03.2019 | 0.016               | 6.33                 | 0.04          |                        | 0.04            |
| 27.03.2019 | 0.018               | 6.47                 | 0.10          |                        | 0.03            |
| 04.04.2019 | 0.066               | 9.13                 | 0.05          |                        | 0.05            |
| 01.05.2019 | 0.176               | 7.36                 | 0.10          |                        | 0.06            |
| 09.05.2019 | 0.093               | 7.95                 | 0.07          |                        | 0.06            |
| 17.05.2019 | 0.055               | 7.64                 | 0.03          |                        | 0.03            |
| 20.05.2019 | 0.005               | 6.51                 | 0.05          |                        | 0.03            |
| 28.05.2019 | 0.065               | 6.91                 | 0.03          |                        | 0.03            |
| 05.06.2019 | 0.012               | 6.33                 | 0.04          |                        | 0.03            |
| 13.06.2019 | 0.047               | 6.47                 | 0.10          |                        | 0.03            |
| 21.06.2019 | 0.004               | 5.36                 | 0.10          |                        | 0.04            |
| 24.06.2019 | 0.156               | 4.97                 | 0.05          |                        | 0.13            |
| 02.07.2019 | 0.020               | 5.31                 | 0.04          |                        | 0.12            |
| 10.07.2019 | 0.05                | 4.92                 | 0.04          |                        | 0.14            |
| 18.07.2019 | 0.06                | 4.97                 | 0.05          |                        | 0.13            |
| 26.07.2019 | 0.06                | 4.97                 | 0.05          |                        | 0.13            |
| 29.07.2019 | 0.05                | 4.97                 | 0.05          |                        | 0.13            |
| 06.08.2019 | 0.05                | 4.97                 | 0.05          |                        | 0.13            |
| 14.08.2019 | 0.04                | 4.97                 | 0.05          |                        | 0.13            |
| 30.08.2019 | 0.04                | 4.97                 | 0.05          |                        | 0.13            |
| 02.09.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 10.09.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 18.09.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 26.09.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 04.10.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 07.10.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 15.10.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 23.10.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 31.10.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 08.11.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 11.11.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 19.11.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 05.12.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 13.12.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 16.12.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
| 24.12.2019 | 0.03                | 4.97                 | 0.05          |                        | 0.13            |
Figure S1. Comparison of relative intensity over m/z range for mass spectrometry fragmentation spectra between MC-LR reference standard (top, orange) and MC-LR detected in Ingbirchworth reservoir in the September sample (bottom, blue) as head to tail plot. The m/z value and the retention time (RT in min) are noted in the title line, HCD 15, 30, 45% stepped normalised collision energy.

Figure S2. Comparison of relative intensity over m/z range for mass spectrometry fragmentation spectra between [D-Asp³]MC-LR reference standard (top, orange) and [D-Asp³]MC-LR detected in Ingbirchworth reservoir in the September sample (bottom, blue) as head to tail plots. The m/z value and the retention time (RT in min) are noted in the title line, HCD 15, 30, 45% stepped normalised collision energy.
Figure S3. Comparison of relative intensity over m/z range for mass spectrometry fragmentation spectra between MC-RR reference standard (top, orange) and MC-RR detected in Ingbirchworth reservoir in the September sample (bottom, blue) as head to tail plots. The m/z value and the retention time (RT in min) are noted in the title line, HCD 15, 30, 45% stepped normalised collision energy.

Figure S4. Comparison of relative intensity over m/z range for mass spectrometry fragmentation spectra between MC-HiIR reference standard (top, orange) and MC-HiIR detected in Ingbirchworth reservoir in the September sample (bottom, blue) as head to tail plots. The m/z value and the retention time (RT in min) are noted in the title line, HCD 15, 30, 45% stepped normalised collision energy.
Figure S5. Comparison of relative intensity over m/z range for mass spectrometry fragmentation spectra between anabaenopeptin A bioreagent (top, orange) and anabaenopeptin A detected in Ingbirchworth reservoir in the September sample (bottom, blue) as head to tail plots. The m/z value and the retention time (RT in min) are noted in the tile line, HCD 15, 30, 45% stepped normalised collision energy.

Figure S6. Comparison of relative intensity over m/z range for mass spectrometry fragmentation spectra between anabaenopeptin B bioreagent (top, orange) and anabaenopeptin B detected in Ingbirchworth reservoir in the September sample (bottom, blue) as head to tail plots. The m/z value and the retention time (RT in min) are noted in the tile line, HCD 15, 30, 45% stepped normalised collision energy.
Figure S7. Comparison of relative intensity over m/z range for mass spectrometry fragmentation spectra between oscillamide Y bioreagent (top, orange) and oscillamide Y detected in Ingbirchworth reservoir in the September sample (bottom, blue) as head to tail plots. The m/z value and the retention time (RT in min) are noted in the tile line, HCD 15, 30, 45% stepped normalised collision energy.

Figure S8. Comparison of relative intensity over m/z range for mass spectrometry fragmentation spectra between anatoxin-a reference standard (top, orange) and oscillamide Y detected in Ingbirchworth reservoir in the August sample (bottom, blue) as head to tail plots. The m/z value and the retention time (RT in min) are noted in the tile line, HCD – 10 eV.
Figure S9 Fragmentation spectrum of Cyanopeptolin CP992 detected in Ingbirchworth reservoir in the September sample at HCD 15, 30, 45% stepped normalised collision energy. Precursor m/z, retention time (RT) and the building block string are noted at the top. The flat structure is shown with annotated building blocks and sites of fragmentation. The table specifies the m/z value and building block fragments that support the identification of this compound.
Figure S10. Fragmentation spectrum of Anabaenopeptilide 202A detected in Ingbirchworth reservoir in the September sample at HCD 15, 30, 45% stepped normalised collision energy. Precursor m/z, retention time (RT) and the building block string are noted at the top. The flat structure is shown with annotated building blocks and sites of fragmentation. The table specifies the m/z value and building block fragments that support the identification of this compound.
Figure S11. Fragmentation spectrum of MC-FL detected in Ingbirchworth reservoir in the September sample at HCD 15, 30, 45% stepped normalised collision energy. Precursor m/z, retention time (RT) and the building block string are noted at the top. The flat structure is shown with annotated building blocks and sites of fragmentation. The table specifies the m/z value and building block fragments that support the identification of this compound.