**DATA PREPARTION AND ANALYSIS INSTRUCTION**

**for the manuscript**

**Phytoplankton group classification by integrating trait information and observed environmental thresholds**

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1. **Data, code and software installation**

Three main elements were sourced for this case study:

* **Data**: Phytoplankton and water quality data were provided by **Sydney Water Corporation (SWC)**. The datasets were uploaded to Zenodo.org via DOI link: **10.5281/zenodo.15164856.** The datasets are **not openly available for direct download** due to access restrictions. However, they can be made available upon reasonable request. To request access, please use the **“Request access”** on this Zenodo and agree to the data sharing license conditions.
* **Code**: R scripts are available on GitHub at the link provided in the manuscript.
* Software: Microsoft Excel, R/RStudio were used.

1. **Data analysis**

With the date items in place, users can proceed with Data Preprocessing (2.1) and the Data Analysis (2.2) from Stage 1 through Stage 4.

* 1. **Data Preprocessing**
* **Phytoplankton Data**: The phytoplankton dataset is provided in Excel format (.xlsx or .csv) under the file name **Data\_HN\_Phytoplankton\_SWC\_13\_3\_2025.csv**.

Data example in the below table:

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Algal Group Description** | **Genus Name** | **Toxic** | **Calendar Date** | **Year** | **Sampling Point Identifier** | **Site Code** | **Cells/mL** | **ASU** | **BioVolume (mm3/L)** |
| Bacillariophyta (Diatom) | Cocconeis | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 7.00 | 5.46 | 0.01 |
| Bacillariophyta (Diatom) | Cymbella | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 3.00 | 1.44 | 0.00 |
| Bacillariophyta (Diatom) | Gomphonema | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 7.00 | 9.77 | 0.02 |
| Bacillariophyta (Diatom) | Navicula | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 8.00 | 3.29 | 0.00 |
| Bacillariophyta (Diatom) | Synedra | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 5.00 | 3.03 | 0.00 |
| Chlorophyta (Green) | Ankistrodesmus | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 74.00 | 23.61 | 0.01 |
| Chlorophyta (Green) | Chlamydomonas | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 34.00 | 5.81 | 0.01 |
| Chlorophyta (Green) | Closterium | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 2.00 | 4.11 | 0.00 |
| Chlorophyta (Green) | Coelastrum | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 10.00 | 1.37 | 0.00 |
| Chlorophyta (Green) | Scenedesmus | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 21.00 | 1.45 | 0.00 |
| Chlorophyta (Green) | Sphaerocystis | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 47.00 | 3.62 | 0.00 |
| Chlorophyta (Green) | Staurastrum | Non-Toxic | 3/01/2006 | 2006 | N42\_SURFAC | N42 | 2.00 | 19.71 | 0.01 |
| … | … | … | … | … | … | … | … | … | … |

* **Water Quality Data**: This dataset is provided in Excel (csv) format under the file name **Data\_HN\_water\_quality\_SWC\_13\_3\_2025.csv**.
* **Next Step – Prepare Input File for Multi-Correlation Analysis**:
  + Open Data\_HN\_Phytoplankton\_SWC\_13\_3\_2025.csv in Excel.
  + Use a **Pivot Table** to calculate the **total daily biovolume** by summing the biovolume of all species for each **date** and **site**. (**Rows**: Date, Site; **Columns**: Site (optional, if you want separate columns for each site), **Values**: Sum of Biovolume (BIOVOL).
  + Export the result as **Total\_BIOVOL**.csv.

|  |  |  |
| --- | --- | --- |
| **Site Code** | **Calendar Date** | **BIOVOL** |
| N18 | 29/07/2008 | 2.374012882 |
| N18 | 12/08/2008 | 1.989492 |
| N18 | 3/09/2008 | 1.435298701 |
| N18 | 23/09/2008 | 1.988031257 |
| N18 | 14/10/2008 | 1.927863749 |
| N18 | 5/11/2008 | 2.066298704 |
| N18 | 25/11/2008 | 1.301655155 |
| N18 | 16/12/2008 | 2.98333389 |
| N18 | 6/01/2009 | 2.549035051 |
| N18 | 27/01/2009 | 3.149 |
| N18 | 17/02/2009 | 11.52599229 |
| N18 | 10/03/2009 | 1.666555795 |
| N18 | 1/04/2009 | 1.9569595 |
| N18 | 21/04/2009 | 0.775864032 |
| N18 | 12/05/2009 | 1.222236847 |
| N18 | 24/06/2009 | 0.694767527 |
| … | … | … |

* + **Then add Water Quality Data to the Table**: Use the **VLOOKUP** (or **XLOOKUP** for newer Excel versions) function to match and import environmental variables from **Data\_HN\_water\_quality\_SWC\_13\_3\_2025.csv** into the **Total\_BIOVOL.csv** table.
  + **Note**: Ensure both files (Total\_BIOVOL.csv and Data\_HN\_water\_quality\_SWC\_13\_3\_2025.csv) have matching columns: **Calendar Date** and **Site Code.** User might need to covert Calendar Date to Datenum, then add Site to create column Site\_Datenum for more accurate adding function.
  + After all parameters are added, save the updated file as **MCA\_data.csv (**See below table).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Site Code** | **Calendar Date** | **BIOVOL** | **Datenum** | **Site\_Datenum** | **TN** | **TP** | **SAL** | **TEMP** | **AMM** | **NIT** | **SIL** | **TURB** | **CHLA** |
| N18 | 29/07/2008 | 2.374012882 | 39658 | N1839658 | 6.00E-01 | 3.40E-02 | NaN | 1.22E+01 | 1.00E-02 | 2.50E-01 | 26 | 1.19E+01 | 26 |
| N18 | 12/08/2008 | 1.989492 | 39672 | N1839672 | 4.80E-01 | 1.70E-02 | NaN | 1.21E+01 | 5.00E-03 | 1.60E-01 | 1.71E+01 | 5.12E+00 | 1.71E+01 |
| N18 | 3/09/2008 | 1.435298701 | 39694 | N1839694 | 3.70E-01 | 2.60E-02 | NaN | 1.38E+01 | 5.00E-03 | 8.00E-02 | 1.24E+01 | 6.30E+00 | 1.24E+01 |
| N18 | 23/09/2008 | 1.988031257 | 39714 | N1839714 | 5.90E-01 | 2.10E-02 | NaN | 1.79E+01 | 1.00E-02 | 3.00E-01 | 1.73E+01 | 7.44E+00 | 1.73E+01 |
| N18 | 14/10/2008 | 1.927863749 | 39735 | N1839735 | 4.70E-01 | 2.60E-02 | NaN | 2.01E+01 | 4.00E-02 | 1.40E-01 | 2.66E+01 | 6.12E+00 | 2.66E+01 |
| N18 | 5/11/2008 | 2.066298704 | 39757 | N1839757 | 3.20E-01 | 2.00E-02 | NaN | 2.24E+01 | 5.00E-03 | 5.00E-03 | 2.04E+01 | 4.06E+00 | 2.04E+01 |
| N18 | 25/11/2008 | 1.301655155 | 39777 | N1839777 | 2.90E-01 | 1.70E-02 | NaN | 23 | 5.00E-03 | 4.00E-02 | 1.34E+01 | 5.50E+00 | 1.34E+01 |
| N18 | 16/12/2008 | 2.98333389 | 39798 | N1839798 | 4.40E-01 | 3.40E-02 | NaN | 2.45E+01 | 5.00E-03 | 5.00E-03 | 2.92E+01 | 8.48E+00 | 2.92E+01 |
| N18 | 6/01/2009 | 2.549035051 | 39819 | N1839819 | 3.60E-01 | 3.60E-02 | NaN | 2.63E+01 | 5.00E-03 | 5.00E-03 | 1.68E+01 | 9.66E+00 | 1.68E+01 |
| N18 | 27/01/2009 | 3.149 | 39840 | N1839840 | 3.30E-01 | 2.40E-02 | NaN | 2.71E+01 | 5.00E-03 | 3.00E-02 | 1.36E+01 | 1.12E+01 | 1.36E+01 |
| N18 | 17/02/2009 | 11.52599229 | 39861 | N1839861 | 5.30E-01 | 3.60E-02 | NaN | 2.43E+01 | 3.00E-02 | 4.00E-02 | 4.33E+01 | 5.36E+00 | 4.33E+01 |
| N18 | 10/03/2009 | 1.666555795 | 39882 | N1839882 | 5.30E-01 | 4.60E-02 | NaN | 2.47E+01 | 1.00E-02 | 8.00E-02 | 2.17E+01 | 1.54E+01 | 2.17E+01 |
| N18 | 1/04/2009 | 1.9569595 | 39904 | N1839904 | 4.00E-01 | 3.20E-02 | NaN | 2.46E+01 | 1.00E-02 | 1.00E-02 | 1.79E+01 | 7.48E+00 | 1.79E+01 |
| N18 | 21/04/2009 | 0.775864032 | 39924 | N1839924 | 5.50E-01 | 4.60E-02 | NaN | 2.05E+01 | 3.00E-02 | 1.90E-01 | 1.25E+01 | 1.07E+01 | 1.25E+01 |
| N18 | 12/05/2009 | 1.222236847 | 39945 | N1839945 | 4.70E-01 | 5.30E-02 | NaN | 1.82E+01 | 5.00E-03 | 6.00E-02 | 2.66E+01 | 1.44E+01 | 2.66E+01 |
| N18 | 24/06/2009 | 0.694767527 | 39988 | N1839988 | 4.30E-01 | 3.40E-02 | NaN | 1.41E+01 | 5.00E-03 | 1.80E-01 | 1.13E+01 | 1.25E+01 | 1.13E+01 |

* + In your table, create a new column for **TN:TP molecular ratio** using the following formula: TNTP = (TN / 14) / (TP / 31).
  + Add a new column called **Site\_Quarter\_Year (SQY),** then use the IF function (or TEXT function) to assign the quarter and year for each site. For example: “N182006Q1” is from site N18, Jan to March 2006 in 2006.
  + **Use Pivot Table to Calculate Mean**: Set **SQY** as the **Row**, Set the **values** to calculate the **mean** of **BIOVOL** and all other environmental variables (e.g., TN, TP, Temperature, etc.).
  + **Output File**: Save the output as **MCA\_data.csv (**See below table).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| SQY | TN | TP | TNTP | SAL | TEMP | AMM | NIT | FRP | TURB | SIL | CHLA | VOL |
| N422006 Q3 | 0.82 | 0.018 | 100.873 | 0.154 | 16.3 | 0.005 | 0.56 | 0.007 | 0.83 | 3.39 | 8.7 | 1.248129 |
| N422006 Q4 | 0.38 | 0.01 | 84.14286 | 0.178 | 22.9 | 0.02 | 0.12 | 0.005 | 0.62 | 1.7 | 3.1 | 0.249592 |
| N422007 Q1 | 0.72 | 0.062 | 25.71429 | 0.143 | 23.8 | 0.01 | 0.27 | 0.014 | 3.01 | 4.96 | 12.3 | 1.190617 |
| N422007 Q2 | 0.61 | 0.01 | 135.0714 | 0.115 | 13.8 | 0.01 | 0.35 | 0.004 | 3.97 | 1.62 | 3.6 | 0.184 |
| N422007 Q3 | 0.81 | 0.029 | 61.55187 | 0.1285 | 14.55 | 0.0225 | 0.43 | 0.0085 | 2.365 | 3.39 | 19.25 | 2.142718 |
| N422008 Q1 | 0.51 | 0.028 | 40.33163 | 0.102 | 25.7 | 0.01 | 0.16 | 0.009 | 4.72 | 5.82 | 11.4 | 2.099816 |
| N422009 Q1 | 0.48 | 0.019 | 55.93985 | 0.0648 | 23.2 | 0.03 | 0.17 | 0.008 | 2.54 | 1.24 | 5.5 | 0.9303 |
| N422010 Q1 | 0.45 | 0.022 | 45.29221 | 0.121 | 25.9 | 0.01 | 0.18 | 0.008 | 2.33 | 2.46 | 8.4 | 1.74763 |
| … | … | … | … | … | … | … | … | … | … | … | … | … |

* **Prepare Input Data for Principal Component Analysis (PCA)**:
  + **Open** the **Data\_HN\_Phytoplankton\_SWC\_13\_3\_2025.csv** (original file) and **Data\_HN\_water\_quality\_SWC\_13\_3\_2025.csv** in Excel.
  + **Join the Water Quality Data to Phytoplankton Data**: Use the **H\_VLOOKUP** (or VLOOKUP if using a standard lookup function) to merge the water quality data with the phytoplankton data based on **matching Date** and **Site** columns (Similar to join data for MCA). The resulting table should now contain both the phytoplankton data (from Data\_HN\_Phytoplankton\_SWC\_13\_3\_2025.csv) and water quality data (from Data\_HN\_water\_quality\_SWC\_13\_3\_2025.csv) for each matching **Date** and **Site**.
  + **Output File**: Save the merged dataset as **PHY\_WQ\_dataset\_PCA.csv** for PCA analysis (See below table).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Calendar Date** | **Year** | **Site Code** | **Cells/mL** | **CellVolume(mm3)** | **Genus Name** | **Algal Group Description** | **TN** | **TP** | **SAL** | **TEMP** | **AMM** | **NIT** | **FRP** | **SIL** | **TURB** |
| 23/03/2006 | 2006 | N75 | 398 | 0.0000002 | Thalassiosira | Bacillariophyta (Diatom) | 4.18 | 0.092 | 0.231 | 22.4 | 0.19 | 2.4 | 0.009 | 2.56 | 4.73 |
| 23/03/2006 | 2006 | N35 | 2 | 0.0000057 | Merotrichia | Chloromonadophyta | NaN | NaN | 0.15 | 24.4 | NaN | NaN | NaN | 1.05 | NaN |
| 23/03/2006 | 2006 | N48 | 7 | 5.67E-06 | Merotrichia | Chloromonadophyta | 1.01 | 0.138 | 0.121 | 22.8 | 0.01 | 0.06 | 0.019 | 44.9 | 13.7 |
| 23/03/2006 | 2006 | N18 | 197 | 6.65E-08 | Actinastrum | Chlorophyta (Green) | NaN | NaN | NaN | 25.5 | NaN | NaN | NaN | 14.1 | NaN |
| 23/03/2006 | 2006 | N48 | 27 | 6.67E-08 | Actinastrum | Chlorophyta (Green) | 1.01 | 0.138 | 0.121 | 22.8 | 0.01 | 0.06 | 0.019 | 44.9 | 13.7 |
| 23/03/2006 | 2006 | N75 | 145 | 6.62E-08 | Actinastrum | Chlorophyta (Green) | 4.18 | 0.092 | 0.231 | 22.4 | 0.19 | 2.4 | 0.009 | 2.56 | 4.73 |
| 23/03/2006 | 2006 | N39 | 14 | 3.14E-07 | Actinotaenium | Chlorophyta (Green) | 0.5 | 0.02 | NaN | NaN | 0.005 | 0.1 | 0.006 | 23.8 | NaN |
| 23/03/2006 | 2006 | N18 | 81 | 1.15E-07 | Ankistrodesmus | Chlorophyta (Green) | NaN | NaN | NaN | 25.5 | NaN | NaN | NaN | 14.1 | NaN |
| 23/03/2006 | 2006 | N26 | 462 | 1.15E-07 | Ankistrodesmus | Chlorophyta (Green) | NaN | NaN | NaN | 25.1 | NaN | NaN | NaN | 37.5 | NaN |
| 23/03/2006 | 2006 | N35 | 29 | 1.14E-07 | Ankistrodesmus | Chlorophyta (Green) | NaN | NaN | 0.15 | 24.4 | NaN | NaN | NaN | 1.05 | NaN |
| 23/03/2006 | 2006 | N39 | 179 | 1.15E-07 | Ankistrodesmus | Chlorophyta (Green) | 0.5 | 0.02 | NaN | NaN | 0.005 | 0.1 | 0.006 | 23.8 | NaN |
| 23/03/2006 | 2006 | N48 | 61 | 1.15E-07 | Ankistrodesmus | Chlorophyta (Green) | 1.01 | 0.138 | 0.121 | 22.8 | 0.01 | 0.06 | 0.019 | 44.9 | 13.7 |
| 23/03/2006 | 2006 | N75 | 36 | 1.14E-07 | Ankistrodesmus | Chlorophyta (Green) | 4.18 | 0.092 | 0.231 | 22.4 | 0.19 | 2.4 | 0.009 | 2.56 | 4.73 |
| 23/03/2006 | 2006 | N18 | 7 | 1.14E-07 | Ankistrodesmus falcatus | Chlorophyta (Green) | NaN | NaN | NaN | 25.5 | NaN | NaN | NaN | 14.1 | NaN |
| 23/03/2006 | 2006 | N26 | 11 | 1.18E-07 | Ankistrodesmus falcatus | Chlorophyta (Green) | NaN | NaN | NaN | 25.1 | NaN | NaN | NaN | 37.5 | NaN |
| 23/03/2006 | 2006 | N35 | 16 | 1.13E-07 | Ankistrodesmus falcatus | Chlorophyta (Green) | NaN | NaN | 0.15 | 24.4 | NaN | NaN | NaN | 1.05 | NaN |
| 23/03/2006 | 2006 | N75 | 5 | 0.00000012 | Ankistrodesmus falcatus | Chlorophyta (Green) | 4.18 | 0.092 | 0.231 | 22.4 | 0.19 | 2.4 | 0.009 | 2.56 | 4.73 |
| 23/03/2006 | 2006 | N39 | 7 | 6.29E-06 | Botryococcus colony | Chlorophyta (Green) | 0.5 | 0.02 | NaN | NaN | 0.005 | 0.1 | 0.006 | 23.8 | NaN |
| 23/03/2006 | 2006 | N75 | 72 | 5.97E-08 | Carteria | Chlorophyta (Green) | 4.18 | 0.092 | 0.231 | 22.4 | 0.19 | 2.4 | 0.009 | 2.56 | 4.73 |
| 23/03/2006 | 2006 | N48 | 61 | 2.15E-07 | Chlamydomonas | Chlorophyta (Green) | 1.01 | 0.138 | 0.121 | 22.8 | 0.01 | 0.06 | 0.019 | 44.9 | 13.7 |
| 23/03/2006 | 2006 | N75 | 18 | 2.17E-07 | Chlamydomonas | Chlorophyta (Green) | 4.18 | 0.092 | 0.231 | 22.4 | 0.19 | 2.4 | 0.009 | 2.56 | 4.73 |
| 23/03/2006 | 2006 | N26 | 23 | 0.0000001 | Chodatella | Chlorophyta (Green) | NaN | NaN | NaN | 25.1 | NaN | NaN | NaN | 37.5 | NaN |
| 23/03/2006 | 2006 | N26 | 4 | 0.0000093 | Closteriopsis | Chlorophyta (Green) | NaN | NaN | NaN | 25.1 | NaN | NaN | NaN | 37.5 | NaN |
| … | … | … | … | … | … | … | … | … | .. | … | … | … | … | … | … |

* **Prepare Input Data for Taxa Indicator Threshold Analysis (TITAN)**:
  + **Convert Phytoplankton Data from Long to Wide Format**: Open **R/RStudio** and run the script **Stage\_1\_Convert\_long\_to\_wide\_phyto\_data.R** to transform the phytoplankton data from long to wide format and calculate **log(abundance + 1)** for each species. Export the result as **Phyto\_HN\_wide\_log.csv.**
  + **Count Values and Remove Low Occurrence Species**: Open **Phyto\_HN\_wide\_log.csv** in Excel., Count the number of values greater than 0 in each column (for each species). Then remove any species (columns) with fewer than **4 occurrences** greater than 0.
  + **Add Water Quality Data**: Use **VLOOKUP** to merge the **water quality data** from **Data\_HN\_water\_quality\_SWC\_13\_3\_2025.csv** into **Phyto\_HN\_wide\_log.csv**. Match the species data with corresponding **Date** and **Site** values.
  + **Save the Final Dataset**: Save the updated file as **HN\_PHY\_WQ\_DATA\_log\_1.csv** (see below table).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Site** | **Date** | **TN** | **TP** | **TN\_TP\_Ratio** | **SAL** | **TEMP** | **AMM** | **NIT** | **FRP** | **SIL** | **TURB** | **T.Anabaena.aphanizomenoides** | **T.Anabaena.bergii** | **T.Anabaena.circinalis** | **T.Anabaenopsis** | **T.Aphanizomenonaceae** | **T.Heterocapsa** | **T.Microcystis** | … |
| N18 | 10/07/2008 | 0.63 | 0.028 | 49.82 | NA | 12.7 | 0.005 | 0.38 | 0.007 | 17 | 8.15 | 0 | 0 | 0 | 0 | 0 | 0 | 2.39794 | … |
| N18 | 29/07/2008 | 0.6 | 0.034 | 39.08 | NA | 12.2 | 0.01 | 0.25 | 0.006 | 26 | 11.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | … |
| N18 | 12/08/2008 | 0.48 | 0.017 | 62.52 | NA | 12.1 | 0.005 | 0.16 | 0.004 | 17.1 | 5.12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | … |
| N18 | 3/09/2008 | 0.37 | 0.026 | 31.51 | NA | 13.8 | 0.005 | 0.08 | 0.006 | 12.4 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0.60206 | … |
| N18 | 23/09/2008 | 0.59 | 0.021 | 62.21 | NA | 17.9 | 0.01 | 0.3 | 0.006 | 17.3 | 7.44 | 0 | 0 | 0 | 0 | 0 | 0 | 0.60206 | … |
| N18 | 14/10/2008 | 0.47 | 0.026 | 40.03 | NA | 20.1 | 0.04 | 0.14 | 0.006 | 26.6 | 6.12 | 0 | 0 | 0 | 0 | 0 | 0 | 2.017033 | … |
| N18 | 5/11/2008 | 0.32 | 0.02 | 35.43 | NA | 22.4 | 0.005 | 0.005 | 0.004 | 20.4 | 4.06 | 0 | 0 | 2.716003 | 0 | 0 | 0 | 2.658965 | … |
| N18 | 25/11/2008 | 0.29 | 0.017 | 37.77 | NA | 23 | 0.005 | 0.04 | 0.004 | 13.4 | 5.5 | 0 | 0 | 0 | 0 | 0 | 0 | 2.017033 | … |
| N18 | 16/12/2008 | 0.44 | 0.034 | 28.66 | NA | 24.5 | 0.005 | 0.005 | 0.005 | 29.2 | 8.48 | 0 | 0 | 2.80618 | 0 | 0 | 0 | 2.515874 | … |
| N18 | 6/01/2009 | 0.36 | 0.036 | 22.14 | NA | 26.3 | 0.005 | 0.005 | 0.005 | 16.8 | 9.66 | 2.658965 | 0 | 0 | 0 | 0 | 0 | 2.017033 | … |
| N18 | 27/01/2009 | 0.33 | 0.024 | 30.45 | NA | 27.1 | 0.005 | 0.03 | 0.004 | 13.6 | 11.2 | 0 | 0 | 0 | 0.60206 | 0 | 0 | 0.60206 | … |
| N18 | 17/02/2009 | 0.53 | 0.036 | 32.6 | NA | 24.3 | 0.03 | 0.04 | 0.005 | 43.3 | 5.36 | 0 | 0 | 0 | 2.737193 | 0 | 0 | 2.39794 | … |
| N18 | 10/03/2009 | 0.53 | 0.046 | 25.51 | NA | 24.7 | 0.01 | 0.08 | 0.011 | 21.7 | 15.4 | 0 | 0 | 2.462398 | 2.271842 | 0 | 0 | 2.462398 | … |
| N18 | 1/04/2009 | 0.4 | 0.032 | 27.68 | NA | 24.6 | 0.01 | 0.01 | 0.009 | 17.9 | 7.48 | 0 | 0 | 2.39794 | 2.017033 | 0 | 0 | 2.271842 | … |
| N18 | 21/04/2009 | 0.55 | 0.046 | 26.48 | NA | 20.5 | 0.03 | 0.19 | 0.015 | 12.5 | 10.7 | 0 | 0 | 0 | 0 | 0 | 0 | 2.271842 | … |
| N18 | 12/05/2009 | 0.47 | 0.053 | 19.64 | NA | 18.2 | 0.005 | 0.06 | 0.009 | 26.6 | 14.4 | 0 | 0 | 0 | 0.60206 | 0 | 0 | 2.748188 | … |
| N18 | 24/06/2009 | 0.43 | 0.034 | 28 | NA | 14.1 | 0.005 | 0.18 | 0.011 | 11.3 | 12.5 | 0 | 0 | 0.60206 | 0 | 0 | 0 | 0.60206 | … |
| N18 | 13/07/2009 | 0.41 | 0.024 | 37.83 | 0.103 | 12.8 | 0.01 | 0.14 | 0.007 | 19.8 | 9.69 | 0 | 0 | 0 | 0 | 0 | 0 | 2.583199 | … |
| N18 | 7/08/2009 | 0.36 | 0.02 | 39.86 | 2.114 | 12.9 | 0.005 | 0.1 | 0.004 | 14.3 | 6.97 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | … |
| N18 | 27/08/2009 | 0.36 | 0.016 | 49.82 | 0.696 | 15.4 | 0.005 | 0.06 | 0.004 | 14.2 | 5.62 | 0 | 0 | 2.39794 | 0 | 0 | 0 | 2.271842 | … |
| …. | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … | … |

* 1. **Data Analysis**

Once the data preparation is complete, the input data is ready for the next step. The following analyses are then conducted:

* + 1. **MCA**
  + Open **RStudio**.
  + Input data: **MCA\_data.csv**.
  + Run the R script: **Stage\_2\_MCA\_Final**. This script will show the correlation matrix and correlation plot.
    1. **Conduct Principal Component Analysis (PCA)**
  + Open **RStudio**.
  + Input data: **PHY\_WQ\_dataset\_PCA.csv**.

Run the R script: **Stage\_2\_PCA\_Final**.

* + 1. **TITAN**
  + Open **RStudio**.
  + **Input Data**: **HN\_PHY\_WQ\_DATA\_log\_1.csv**.
  + Open the R Script: **Stage\_3\_TITAN**.
    - **Run lines 1–692** to perform **TITAN** for 10 variables and save the results. This process may take up to 4 days for each variable.
    - **Run lines 694–907** to export **“Change Point 10th and 90th”** and create input files for **K-prototype clustering**: Clustering\_data\_4.csv (with **TN, TP, TNTP, and TEMP**) and Clustering\_data\_all.csv (with **all variables**)
    - **Run lines 913–1062** to perform **TITAN** for 2 species (as per review suggestions) and update the new thresholds for the species in **Clustering\_data\_4.csv**.
    - Next, **check and plot the purity of TITAN** by running code from **lines 1103–1479**.
    - Add **species traits** to **Clustering\_data\_4.csv**. Species traits can be seen in the Supplementary Table S1.
    - The final output file is **K\_prototype\_input.csv** (**Supplementary Table S1).**
    1. **Classification – K-prototype**
  + Open **RStudio**
  + Input data: **K\_prototype\_input.csv; Classification\_trait\_thresholds.RData** (for traits and revised species name after 1st revision)
  + **Open the R Script**: **Stage\_4\_Classification**.
    - **Run the lines 1- 31** to read the input data and assign categorical variables.
    - **Run the line 37 - 42** to select the optimal number of clusters based on the **Elbow plot**.
    - **K-Prototype Classification**: Since **5 clusters** were selected, run the K-prototype classification (line **48**).
    - **Assign new names** to the clusters (line **51 - 61**).
    - **Summary of Clusters**: Use **summary(cluster)** to display the summary of the cluster characteristics.
    - **Visualize Clusters**: Add other thresholds and visualize the clusters using plotting (**run line 62 – 258**).
    - **Test Normality**: To determine which groups differ significantly from each other, first, run **shapiro.test** (line **262 - 283**) to check the normality of the thresholds.
    - Since the p-value of all Shapiro tests is **< 0.05**, indicating non-normal distribution, proceed with non-parametric tests.
    - Then **Kruskal-Wallis Test**: Run **kruskal.test** to assess whether there are statistically significant differences between the thresholds of the clusters (**Line 288 – 304)**.
    - **Post-hoc Tests**: If significant differences are observed, run **kwAllPairsConoverTest** with the **"holm"** method, and **dunnTest** with the **method="bonferroni"** to identify which groups differ (line **306 - 367**).
    - **Taxonomic Group Differences**: Run **kruskal.test** (Line **370 – 599)** to assess differences among taxonomic groups contributing to different functional groups (see **Supplementary Table S5, Text S2**).
    - **Levene's Test**: Use **Levene's Test** (line **601 – 799)** to quantify group homogeneity (see **Supplementary Text S3**).
    1. **Sensitivity Analysis (for Classification)**
  + Open **RStudio**.
  + **Input Data**:
    - **K\_prototype\_input.csv** (same as classification with 4 variables).
    - **K\_prototype\_classification\_input\_all\_trait.csv** (file containing other threshold values same as Clustering\_data\_all.csv).
    - **Trait\_check.csv** (file containing revised species names and traits in Supplementary Table S1).
  + Open the R script: **Stage\_4\_Sensitivity.R**. This script will export results of 4 test in csv file.