Metadata for Wagner et al. L&O

**Table 1.** Description of the fields needed to describe the creation of your dataset.

|  |  |
| --- | --- |
| **Title of dataset** | **Diazotrophy modulates cyanobacteria stoichiometry through functional traits that determine bloom magnitude and toxin production** |
| **Abstract** | Harmful cyanobacterial blooms are an increasing threat to water quality. The interactions between two eco-physiological functional traits of cyanobacteria, diazotrophy (nitrogen (N)-fixation) and N-rich cyanotoxin synthesis, have never been examined in a stoichiometric explicit manner. We explored how a gradient of resource N:phosphorus (P) affects the biomass, N, P stoichiometry, light-harvesting pigments, and cylindrospermopsin production in a N-fixing cyanobacterium, Aphanizomenon. Low N:P *Aphanizomenon* cultures produced the same biomass as populations grown in high N:P cultures. The biomass accumulation determined by carbon, indicated low N:P *Aphanizomenon* cultures did not have a N-fixation growth tradeoff, in contrast to some other diazotrophs that maintain stoichiometric N homeostasis at the expense of growth. However, N-fixing *Aphanizomenon* populations produced less particulate cylindrospermopsin and had undetectable dissolved cylindrospermopsin compared to non-N-fixing populations. The pattern of low to high cyanotoxin cell quotas across an N:P gradient in the diazotrophic cylindrospermopsin producer is similar to the cyanotoxin cell quota response in non-diazotrophic cyanobacteria. We suggest that diazotrophic cyanobacteria may be characterized into two broad functional groups, the N-storage-strategists and the growth-strategists, which use N-fixation differently and may determine patterns of bloom magnitude and toxin production in nature. |
| **Keywords** | Ecological stoichiometry, harmful algal blooms, cyanotoxin, nitrogen fixation, light-harvesting pigments |
| **Lead author for the dataset** | Nicole D. Wagner |
| **Title and position of lead author** | Assistant Professor |
| **Organization and address of lead author** | Department of Biological Sciences, Oakland University, Rochester MI, 48326, USA |
| **Email address of lead author** | Nicolewagner@oakland.edu |
| **Additional authors or contributors to the dataset** | Felicia S. Osburn, Raegyn B. Taylor, Jeffrey A. Back, C. Kevin Chambliss, Bryan W. Brooks, and J. Thad Scott |
| **Organization associated with the data** | Baylor University |
| **Funding** | Title: Developing a predictive understanding of harmful cyanobacteria growth, toxins production, and comparative toxicity across environmentally important gradients of N:P and salinity Lead P.I. **Geoffrey Scott; Project leaders: Bryan Brooks and J. Thad Scott** National Institutes of Health, 1P01ES028942 |
| **License** |  |
| **Geographic location – verbal description** | Baylor University, Waco, McClennan County, TX, USA |
| **Geographic coverage bounding coordinates** | 31.5489° N, 97.1131° W |
| **Time frame - Begin date** | September 2020 |
| **Time frame - End date** | November 2022 |
| **General study design** | Laboratory bioassay experiment. *Aphanizomenon* flos-aquae(PCC 7905) was grown under 11 Nitrogen:Phosphorus media conditions (1, 2, 4, 8, 12, 16, 20, 30, 50, 75 and 100 by mol) for 37 days. Cultures were grown in incubators held at 26°C with a light intensity of 140 µmol m-2 s-1 on a 14h:10h light:dark cycle. Every other day cultures were shaken and rotated to prevent settling and incubator placement effects. We measured *in-vivo* chlorophyll a every 3-4 days to track growth. Subsampling for particulate carbon and nitrogen was done after 10, 17, 21, 25, 29, 33 days of growth. After 37 days of growth, we sampled for particulate carbon, nitrogen, phosphorus, cylindrospermopsin, chlorophyll a, and phycobilin pigments on to 24 mm glass fiber filters (GF/F). The filtrate was saved for dissolved nitrogen (nitrate, nitrite, ammonium), phosphorus, and total dissolved nitrogen and phosphorus. A subsample of each culture was preserved in Lugol’s iodine and counted on a compound microscope at 400x magnification to obtain cell densities. We used a mass balance approach to determine gross-nitrogen-fixation rates. |
| **Methods description** | Methods done per general study design above. |
| **Laboratory, field, or other analytical methods** | *In vivo* chlorophyll-a– performed using a Tuner Designs fluorometer with the *in vivo* chlorophyll a module  Particulate carbon, nitrogen, and phosphorus - sampled using 0.7µm GF/F Whatman filters. Particulate carbon and nitrogen filters were dried at 60 °C for 24 hours, and then analyzed simultaneously on an elemental analyzer as described by Wagner et al (2019). Particulate phosphorus was analyzed by hot persulfate digestion (3% *w/v*) and analyzed using the molybdate blue method (APHA 2002).  Chlorophyll a was analyzed according to EPA method 445.0. Filters were extracted in 90% acetone:water over night at 4°C and analyzed on a Tuner Designs Trilogy fluorometer using the acid chlorophyll a module.  Phycobilin pigments were analyzed as previously described by Wang et al. (2021). Briefly, filters were placed in 5 mL of 0.1M phosphate buffer with two rounds of freeze-thaw cycle to promote cell lysis. After filters were sonicated for 7 min and stored at 4°C overnight. Phycobilin pigments were read on a UV/Visible spectrophotometer at 625, 615, and 562 nm. Concentrations were calculated as in Wang et al. 2021  Total and dissolved cylindrospermopsin – Particulate and dissolved cylindrospermopsin were extracted and analyzed using an isotope dilution method coupled with LC-MS/MS as described in Haddad et al. (2019) and implemented in Osburn et al. (2022). Total cylindrospermopsin was calculated by the sum of particulate and dissolved. |
| **Taxonomic species or groups** | *Aphanizomenon flos-aquae* (PCC 7905) |
| **Quality control** | Samples were handled and ran per QA/QC guidelines |
| **Additional information** | None |
|  |  |

**Table 2A.** Data dictionary: description of the variables (i.e., columns) in EACH dataset.

Dataset filename: Wagner\_Apha\_all\_data\_stats\_L\_O.csv

Dataset description: All data generated on day 37 of the experiment.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Column name** | **Description** | **Units** | **Code explanation** | **Data format** | **Missing data code** |
| *The name of the variable in the dataset; avoid special characters, dashes and spaces* | *A detailed description of the variable* | *Units the variable is measured in* | *If you use codes in your column, please explain each code, such as: LR = Little Rock Lake; A=sample; etc.* | *State exactly how the data are stored; for dates, state how it is formatted, including time zone, etc.* | *If data are missing, indicate how they are stored, such as NULL, NA, blank cell, etc.* |
| NP | Nitrogen:phoshorus of treatment | molar | None | Numeric | Blank cell |
| Rep | Replicate identification | No units | None | Character | Blank cell |
| N\_added | Concentration of nitrate added to each treatment | mg/L | None | Numeric | Blank cell |
| Cells\_L | Cell density of each bioassay culture | Cells/L | None | Numeric | Blank cell |
| N\_mg\_L | Particulate nitrogen concentration | mg/L | None | Numeric | Blank cell |
| C\_mg\_L | Particulate carbon concentration | mg/L | None | Numeric | Blank cell |
| P\_mg\_L | Particulate phosphorus concentration | mg/L | None | Numeric | Blank cell |
| CP\_mol | Carbon:phosphorus | molar | None | Numeric | Blank cell |
| CN\_mol | Carbon:nitrogen | molar | None | Numeric | Blank cell |
| NP\_mol | Nitrogn:phosphorus | molar | None | Numeric | Blank cell |
| DIN\_mg\_L | Dissolved inorganic nitrogen (nitrate + nitrite + ammonium) | mg/L | None | Numeric | Blank cell |
| TDN\_mg\_L | Total dissolved nitrogen | mg/L | None | Numeric | Blank cell |
| Organic\_N\_mg\_L | Total organic N = Particulate N + TDN -DIN for the N:P 1-20 treatments only. Above N:P 20 particulate N is Organic N | mg/L | None | Numeric | Blank cell |
| PO4\_ug\_L | Dissolved phosphate | µg/L | None | Numeric | Blank cell |
| TDP\_ug\_L | Total dissolved phosphorus | µg/L | None | Numeric | Blank cell |
| NP\_media\_mol | Dissolved Nitrogen:phosphorus in the media after 37 days of growth | molar | None | Numeric | Blank cell |
| N-fix\_ug\_L\_h | Nitrogen fixation rates Above N:P 30 all rates are 0 | µg/L/h | None | Numeric | Blank cell |
| N\_quota\_pg\_cell | Nitrogen cell quota | pg/cell | None | Numeric | Blank cell |
| C\_quota\_pg\_cell | Carbon cell quota | pg/cell | None | Numeric | Blank cell |
| P\_quota\_pg\_cell | Phosphorus cell quota | pg/cell | None | Numeric | Blank cell |
| CYN\_ug\_L\_part | Particulate cylindrospermopsin | µg/L | None | Numeric | Blank cell |
| CYN\_ug\_L\_Dis | Dissolved cylindrospermopsin | µg/L | None | Numeric | Blank cell |
| Total\_CYN\_ug\_L | Total cylindrospermopsin | µg/L | None | Numeric | Blank cell |
| CYN\_fg\_cell | Total cylindrospermopsin cell quota | fg/cell | None | Numeric | Blank cell |
| CYN\_Dis\_fg\_cell | Dissolved cylindrospermopsin cell quota | fg/cell | None | Numeric | Blank cell |
| CYN\_Part\_fg\_cell | Particulate cylindrospermopsin cell quota | fg/cell | None | Numeric | Blank cell |
| CYN\_Percent\_Diss | Percent dissolved of the total cylindrospermopsin | % | None | Numeric | Blank cell |
| Chla\_ug\_L | Chlorophyll a | µg/L | None | Numeric | Blank cell |
| Chla\_pg\_cell | Chlorophyll a cell quota | pg/cell | None | Numeric | Blank cell |
| PC\_mg\_L | Phycocyanin concentration | mg/L | None | Numeric | Blank cell |
| APC\_mg\_L | Allophycocyanin concentration | mg/L | None | Numeric | Blank cell |
| PE\_mg\_L | Phycoerythrin concentration | mg/L | None | Numeric | Blank cell |
| Total\_PBP\_mg\_L | Total phycobilin pigment concentration | mg/L | None | Numeric | Blank cell |
| PBP\_pg\_cell | Total phycobilin pigment cell quota | pg/cell | None | Numeric | Blank cell |

**Table 3.**

Dataset filename: Wagner\_Apha\_RFU.xlx

Dataset description: All *in vivo* fluorescence data obtained throughout experiment.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Column name** | **Description** | **Units** | **Code explanation** | **Data format** | **Missing data code** |
| *The name of the variable in the dataset; avoid special characters, dashes and spaces* | *A detailed description of the variable* | *Units the variable is measured in* | *If you use codes in your column, please explain each code, such as: LR = Little Rock Lake; A=sample; etc.* | *State exactly how the data are stored; for dates, state how it is formatted, including time zone, etc.* | *If data are missing, indicate how they are stored, such as NULL, NA, blank cell, etc.* |
| Day | Day of experiment when RFU was taken | No units | None | Numeric | Blank cell |
| NP | Nitrogen:phoshorus of the culture | mol | None | Numeric | Blank cell |
| Rep | Replicate identification | No units | None | Character | Blank cell |
| RFU | In-vivo measured chlorophyll-a | Relative fluorescence units | None | Numeric | Blank cell |

**Table 4.**

Dataset filename: Wagner\_C\_N\_growth\_L\_O.xlx

Dataset description: All particulate nitrogen, carbon, and growth rate data obtained throughout experiment.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Column name** | **Description** | **Units** | **Code explanation** | **Data format** | **Missing data code** |
| *The name of the variable in the dataset; avoid special characters, dashes and spaces* | *A detailed description of the variable* | *Units the variable is measured in* | *If you use codes in your column, please explain each code, such as: LR = Little Rock Lake; A=sample; etc.* | *State exactly how the data are stored; for dates, state how it is formatted, including time zone, etc.* | *If data are missing, indicate how they are stored, such as NULL, NA, blank cell, etc.* |
| NP | Nitrogen:phoshorus of the culture | mol | None | Numeric | Blank cell |
| Rep | Replicate identification | No units | None | Character | Blank cell |
| Day | Day of experiment when RFU was taken | No units | None | Numeric | Blank cell |
| N\_mg\_L | Particulate nitrogen concentration | mg/L | None | Numeric | Blank cell |
| C\_mg\_L | Particulate carbon concentration | mg/L | None | Numeric | Blank cell |
| Growth\_rate | Growth rate of each culture | Per day | None | Numeric | Blank cell |

**Notes and Comments:** No data derived from other sources was used. No scripts were submitted.