# Introduction

1. Eutrophication can have serious consequences on aquatic ecosystem health including decreased levels of dissolved oxygen, formation of toxic compounds, changes in abundance and composition of various aquatic organisms, and overall loss of biodiversity (Camargo & Alonso, 2006).
   1. Anthropogenically caused nutrient enrichment is one of the biggest threats to freshwaters today (Smith & Schindler, 2009) and can lead to more frequent and intense harmful algal blooms, one of the greatest risks to freshwaters biodiversity across the world (Reid et al., 2019).
   2. In the United States, an estimated $2.2 billion in losses because of eutrophication is likely an underestimate of the actual amount. Costs are associated with recreation, fisheries, property values, loss of biodiversity, and drinking water treatment (Dodds et al., 2008).
      1. And determining economic value of freshwater is difficult with many important factors often excluded from these analyses (Keiser et al., 2019).
   3. Many freshwaters across the United States are generally at serious risk of or are already plagued by eutrophication.
      1. Median TN and TP concentrations in lakes exceeded reference values across all ecoregions in 2008 study (Dodds et al., 2008).
      2. In streams, chlorophyll-a concentrations were found to be substantially higher above the thresholds of 30 µg/L of P and 40 µg/L of N (Dodds et al., 2011).
2. Enrichment of N and P are mainly responsible for eutrophication (Wetzel, 2001). The elements are not mutually exclusive, however, as their cycles are coupled in the environment (Oviedo-Vargas et al., 2013); and studying the relative abundances may unfold large-scale patterns that would otherwise be unseen.
   1. Reductions in P pollution in large lakes may lead to the accumulation of N (Finlay et al., 2013), negating the attempt to decrease nutrient pollution.
   2. Large-scale nutrient stoichiometry integrates biogeochemical processes and serves as the backdrop for many smaller-scale processes to occur (Sterner & Elser, 2002).
      1. Ecosystem stoichiometry varies with temporal scale and can be impacted by things such as biotic dynamics, precipitation, geological weathering, anthropogenic influences (Sterner & Elser, 2002).
   3. Although single nutrient concentration patterns are relatively well-known across regions in the US, nutrient stoichiometry is much more difficult to predict (Collins et al., 2017).
3. Behavior of nutrients in freshwaters can be influenced depending on a variety of factors including the surrounding landscape, legacy storage (Lin et al., 2021), precipitation, biogeochemistry, source water (Basu et al., 2011), and residence time (Maranger et al., 2018).
   1. Omernik’s development of ecoregions provides a qualitative understanding of spatial patterns and regional homogeneities that can be used to inform freshwater researchers (Omernik, 1987).
   2. Although difficult to predict, nutrient stoichiometry does show some patterns that may be useful for assessing trophic status of lakes.
      1. Additions of N and P to lakes has demonstrated much greater impact on productivity in lakes than addition of a single element (Elser et al., 2011).
      2. TN:TP ratios tend to be high in oligotrophic lakes and low in eutrophic lakes, indicating potential shifts in limitation from P to N as trophic status increases (Downing & McCauley, 1992).
         1. TN:TP can be used to indicate nutrient deficiency based on the Redfield ratio, the molar ratio of 106C:16N:1P, at which marine phytoplankton exhibit balanced growth (Redfield, 1958).
      3. Generally, increased residence time correlates with increased C:N, C:P, and N:P. Residence time may also promote burial of P and lead to higher rates of primary productivity (Maranger et al., 2018).
4. In this study, we use US Environmental Protection Agency (EPA) National Lakes Assessment (NLA) data to evaluate patterns of nutrient stoichiometry in relation to trophic status in lakes across the US, as balancing nutrient stoichiometry may assist in eutrophication remediation (Stutter et al., 2018).
   1. Intended to “support efforts to assess nutrient water quality and more effectively protect and restore waters from nutrient pollution.” (wording from challenge description)
   2. We aim to answer the following questions:
      1. How does nutrient limitation/enrichment vary across ecoregions and what are the underlying mechanisms?
      2. Is trophic status (based on chlorophyll) more influenced by nitrogen or phosphorus and how/why does this relationship vary spatially?
      3. What are the trends of stoichiometry and trophic levels across ecoregional and the national scale?

# Methods

1. Data
   1. US EPA NLA data 2007, 2012, 2017
   2. # lakes sampled
      1. 2007: # lakes surveyed = 1156, 95 resampled in same year, 124 reference lakes
      2. 2012: # lakes surveyed = 1038, 100 resampled in same year, 0 reference lakes
      3. 2017: # lakes surveyed = 1112, 97 resampled in same year, 108 (hand) reference lakes?
      4. Lakes in 2007 and 2012 = 364
      5. Lakes in 2012 and 2017 = 473
      6. Lakes in 2007 and 2017 = 282
      7. Lakes in all 3 years = 234
   3. Lake sizes sampled
      1. In 2007, lakes greater than 4 ha were sampled. This changed in the 2012 and 2017 surveys and lakes with surface area > 1 ha and 1-m deep were sampled
         1. Sampling programs often exhibit similar biases including when and which lakes are sampled. Most lake data are collected throughout in the summer and from large lakes (>20 ha) (Stanley et al., 2019). – NLA data follows the collection during summer, but breaks away from the large lakes sampling only by including smaller lakes (<20 ha).
   4. Lakes sampled during the summer (May-September, with a handful of sampling events in October – 4 in 2017 and 9 in 2007) in each year
2. Site selection
   1. Generalized Random Tessellation Stratified survey design (p. 3 technical doc) to randomly choose sampling sites. (USEPA, 2022)
      1. Stratification based on omernik level-3 aggregated ecoregions, state, and lake size
      2. Each lake is assigned a weight to indicate the # lakes it represents
         1. NLA adjusted site weights will be used to broaden the results to regional and national extents
3. Sampling and laboratory methods
   1. Standardized sampling protocols p.37 manuals (USEPA, 2007b, 2011, 2017a)
      1. Water was collected using an integrated sampler within the euphotic zone (up to 2 m).
      2. Chlorophyll sample is placed in a dark 2L bottle and stored on ice until filtration occurred -- Chlorophyll samples filtered in the field with 0.4 µm pore size polycarbonate filters??? Double check this.
      3. Nutrients sample is placed ino a 250 mL bottle and sulfuric acid is added to stabilize the sample at pH <2 and stored on ice
   2. Standardized lab protocols p.51 manuals (USEPA, 2007a, 2012, 2017b)
      1. Shipped overnight to approved laboratories and processed within 24 hours
         1. Laboratory processing procedures must maintain quality assurance/control outlined by the EPA.
      2. Chlorophyll a is analyzed via extraction in 90% acetone followed by fluorometry
      3. TN and TP (no3 and nh4) analyzed via persulfate digestion then automaticed colorimetric analysis
4. Trophic state calculation
   1. p.80 technical doc
5. Statistical/Data analyses
   1. R programming (R Core Team, 2022)
   2. tidyverse (Wickham et al., 2019)
   3. Spsurvey (Dumelle et al., 2022)
      1. Calculated change in trophic levels at the aggregated ecoregional scale and national scale using change\_analysis function in the R package spsurvey
         1. This analysis and package uses the stratified randomized weighting of lakes p 133 tech doc
   4. More TBD as results come in
   5. Use N:P to assess nutrient limitation and stoichiometry at national and ecoregional scales
   6. Analyze nutrient limitation in relation to trophic state
   7. Analyze stoichiometric shifts across time to evaluate the condition of waters

# Results – very preliminary – I need help determining what tests to run and what else to look at

1. How does nutrient limitation/enrichment vary across ecoregions and what are the underlying mechanisms?
   1. Figure showing N limitation and P limitation + citations used for justification (e.g. Bergstrom, McCauley)
   2. Sp survey of limitation shifts over time – national, ecoregional
   3. Trophic status in N-limited, P-limited for current data (2017)
      1. Ecoregional, national
2. Is trophic status (based on chlorophyll) more influenced by nitrogen or phosphorus and how/why does this relationship vary spatially?
   1. TN:TP ratio vs TS
      1. Ecoregion, nationally (all data?)
   2. TN vs TS
      1. Ecoregion, nationally (all data?)
   3. TP vs TS
      1. Ecoregion, nationally (all data?)
3. What are the trends of stoichiometry and trophic levels across ecoregional and the national scale?
   1. Trophic status across ecoregions, national
   2. Look at urban vs. non urban
   3. % development and % ag
   4. Elevation

# Discussion

1. Summarize aim and main findings
2. Link findings to literature – some potentially useful things
   1. The strongest stoichiometric imbalances occur in waters influenced by urbanization and agriculture (Stutter et al., 2018).
      1. Reasoning ^ could be related to differences in organic carbon sources. Forests, wetlands, sewage effluent, and agriculture all supply large quantities of organic carbon to freshwaters. However, forests and wetlands likely provide higher quality, beneficial organic carbon while the opposite is likely true in urban and agricultural areas (Stutter et al., 2018).
3. Regional scale processes can influence nutrient composition in lakes, including N deposition (Burns, 2004) and vegetation and soil characteristics (Kopáček et al., 2000).
4. Limitations and strengths
   1. Balancing stoichiometry alone to mitigate eutrophication is not a solution, rather it should be complimentary to controlling nutrient sources (Stutter et al., 2018).
5. Implications
   1. Potential opening: Freshwater resources are critical to human health and food provisioning, industries, ecosystem function, and recreational and cultural experiences. Eutrophication is one of the world’s top threats to biodiversity (Reid et al., 2019), is commonly caused by anthropogenic activities (Smith & Schindler, 2009), and can have serious consequences on both aquatic ecosystem and human health (Camargo & Alonso, 2006), with economic losses over $2.2 billion (Dodds et al., 2008).
   2. As of the writing of this manuscript, the US Environmental Protection Agency (EPA) has been developing nutrient criteria in US waters to control pollution and plan to amend the Clean Water Act with updated information. They have developed guidelines with various models that incorporate regional specifics so that states may develop their own nutrient thresholds (USEPA, 2021).
      1. Further considering stoichiometry in addition to absolute nutrient concentrations may lead to better protections because of the coupled nature of N and P. For example, reductions in P loading in large lakes across the world resulted in rising nitrate concentrations (Finlay et al., 2013).
6. Conclusion
   1. Potential opening: Lakes and rivers have been extensively altered by climate change, hydrological modifications, land-use, and chemical and nutrient inputs (Carpenter et al., 2011). Nutrients are of particular interest due to their necessity in ecosystem functioning and their simultaneous capacity to pollute. Understanding coupled nutrient cycling and the regional stoichiometric differences of eutrophication is a fundamental step toward solutions.

Basu, N. B., Thompson, S. E., Suresh, P., & Rao, C. (2011). Hydrologic and biogeochemical functioning of intensively managed catchments: A synthesis of top-down analyses. *Water Resources Research*, *47*(10). https://doi.org/10.1029/2011WR010800

Burns, D. A. (2004). The effects of atmospheric nitrogen deposition in the Rocky Mountains of Colorado and southern Wyoming, USA-a critical review. *Environmental Pollution (Barking, Essex : 1987)*, *127*(2), 257–269. https://doi.org/10.1016/S0269-7491(03)00264-1

Camargo, J. A., & Alonso, Á. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International*, *32*(6), 831–849. https://doi.org/10.1016/J.ENVINT.2006.05.002

Carpenter, S. R., Stanley, E. H., & Zanden, M. J. vander. (2011). State of the World’s Freshwater Ecosystems: Physical, Chemical, and Biological Changes. *Https://Doi.Org/10.1146/Annurev-Environ-021810-094524*, *36*, 75–99. https://doi.org/10.1146/ANNUREV-ENVIRON-021810-094524

Collins, S. M., Oliver, S. K., Lapierre, J. F., Stanley, E. H., Jones, J. R., Wagner, T., & Soranno, P. A. (2017). Lake nutrient stoichiometry is less predictable than nutrient concentrations at regional and sub-continental scales: In *Ecological Applications* (Vol. 27, Issue 5, pp. 1529–1540). https://doi.org/10.1002/eap.1545

Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J., Schloesser, J. T., & Thornbrugh, D. J. (2008). Eutrophication of U.S. Freshwaters: Analysis of Potential Economic Damages. *Environmental Science and Technology*, *43*(1), 12–19. https://doi.org/10.1021/ES801217Q

Dodds, W. K., Smith, V. H., & Lohman, K. (2011). Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Https://Doi.Org/10.1139/F02-063*, *59*(5), 865–874. https://doi.org/10.1139/F02-063

Downing, J. A., & McCauley, E. (1992). The nitrogen : phosphorus relationship in lakes. *Limnology and Oceanography*, *37*(5), 936–945. https://doi.org/10.4319/LO.1992.37.5.0936

Dumelle, M., Kincaid, T. M., Olsen, A. R., & Weber, M. H. (2022). *spsurvey: Spatial Sampling Design and Analysis*.

Elser, J. J., Marzolf, E. R., Goldrnan, C. R., Marnoif, E. R., & Goldman, C. 8. (2011). Phosphorus and Nitrogen Limitation of Phytoplankton Growth in the Freshwaters of North America: A Review and Critique of Experimental Enrichments. *Https://Doi.Org/10.1139/F90-165*, *47*(7), 1468–1477. https://doi.org/10.1139/F90-165

Finlay, J. C., Small, G. E., & Sterner, R. W. (2013). Human influences on nitrogen removal in lakes. *Science*, *342*(6155), 247–250. https://doi.org/10.1126/SCIENCE.1242575

Keiser, D. A., Kling, C. L., & Shapiro, J. S. (2019). The low but uncertain measured benefits of US water quality policy. *Proceedings of the National Academy of Sciences*, *116*(12), 5262–5269. https://doi.org/10.1073/pnas.1802870115

Kopáček, J., Stuchlík, E., Straškrabová, V., & Pšenáková, P. (2000). Factors governing nutrient status of mountain lakes in the Tatra Mountains. *Freshwater Biology*, *43*(3), 369–383. https://doi.org/10.1046/J.1365-2427.2000.00569.X

Lin, J., Compton, J. E., Hill, R. A., Herlihy, A. T., Sabo, R. D., Brooks, J. R., Weber, M., Pickard, B., Paulsen, S. G., & Stoddard, J. L. (2021). Context is Everything: Interacting Inputs and Landscape Characteristics Control Stream Nitrogen. *Environmental Science & Technology*. https://doi.org/10.1021/acs.est.0c07102

Maranger, R., Jones, S. E., & Cotner, J. B. (2018). Stoichiometry of carbon, nitrogen, and phosphorus through the freshwater pipe. *Limnology and Oceanography Letters*, *3*(3), 89–101. https://doi.org/10.1002/LOL2.10080

Omernik, J. M. (1987). Ecoregions of the Conterminous United States. *Annals of the Association of American Geographers*, *77*(1), 118–125. https://doi.org/10.1111/J.1467-8306.1987.TB00149.X

Oviedo-Vargas, D., Royer, T. v., & Johnson, L. T. (2013). Dissolved organic carbon manipulation reveals coupled cycling of carbon, nitrogen, and phosphorus in a nitrogen-rich stream. *Limnology and Oceanography*, *58*(4), 1196–1206. https://doi.org/10.4319/LO.2013.58.4.1196

R Core Team. (2022). *R: A Language and Environment for Statistical Computing*. https://www.R-project.org/

Redfield, A. C. (1958). THE BIOLOGICAL CONTROL OF CHEMICAL FACTORS IN THE ENVIRONMENT. *American Scientist*, *46*(3), 230A – 221. http://www.jstor.org/stable/27827150

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, *94*(3), 849–873. https://doi.org/10.1111/brv.12480

Smith, V. H., & Schindler, D. W. (2009). Eutrophication science: where do we go from here? *Trends in Ecology & Evolution*, *24*(4), 201–207. https://doi.org/10.1016/J.TREE.2008.11.009

Stanley, E. H., Collins, S. M., Lottig, N. R., Oliver, S. K., Webster, K. E., Cheruvelil, K. S., & Soranno, P. A. (2019). Biases in lake water quality sampling and implications for macroscale research. *Limnology and Oceanography*, *64*(4), 1572–1585. https://doi.org/10.1002/lno.11136

Sterner, R. Warner., & Elser, J. J. (2002). *Ecological stoichiometry : the biology of elements from molecules to the biosphere*.

Stutter, M. I., Graeber, D., Evans, C. D., Wade, A. J., & Withers, P. J. A. (2018). Balancing macronutrient stoichiometry to alleviate eutrophication. *Science of The Total Environment*, *634*, 439–447. https://doi.org/10.1016/J.SCITOTENV.2018.03.298

USEPA. (2021). Ambient Water Quality Criteria Recommendations for Lakes and Reservoirs of the Conterminous United States: Information Supporting the Development of Numeric Nutrient Criteria. *U.S. Environmental Protection Agency, Washington, DC*. https://www.epa.gov/nutrient-policy-data/ambient-water-quality-criteria-address-nutrient-pollution-lakes-and-reservoirs

USEPA. (2007a). Survey of the Nation’s Lakes: Integrated Quality Assurance Project Plan. EPA/841-B-07-003. *U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC*. https://www.epa.gov/national-aquatic-resource-surveys/national-lakes-assessment-2007-quality-assurance-project-plan

USEPA. (2007b). Survey of the Nation’s Lakes. Field Operations Manual. EPA 841-B-07- 004. *U.S. Environmental Protection Agency, Washington, DC*. https://www.epa.gov/national-aquatic-resource-surveys/national-lakes-assessment-2007-field-operations-manual

USEPA. (2011). 2012 National Lakes Assessment. Field Operations Manual. EPA 841-B-11-003. *U.S. Environmental Protection Agency, Washington, DC*. https://www.epa.gov/national-aquatic-resource-surveys/national-lakes-assessment-2012-field-operations-manual

USEPA. (2012). 2012 National Lakes Assessment. Laboratory Operations Manual. EPA-841-B-11-004. *U.S. Environmental Protection Agency, Washington, DC*. https://www.epa.gov/national-aquatic-resource-surveys/national-lakes-assessment-2012-laboratory-operations-manual

USEPA. (2017a). National Lakes Assessment 2017. Field Operations Manual. EPA 841-B-16-002. *U.S. Environmental Protection Agency, Washington, DC*. https://www.epa.gov/national-aquatic-resource-surveys/national-lakes-assessment-2017-field-operations-manual

USEPA. (2017b). National Lakes Assessment 2017. Laboratory Operations Manual. V.1.1. EPA 841‐B‐16‐ 004. *U.S. Environmental Protection Agency, Washington, DC*. https://www.epa.gov/national-aquatic-resource-surveys/national-lakes-assessment-2017-laboratory-operations-manual

USEPA. (2022). National Lakes Assessment 2017: Technical Support Document. EPA 841‐R‐22‐001. *U.S. Environmental Protection Agency, Office of Water and Office of Research and Development*. . https://www.epa.gov/national‐aquatic‐ resource‐surveys/national‐lakes‐assessment‐2017‐technical‐support‐document

Wetzel, R. G. (2001). *Limnology* (3rd ed.). Academic Press.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., … Yutani, H. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686