# Introduction

Eutrophication can have serious consequences on aquatic ecosystem health including decreased levels of dissolved oxygen, formation of toxic compounds, changes in abundance and community composition of organisms, and overall loss of biodiversity (Camargo & Alonso, 2006). 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 freshwater biodiversity across the world (Reid et al., 2019). In the United States, an estimated $2.2 billion in losses because of eutrophication is likely an underestimate of the actual amount (Dodds et al., 2008) and determining economic value of freshwater is difficult with many important factors often excluded from these analyses (Keiser et al., 2019). Many freshwaters across the United States are generally at serious risk of or are already plagued by eutrophication. Median TN and TP concentrations in lakes exceeded reference values in a 2008 study (Dodds et al., 2008) and in streams, chlorophyll-a concentrations were observed at substantially higher values when nutrient concentrations surpassed the thresholds of 30 µg/L of P and 40 µg/L of N (Dodds et al., 2011).

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. Large-scale nutrient stoichiometry integrates biogeochemical processes and serves as the backdrop for many smaller-scale processes to occur. Ecosystem stoichiometry varies temporally and can be impacted by things such as biotic dynamics, precipitation, geological weathering, anthropogenic influences (Sterner & Elser, 2002). 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).

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). 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). Although difficult to predict, nutrient stoichiometry does show some patterns that may be useful for assessing trophic status of lakes. 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). Generally, increased residence time correlates with increased ratios as residence time may also promote burial of P and lead to higher rates of primary productivity (Maranger et al., 2018). N:P 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). And N:P ratios have been used to indicate nutrient deficiency based on the Redfield ratio, the molar ratio of 106C:16N:1P (Redfield, 1958), as well as through experimental determinations of ratio thresholds (e.g., Bergström, 2010; Downing & McCauley, 1992; Ptacnik et al., 2010).

In this study, we use US Environmental Protection Agency (EPA) National Lakes Assessment (NLA) data to evaluate patterns of nutrient stoichiometry and limitation in relation to trophic status in lakes across the US. This research is intended to support efforts to assess nutrient water quality and more effectively protect and restore waters from nutrient pollution. The NLA data are specifically designed to assess lakes across the US, rather than the individual lakes sampled by using population weight estimates (USEPA, 2022b). Additionally, while most lake data are biased toward large lakes (>20 ha) (Stanley et al., 2019), NLA data are sampled from both natural lakes and reservoirs as small as 1-4 ha. These reasons make these data appropriate for broad assessment of US waters. For this study, we aim to answer the following questions: what are the trends of nutrient stoichiometry and trophic levels across scales of space (ecoregional and national) and time (3 survey years)? Is trophic level (based on chlorophyll-a concentration) more influenced by nitrogen or phosphorus? And how does nutrient limitation relative to trophic state vary spatially and temporally?

# Methods

## NLA lakes and methods

NLA data from survey years 2007, 2012, and 2017 were used in the analyses (USEPA, 2010, 2016, 2022a). In 2007, 1156 lakes were surveyed, 95 were resampled in the same year and 124 were considered reference lakes. In 2012, 1038 lakes were surveyed and 100 were resampled in the same year. In 2017, 1112 lakes were sampled, 97 were resampled in the same year, and 108 were considered reference lakes. 364 lakes were sampled in both 2007 and 2012, 473 were sampled in both 2012 and 2017, and 234 lakes were sampled all three years. 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 included.

### Site selection

The EPA used a Generalized Random Tessellation Stratified survey design to randomly choose sampling sites. Stratification was based on Omernik level-3 aggregated ecoregions, state, and lake size. Each lake is assigned a weight to indicate the number of lakes it represents with error. The NLA site weights are intended to broaden results to regional and national extents (USEPA, 2022b).

### Sampling and laboratory methods

Lakes were sampled during the summer month (May-September) of each survey year. In 2007 there were 9 sampling events in October and in 2017, there were 4 sampling events in October. The EPA used standardize sampling protocols in each survey year. Water was collected using an integrated sampler within the euphotic zone or up to 2m depth. Chlorophyll samples were stored in a dark 2L bottle and stored on ice until filtration with a 0.4 µm pore size polycarbonate filters. Nutrient samples were stored in 250 mL bottles on ice and sulfuric acid was added to stabilize samples at pH <2. More on these standardize sampling procedures can be found in the NLA field operations manuals (USEPA, 2007b, 2011, 2017a).

Samples were shipped overnight to approved laboratories and processed within 24 hours of receipt. Samples are analyzed for chlorophyll-a via extraction in 90% acetone followed by fluorometry. Total nutrients are analyzed via persulfate digestion then automated colorimetric analysis. Laboratory processing procedures must maintain quality assurance/control outlined by the EPA. More information about these processes and quality assurance can be found in the NLA laboratory operations manuals (USEPA, 2007a, 2012, 2017b).

### Trophic state determination

The NLA uses chlorophyll-a concentration as a proxy for trophic state, while acknowledging that trophic state is actually determined by a variety of characteristics including nutrients, climate, morphometry, etc. Oligotrophic lakes have chlorophyll-a concentrations ≤ 2 µg L-1. Mesotrophic lakes have concentrations greater > 2 µg L-1 and ≤ 7 µg L-1. Eutrophic lakes have concentrations > 7 µg L-1 and ≤ 30 µg L-1. And hypereutrophic lakes have concentrations > 30 µg L-1 (USEPA, 2022b).

## Data analyses

### Limitation calculation

Nutrient limitation for lakes can fall into three categories: P-limited, N-limited, or co-nutrient limited. To calculate limitation, we used the full dataset median nutrient concentrations in each ecoregion as a threshold of healthy nutrient level. Additionally, we used the logged average molar N:P ratio in each ecoregion and in each survey year. Median N and median P concentrations were not statistically different from the reference lake medians (p = 0.80 and p = 0.70), respectively). Average molar N:P was used as a threshold similar to the method in Downing and McCauley (1992), where they found N limitation occurring in lakes with N:P less than the world lake average N:P. Here, we averaged by ecoregion and survey year to account for the large variation in stoichiometric data that occur across the data (mean = 59.49, standard deviation = 113.49).

### Statistical analyses

All data analyses were performed in the R programming language (R Core Team, 2022) with heavy reliance on the tidyverse package suite for data wrangling and visualization (Wickham et al., 2019) and maps were created using the sf package (Pebesma, 2018). To assess temporal shifts, we used the change.anlaysis function in the spsurvey package (Dumelle et al., 2022). Using this, we assessed changes from 2007-2012 and 2012-2017 in nutrient stoichiometry within nutrient limitation and trophic statuses and changes in nutrient limitation within trophic status. Each of these were calculated at the national scale and the ecoregional scale. Reference lakes were not included in these analyses as they were not part of the random stratified sampling design and were not assigned weights. The change.analysis uses the lakes weights as defined by the stratified random survey design and provides the difference between response variables over chosen survey years along with the standard error. The change is statistically significant when the error bars do not cross zero (USEPA, 2022b).

To assess whether TN or TP was a better predictor of trophic state, chlorophyll-a was used as a proxy for trophic state. Both the response and predictor variables were logged, and mixed linear models were used. The r2 values were compared to determine which nutrient predicted chlorophyll-a better.

# Results

<https://docs.google.com/presentation/d/1Jn110Yb21PLA5kqE37ZVUrIQP30fNfXc/edit#slide=id.p1>

# Discussion

1. Summarize aim and main findings
2. Link findings to literature – some potentially useful things
   1. balancing nutrient stoichiometry may assist in eutrophication remediation (Stutter et al., 2018).
   2. 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. 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.
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).
   2. Use of TN, TP to determine limitation has been questioned in the past
   3. Limitation, trophic state likely depend on a lot of criteria – here we are using nutrient and chlorophyll concentrations as proxy measurements
      1. Many limitation calculations rely on experimental data to understand when additions of nutrients result in growth.
      2. Nutrient sources p940 downing and mccauley
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.

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