# Introduction

1. Enrichment of nitrogen (N) and phosphorus (P) are mainly responsible for eutrophication (Wetzel, 2001), one of the biggest, often anthropogenically-caused threats to freshwaters (Smith & Schindler, 2009) and biodiversity (Reid et al., 2019) across the globe.
   1. Eutrophication can have serious consequences on aquatic ecosystem health including decreased oxygen, formation of toxins, and changes in organismal communities (Camargo & Alonso, 2006).
   2. In the United States (US), an estimated $2.2 billion in annual losses because of eutrophication is likely an underestimate of the actual amount (Dodds et al., 2008)
      1. as determining economic value of freshwater is difficult with many important factors often excluded from these analyses (Keiser et al., 2019).
   3. Furthermore, many freshwaters across the US are generally at serious risk of or are already plagued by eutrophication (Dodds et al., 2008).
2. There is an ongoing debate about whether N or P is more important in causing eutrophication or which is the main limiting nutrient, and many studies focus solely on P or argue that P should be the top management priority (Carpenter, 2008; Ngatia & Taylor, 2019).
   1. Reasons supporting the P paradigm include factors like N-fixing bacteria that can increase N availability (Schindler et al., 2008), accumulation of P in soils and freshwater sediments (Bennett et al., 2001), potential for internal loading (Sun et al., 2022), past successful P reduction efforts (Foy, 2005), and geographic extent (e.g. focus on the northeast and Midwest US in (Liang et al., 2020).
   2. Furthermore, there is belief that P is primarily the limiting nutrient in freshwaters, while N is limiting in oceans (Correll, 1999), and that N-limitation in freshwaters is merely a result of P enrichment in eutrophic waters (Havens, 1995).
3. Despite the paradigm surrounding P, the importance of N is not unknown. In fact, some believe that most northern-hemisphere lakes were N-limited prior to an influx of N-deposition resulting in eutrophication and a shift toward P-limitation (Bergström & Jansson, 2006).
   1. Focusing on eutrophication management, P reduction alone is no longer an adequate solution. Rather, there is increased need for research on nutrient amounts, ratios, and N’s impact on eutrophication (Yao et al., 2018).
      1. Reductions in P pollution in large lakes may lead to accumulation of N (Finlay et al., 2013), negating the attempt to mitigate nutrient pollution.
      2. In the Western US, N-deposition can significantly alter ecosystems (Fenn et al., 2003).
      3. Projected precipitation patterns under a changing climate will only likely increase N loading into freshwaters worldwide (Sinha et al., 2017).
   2. Experiments have demonstrated how both nutrients together have an impact on productivity significantly more than additions of a single nutrient and how stoichiometrically imbalanced food can have detrimental impacts (Elser et al., 2011; Redoglio et al., 2022).
4. Because their cycles are coupled in the environment (Oviedo-Vargas et al., 2013), studying N and P in together terms of relative abundances may unfold large-scale patterns that would otherwise be unseen.
   1. Nutrient limitation can be defined using Liebig’s law of minimum, which states that organismal growth is limited by the resource or nutrient in lowest supply. And the relative abundance of these nutrients indicate the balance of the supply (Sterner & Elser, 2002).
      1. Limitation directly connects to eutrophication through nutrient drivers of primary productivity.
   2. Just as biological processes impact availability of nutrient supply and regulate global biogeochemical processes (Reiners, 1986), so too are the nutrient supply pools important as nutrient supply can limit biological function and growth (Sterner & Elser, 2002).
   3. Large-scale nutrient stoichiometry integrates biogeochemical processes like biotic dynamics, precipitation, geological weathering, and anthropogenic influences; and serves as the backdrop for many smaller-scale processes to occur (Sterner & Elser, 2002).
      1. Balancing nutrient stoichiometry may assist in eutrophication management, rather than focusing on a sole nutrient (Stutter et al., 2018).
5. In this study, we use US Environmental Protection Agency (EPA) National Lakes Assessment (NLA) data to evaluate differences in nutrient limitation across US lakes between survey years 2007 and 2017.
   1. This research is intended to support efforts to assess nutrient water quality and more effectively protect and restore waters from nutrient pollution.
   2. 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).
   3. Using these broad scale survey data, we aim to answer the following questions:
      1. Which nutrient correlates with eutrophication in lakes across ecoregions of the US?
      2. Which nutrient is limiting in lakes across ecoregions of the US?
      3. Where and when are shifts in nutrient limitation occurring?

# Methods

## NLA lakes and methods

NLA data from survey years 2007 and 2017 were used in the analyses (USEPA, 2010, 2022a). In 2007, 1156 lakes were surveyed, 95 were resampled in the same year and 124 were considered reference lakes. And in 2017, 1112 lakes were sampled, 97 were resampled in the same year, and 108 were considered reference lakes. 282 lakes were sampled in both survey years. In 2007, lakes greater than 4 ha were sampled. This changed in the later 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 (USEPA, 2022b). Stratification was based on Omernik level-3 aggregated ecoregions, state, and lake size. Discretizing the dataset into Omernik’s 9 aggregated ecoregions provides a qualitative understanding of spatial patterns and regional homogeneities (Omernik, 1987). 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, 2007a, 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, 2007b, 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-limitation, N-limitation, or co-nutrient limitation. To calculate the potential limitation category, we used both a nutrient concentration and a molar nutrient ratio. Page84 of usepa 2000 doc….

we used the 25th percentile total nutrient concentration in each ecoregion as a threshold of healthy nutrient level. These data were not statistically different from the reference lake median nor 75th percentile total nutrient concentrations for N and P (p = 0.218 and p = 0.177, respectively) (USEPA et al., 2000).

To calculate the potential limitation category, 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 nutrient stoichiometry 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

# Discussion

# References

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