# 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, which illustrates balanced growth of marine algal cells have a 106C:16N:1P molar ratio (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 (Carpenter et al., 2011).
   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
   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 (citation p. 3 technical doc) to randomly choose sampling sites.
      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
3. Sampling and laboratory methods
   1. Standardized sampling and laboratory protocols
4. Trophic state calculation
   1. p.80 technical doc
5. Statistical analyses
   1. R programming (cite)
   2. Ggploting (cite)

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