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The effect of freshwater aquaculture on epilimnetic phosphorus, nitrogen, and chlorophyll-a in oligotrophic boreal shield lakes

## Introduction

Inland seafood production, such as freshwater aquaculture, has been proposed as a sustainable solution as we work towards global food security (Costello et al., 2020; Garlock et al., 2022). In Canada, freshwater aquaculture is primarily based on rainbow trout (Oncorhynchus mykiss) production with the bulk of production (66% from 1986-2015) being conducted in Ontario (Rennie et al., 2019). However, the expansion of freshwater aquaculture has been limited due to the many unknowns surrounding environmental impacts and nutrient loading (Yan, 2005). Phosphorus is an essential nutrient that drives the growth of aquatic plants and algae. It is considered a limiting nutrient in aquatic ecosystems, and is introduced through natural processes such as rock and soil erosion in small quantities (ECCC, 2010). Nitrogen is another nutrient essential for plant growth, whose effects are closely related to phosphorus. Eutrophication is a process associated with increased phosphorus and nitrogen loads. Several studies hypothesize that cage aquaculture may increase lake productivity because aquaculture wastes from leftover fish feed and extra fish waste contain phosphorus and nitrogen (Bristow et al., 2008; Findlay et al., 2009). Chlorophyll-a is often used as a proxy for algae biomass, allowing it to be used to determine the effects of aquaculture on lake primary productivity (Findlay et al., 2009). The purpose of this project is to determine how phosphorus, nitrogen, and chlorophyll-a levels are impacted by the introduction of an aquaculture farm into a freshwater lake. We hypothesize that rainbow trout aquaculture in freshwater lakes will increase phosphorus, nitrogen, and chorlophyll a concentrations in the epilimnion layer, as aquaculture involves large nutrient inputs which can increase productivity of primary producers such as algae.

## Methods

The data consists of water chemistry data collected from Lake 375 of the IISD-Experimental Lakes Area (ELA) in Ontario, Canada. An experiment to determine the whole-ecosystem impacts of freshwater aquaculture was conducted in Lake 375 between 2003-2007. Water samples were taken from the thermal epilimnion from 1982-2022 (Sandilands & Fafard, 2020). The data set was initially explored using time series and histogram plots of each variable. A new column representing the stage of aquaculture treatment for a given year was added (pre-2003 "Before", 2003-2007 "During", post-2007 "After"). The initial dataset was subsetted to remove all years in the after the aquaculture due to the lack of data. The subsetted data set was used for all statistical analyses. Following the initial data exploration, ANOVAs, Linear Mixed Models (LMM), and ARIMAs were run for model selection. For ANOVAs and LMMs the dependent variable was the measured concentration for each characteristic, and the independent variable was the treatment. The LMMs were run with year as a random variable. The ARIMAs proved to have the lowest AICc scores, and the LLMs had lower or equal AICc scores to the ANOVAs. Observing this, we used a permutation test to remove the impact of dependency and account for the non-normal distribution for each variable. We selected variables related to our hypotheses and ran LMMs, Permutation Tests, and ARIMAs. The permutation tests for each variable isolated the result value and its corresponding treatment. The treatment was reshuffled 1000 times with replacement, and the difference in means of the before and during treatments were plotted in a histogram to visualize a null distribution and compared to the difference in means from the dataset. For the ARIMAs, the "Forecast" package in R was used.

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The "auto. arima" function was used to fit the best ARIMA model given the data. The forecast was plotted 20 timesteps into the future.

## **Results**

The LMMs generated for all three variables reported that the aquaculture experiment had a significant effect (p < 0.001) on mean nutrient or chlorophyll-a concentration (Figure 1A, Figure 2A, Figure 3A). For particulate phosphorus (partP), the LMM reported that relative to the lake before aquaculture, partP concentrations are higher by 3.81712µg/L during aquaculture (Figure 1A). The random effect of year on partP accounted for 17% of the variation between partP observations (Figure 1A). According to the LMM, particulate nitrogen (partN) concentrations were predicted to increase by 42.0569µg/L while aquaculture took place (Figure 2A). The random effect of year contributed to 4.7% of the variation between observations for partN (Figure 2A). Likewise, CHLA according to the LMM, concentrations are 2.53238ug/L higher during aquaculture (Figure 3A). Similar to partN, the random effect of year contributed only a small portion (1.4%) to the variation seen in the observations (Figure 3A). All permutations demonstrated a significant difference in the true difference of means between the two groups compared to the null distribution (Figure 1B, Figure 2B, Figure 3B). ARIMA forecasting for all variables indicated a marked increase in concentration when given observations from before and during aquaculture experiment, in comparison to the predictions generated when only observations from before the experiment were considered (Figure 1C, Figure 2C, Figure 3C). PartP and CHLA both increased in concentration but there was no increasing or decreasing trend associated with the future prediction (Figure 1C(ii), Figure 2C(ii)). In contrast, the ARIMA predicted that partN concentrations would increase over time (Figure 2C(ii)).

## **Discussion**

Lake 375 is an oligotrophic and dimictic lake that is low in nutrients, is very cold and deep, and undergoes complete mixing twice a year when the thermal stratification between the surface and bottom layers become negligible (Azevdeo et al., 2011). These characteristics result in the productivity of the lake becoming severely limited by nutrient load, and many lake phytoplankton species have adapted to severe P and N deficiencies as a result (Hecky et al., 1993). Excessive input of P into these lakes creates risks of cyanobacteria blooms and may result in eutrophication (Molot et al., 2021). Despite the significant effects found in the tests of P, N, and CHLA, algae populations were lower than expected and eutrophication did not occur in Lake 375 (Findlay et al., 2009). Algal blooms only occurred during the seasonal thermal mixing, and the intensified effects only lasted a few days (Paterson et al., 2010). This can be attributed to the rapid sedimentation of the introduce P, which became unavailable to phytoplankton at the epilimnion during the stratified season, and thus do not have the resources available to cause a bloom (Azevdeo et al., 2011; Paterson et al., 2010). Even if eutrophication did not occur, there were still many effects of the aquaculture that damaged the lake ecosystem. Species were adapting to the influx in nutrients, as the  $\Box^{13}$ C and  $\Box^{15}$ N signatures of the native species were shifting towards that of the aquaculture waste, and the minnows were progressively consuming more feed as the study progressed (Kullman et al., 2009). It was also noted that the high concentrations of additional nutrients from the aquaculture had lethal effects on benthic species. Experiments showed 100% mortality in Sphaerium simile exposed to the sediment sourced directly underneath the cage (Kullman et al., 2007).

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# **Appendix**

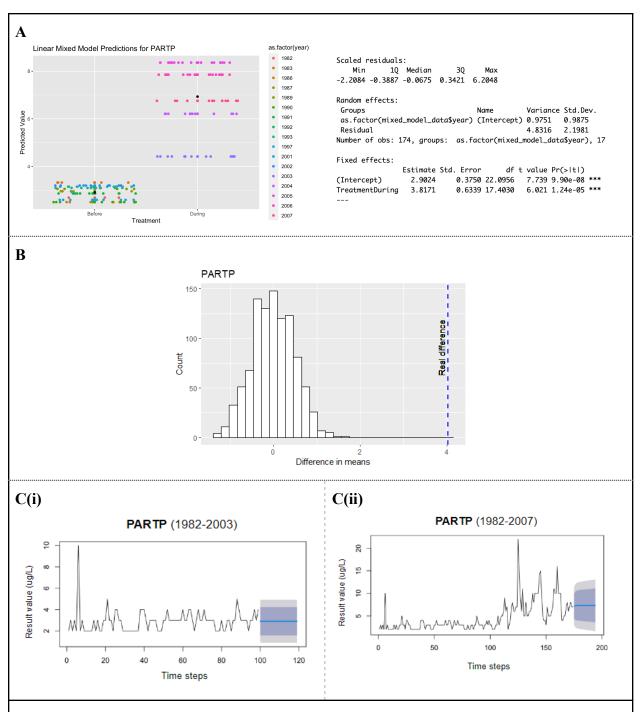


Figure 1. Data analysis for particulate phosphorus concentrations in the epilimnion layer of Lake 375. A) Linear mixed model depicting effect of Treatment (before aquaculture, after aquaculture) on concentration of partP in the epilimnion layer of Lake 375. Year was included as a random effect. Treatment has a significant effect on partP concentration (p < 0.001) with a coefficient of 3.81712. Year accounts for 17% of variation in partP observations. B)

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Comparison of difference of means in the concentration of partP in years during aquaculture and in the years before aquaculture. This difference was compared with the null distribution to determine significance. Real difference of means = 4.02. C) ARIMA forecasting for partP. C(i) is the prediction made when the model is only provided observations from before the aquaculture experiment. C(ii) is the prediction made when the model is provided with observations from before and during the aquaculture experiment. ARIMA predicts 20 timesteps into the future for both models.

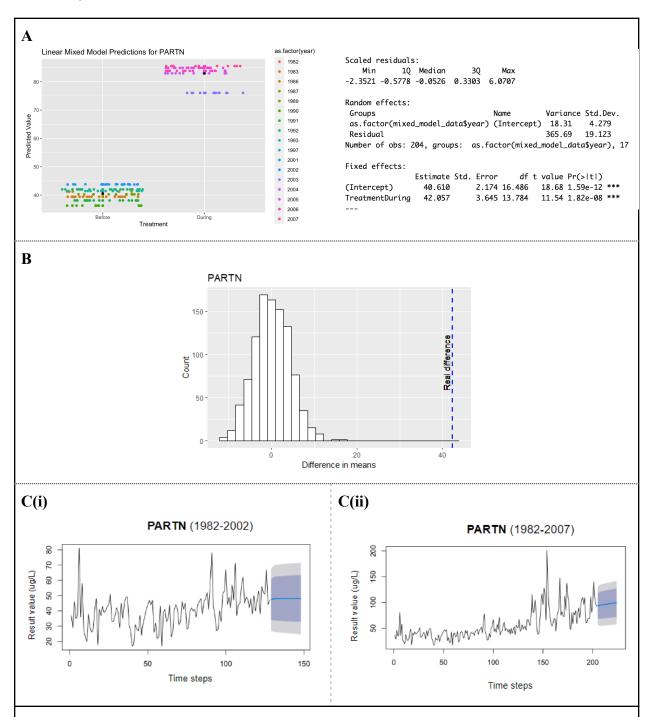


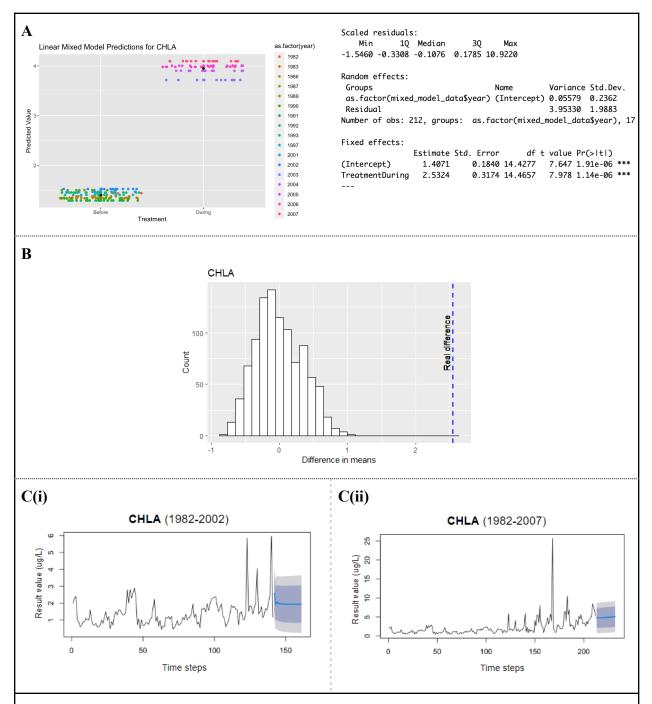
Figure 2: Data analysis for particulate nitrogen concentrations in the epiliminion layer of Lake 375. A) Linear mixed model depicting effect of Treatment (before aquaculture, after aquaculture) on concentration of partN in the epilimnion layer of Lake 375. Year was included as a random effect. Treatment has a significant effect on partN concentration (p < 0.001) with a coefficient of 42.0569. Year accounts for 4.7% of variation in partN observations. B) Comparison of difference of means in the concentration of partN in years during aquaculture and in the years before aquaculture. This difference was compared with the null distribution to

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determine significance. Real difference of means = 42.3. C) ARIMA forecasting for partN. C(i) is the prediction made when the model is only provided observations from before the aquaculture experiment. C(ii) is the prediction made when the model is provided with observations from before and during the aquaculture experiment. ARIMA predicts 20 timesteps into the future for both models.

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**Figure 3: Data analysis for chlorophyll-a concentrations in the epiliminion layer of Lake 375. A)** Linear mixed model depicting effect of Treatment (before aquaculture, after aquaculture) on concentration of CHLA in the epilimnion layer of Lake 375. Year was included as a random effect. Treatment has a significant effect on CHLA concentration (p < 0.001) with a coefficient of 2.53238. Year accounts for 1.4% of variation in CHLA observations. **B)** Comparison of difference of means in the concentration of CHLAin years during aquaculture and in the years before aquaculture. This difference was compared with the null distribution to determine significance. Real difference of means = 2.55. **C)** ARIMA

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forecasting for CHLA. C(i) is the prediction made when the model is only provided observations from before the aquaculture experiment. C(ii) is the prediction made when the model is provided with observations from before and during the aquaculture experiment. ARIMA predicts 20 timesteps into the future for both models.