EFFECTS OF NITRATE LEVELS ON THE DISSOLVED OXYGEN LEVELS IN THE OTTAWA RIVER

to

The GLOBE Program

by

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<u>Abstract</u>

Dissolved oxygen is crucial to aquatic life as organisms need it to breathe and survive in their environment, and is also a way to determine the condition of an ecosystem due to this fact. Nitrate is also critical for aquatic animals as they serve as a vital nutrient for aquatic plants, which produce oxygen through photosynthesis, and are an important food source for many freshwater animals. However, eutrophication can sometimes occur with excess nitrate levels and, thus, the concentration of nitrates are a vital aspect of an aquatic ecosystem's health. This study examines the relationship of nitrates on dissolved oxygen levels, and predicts that after nitrate levels cross 1 mg/L, the dissolved oxygen levels will drop. The study included 6 total samples, 3 of which were above 20ºC and 3 below 20ºC. All samples were taken from the Ottawa River. Overall, an inverse relationship between nitrates and dissolved oxygen levels were determined from the data collected.

Introduction

River ecosystems house a complex array of organisms, from fish to plants to other animals; they are, without a doubt, essential to the survival of many species (NOAA, 2024). As such, maintaining a proper balance ensures the ecosystem thrives. Out of the many factors concerning a river ecosystem, nitrates are one such factor that directly impacts aquatic plant growth, therefore indirectly impacting an aquatic ecosystem (EPA, 2012). This study aims to further explore the effects of nitrates on the amount of dissolved oxygen produced in the Ottawa River. Our study predicts that if the amount of nitrates exceeds 1 mg/L (the threshold for a natural amount of ammonia/nitrates in rivers), then the amount of dissolved oxygen will decrease, as the excessive amount of plant growth can be harmful to an ecosystem, thus decreasing the dissolved oxygen level.

Nitrates are one form of nitrogen found in terrestrial and aquatic ecosystems that serve as a nutrient to plants (EPA, 2012). They are fixed from atmospheric nitrogen into ammonia, which is then changed into nitrites and finally nitrates, the form of nitrogen plants can utilize. However, nitrates can also be introduced into an ecosystem through wastewater treatment plants, runoff from fertilized soil, runoff from animal manure storage, and things of that nature (EPA, 2012). In excess, nitrates can ultimately accelerate a process called eutrophication, potentially creating a hypoxic environment in the river, as well as becoming toxic to mammals (EPA, 2012). As a result, the amount of nitrates present in an ecosystem can change the levels of dissolved oxygen, temperature, and other indicators (EPA, 2012).

As mentioned, the levels of dissolved oxygen can be affected by the amount of nitrates present in an ecosystem. Dissolved oxygen describes the concentration of gaseous oxygen

present in water (EPA, 2024). Oxygen can enter a body of water in essentially two ways. One is by first-hand absorption of oxygen in the atmosphere (EPA, 2024). Additionally, water absorbs oxygen that is given off by plants during photosynthesis, which is crucial to aquatic life (EPA, 2024). This component can additionally be affected by temperature, rising as temperatures lower and vice versa (USGS, 2018). Dissolved oxygen is a great, versatile marker as it is used by all forms of aquatic life (USGS, 2018). Essentially, dissolved oxygen is an important indicator of the amount of oxygen available in an aquatic ecosystem for organisms and can indicate the overall health of an ecosystem.

The level of nitrates in an aqueous ecosystem can affect its overall well-being. Excess amounts of nitrates in an aquatic ecosystem lead to a phenomenon known as eutrophication. Nitrates, being a nutrient to plants, can stimulate algae growth and subsequently algae blooms. When algae die and decompose, oxygen is taken from the ecosystem and used, creating a hypoxic environment (USGS, 2018). This process decreases the amount of dissolved oxygen in an ecosystem. Eutrophication creates what's known as "dead zones", where oxygen levels are decreasing as it's being used, yet plants are unable to photosynthesize to make up for the loss, as the algae sit on top of the water (EPA, 2024). This, in turn, makes it impossible for aquatic life to survive and throws the ecosystem into havoc.

Research regarding the effects of dissolved oxygen on nitrates has been attempted. For example, a study done by Stenstrom and Poduska (1979) showed an inverse relationship between the two, with dissolved oxygen as the independent variable. Additionally, research done by Moore (2022) used nitrate levels as a proxy to determine ecosystem health based on the amount of dissolved oxygen. Hence, more research should be conducted to examine the effects of

nitrates on dissolved oxygen directly. This study is undertaken in the following context. The nitrate, dissolved oxygen, and temperature levels in the Ottawa River, Ohio, were recorded in the months of November and December. The data collected will be used to determine the effects of nitrate concentrations on dissolved oxygen in a river ecosystem.

Methods and Materials

Materials:

- ≥ 50 mL of the sample water

- CHEMets Kits (Dissolved Oxygen K-7512)

- La Motte Nitrate Nitrogen Low Range Comparator (Code 3615-01)

- 1 Thermometer

Methodology:

- Temperature

  1. Fill a container with collected sample water.

  2. Put the thermometer into the container with sample water, making sure it doesn't touch the glass.

  3. Wait 30 seconds or until the reading is stable.

  4. Record the temperature in degrees Celsius.

- Nitrates (LOW RANGE: 0-1.0 ppm Nitrate Nitrogen)

  1. Fill the water sampling bottle (0688) with sample water.

  2. Slide the Nitrate-Nitrogen Low Range Comparator Bar (3614-01) into the Low Range Comparator Viewer (1102). Fill one test tube (0898) to the 10mL line with sample water. Place in Low Range Comparator.

3. Fill one test tube (0898) to the lower line (5 mL) with sample water.

4. Dilute to second line with *Mixed Acid Reagent (V-62798). Cap and mix.

5. Wait 2 minutes.

6. Use the 0.1g spoon (0699) to add one level measure (avoid any excess) of *Nitrate Reducing Reagent (V-6279).

7. The mixing procedure is extremely important. Cap tube. Invert the tube slowly and completely 30 times in 1 minute to ensure complete mixing.

8. Wait 10 minutes.

9. Insert test tube into Low Range Comparator (1102). Match sample color to a color standard. Record as ppm Nitrate-Nitrogen.

   NOTE: To convert to nitrate, multiply by 4.4. Record as ppm Nitrate.

- Nitrates (HIGH RANGE: 0-10.0 ppm Nitrate Nitrogen)

  1. Use the 0.5 mL pipette (0353) to add 0.5 mL of the water sample to a test tube (0898).

  2. Slide the Nitrate-Nitrogen Low Range Comparator Bar (3614-01) into the Low Range Comparator Viewer (1102). Fill one test tube (0898) to the 10mL line with sample water. Place in Low Range Comparator.

  3. Add Distilled Water to the lower line (5 mL).

  4. Dilute to second line with *Mixed Acid Reagent (V-6278). Cap and mix.

  5. Wait 2 minutes. Use the 0.1g spoon (0699) to add one level measure (avoid any excess) of *Nitrate Reducing Reagent (V-6279).

6. The mixing procedure is extremely important. Cap tube. Invert the tube slowly and completely 30 times in 1 minute to ensure complete mixing.

7. Wait 10 minutes.

8. Insert test tube into Low Range Comparator (1102). Match sample color to a color standard. Multiply the reading by 10. Record as ppm Nitrate-Nitrogen.

   NOTE: To convert to nitrate, multiply by 4.4. Record as ppm Nitrate.

   *Reagent is a potential health hazard.

- Dissolved Oxygen (Indigo Carmine Method 1-12 ppm)

   1. Fill the sample cup to the 25 mL mark with the sample to be tested.

   2. Place the ampoule, tip first, into the sample cup. Snap the tip by putting the tip into the crevice and pushing down until it snaps. The ampoule will fill, leaving a bubble for mixing.

   3. To mix the ampoule, invert it several times, allowing the bubble to travel from end to end.

   4. Dry the ampoule. Obtain a test result 2 minutes after snapping the tip.

   5. Obtain a test result by placing the ampoule between the color standards until the best color match is found.

Presentation of Data and Results

Raw Data Table

Date	Dissolved Oxygen (in ppm mg/L)	Temperature (in ºC)	Nitrates (in ppm mg/L)
5/15/2001	7.7	16.0	0.68
11/26/2024	5.0	6.00	1.32
12/02/2024	7.0	1.00	0.88
7/17/2008	8.8	22.1	0.30
6/30/2019	8.0	24.0	0.00
5/31/2023	5.5	22.3	0.00

Figure 1

Figure 2

Dissolved Oxygen Level vs. Nitrate Level
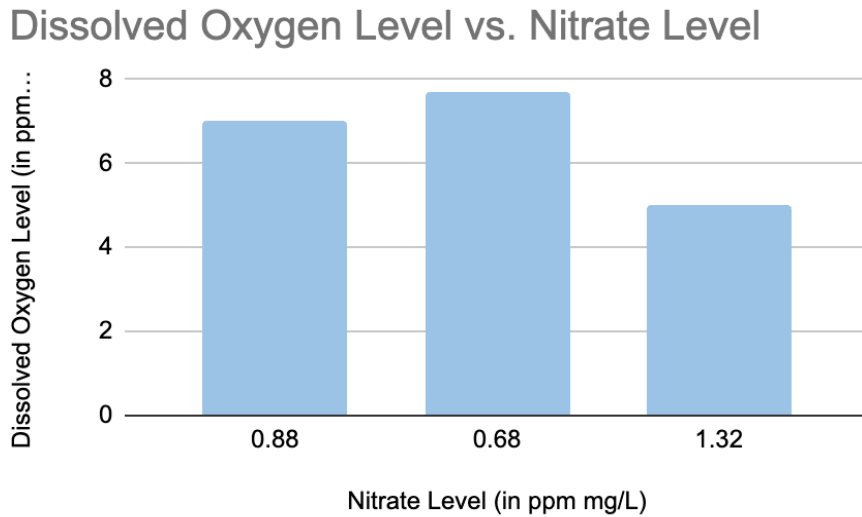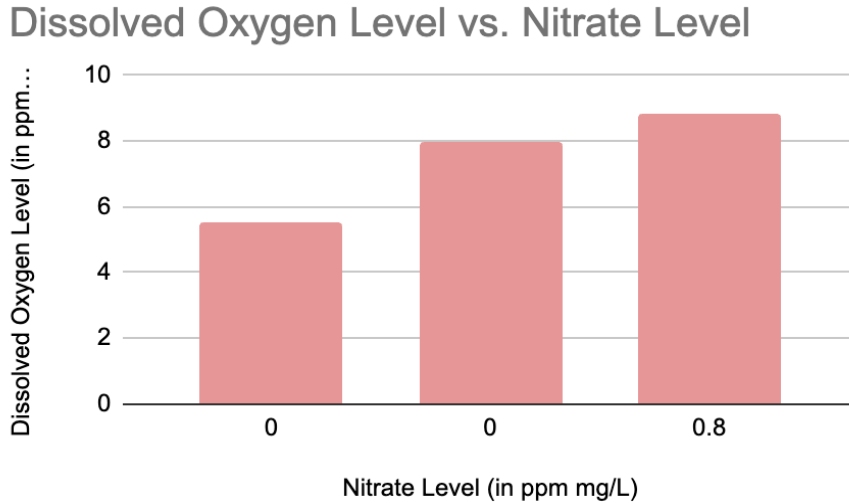
Figure 1 shows the relationship between dissolved oxygen and nitrate levels in samples under 20ºC, while Figure 2 represents the same relationship with samples over 20ºC. Research shows that 20ºC to 25ºC is the range of dissolved oxygen's critical temperature, where the level of dissolved oxygen changes dramatically due to temperature alone. Therefore, the data set was divided into a set below 20ºC and above 20ºC for a more accurate analysis. Using three samples for each graph, a relationship can be determined.

In tested samples under 20ºC, the first had a nitrate level of 0.88 ppm mg/L and 7.0 ppm mg/L of dissolved oxygen. In the second, the sample's nitrate level was at 0.68 ppm mg/L, and its dissolved oxygen was 7.7 ppm mg/L, the highest of that temperature range tested. The last sample contained 1.32 ppm mg/L of nitrates and 5.0 ppm mg/L of dissolved oxygen. The trends of these samples represent an inverse relationship between dissolved oxygen and nitrate levels.

In samples over 20ºC, the first and second samples both had 0 ppm mg/L of nitrates, but

they were tested at different temperatures, with the former at 22.3ºC and the latter at 24ºC. The

last sample was tested to have 0.8 ppm mg/L of nitrates and 8.8 ppm mg/L of dissolved

oxygen. The graph's trend shows a direct relationship between dissolved oxygen and nitrate

levels.

Statistical Analysis of Data

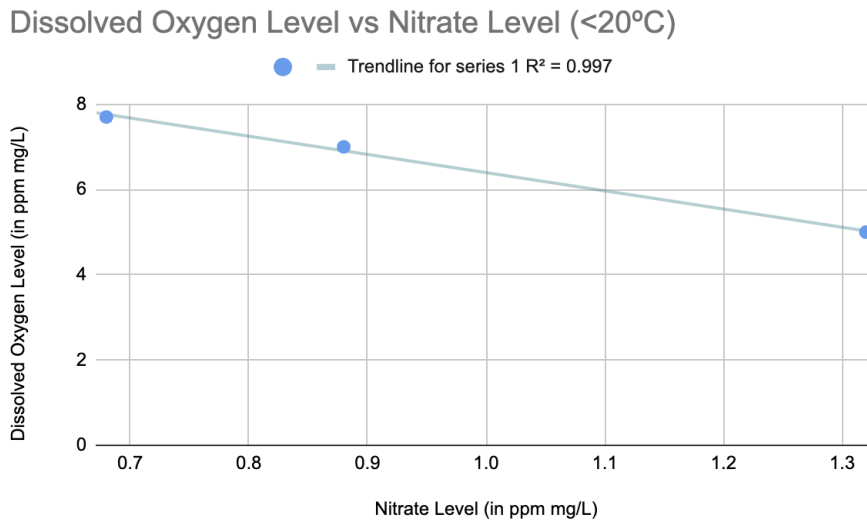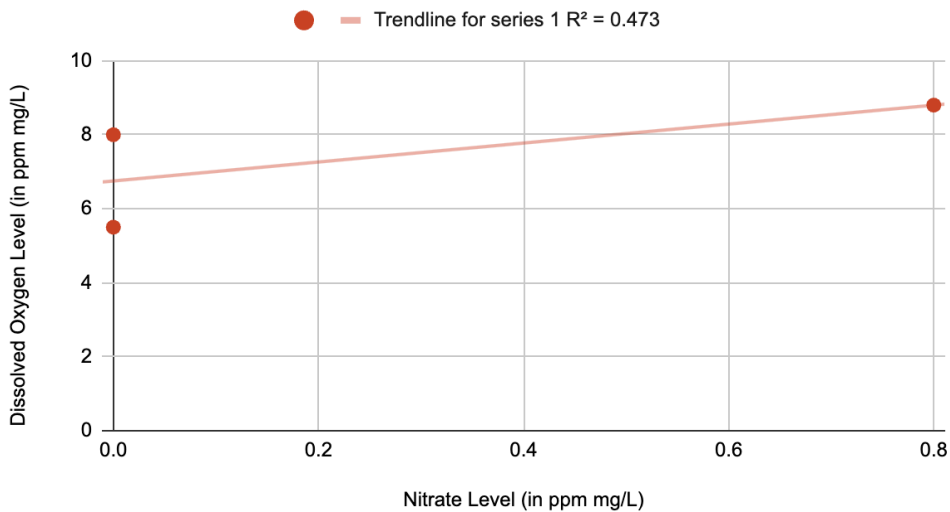**Figure 3**

Dissolved Oxygen Level vs Nitrate Level (<20ºC)

Figure 4

The results suggest a direct correlation between nitrate level and dissolved oxygen in temperatures above 20°C (typically with nitrate levels below 1 mg/L), and an inverse relationship in temperatures below 20°C (typically with higher nitrate levels, close to or above 1 mg/L). To further examine these results, a scatter plot was made, and a line of best fit was graphed to determine the strength of the relationship. As shown in Figure 3, the line of best fit showcases a negative downward trend, indicating an inverse relationship between the independent and dependent variables. Therefore, as the nitrate concentration got closer to and surpassed 1 mg/L, the dissolved oxygen level decreased. This was shown to be a scientifically significant relationship, with an r^2 value of 0.99. This essentially tells us that 99% of the variability in the data is explained by the independent variable. Figure 4 shows an upward slope regarding the line of best fit. Based on the line, as the nitrate concentration started

approaching 1 mg/L, the dissolved oxygen level also increased. However, the r^2 value was 0.47, showing a nearly low correlation between the independent variable and dependent variable. This could have been due to the limited power of a small sample size, as well as possible errors during measurements.

This study predicted that if nitrate levels rose above 1 mg/L, dissolved oxygen levels would decrease. Overall, the data collected seems to support this hypothesis. At higher nitrate levels, there is an inverse trend between the variables, and at lower nitrate levels, a direct correlation is shown. However, only the inverse relationship is considered to be statistically significant, and more research is needed to be conducted on nitrate levels below 1 mg/L to draw a definite conclusion. The results of how nitrate levels over 1 mg/L affect dissolved oxygen support previous literature and research conducted.

Conclusion

In this project, the data collected provided evidence that nitrate levels affect dissolved oxygen levels inversely in nitrate levels over 1 mg/L. Evidence suggests this relationship as nitrate levels tested in samples under 1 mg/L tended to have an increase in dissolved oxygen levels with a lower nitrate concentration. This relationship was shown to be statistically significant, further supporting the correlation between the independent and dependent variables. In a sample tested with over 1 mg/L of nitrates, the level of dissolved oxygen was drastically less than samples with under 1 mg/L of nitrates. In fact, the sample had the lowest amount of dissolved oxygen, albeit the higher nitrate levels. However, this relationship nearly had a low correlation.

Discussion

The relationship of nitrates and dissolved oxygen is shown to be an inverse one in nitrate levels near or above 1 mg/L. This relationship is key to understanding the health of an ecosystem, as well as possible factors that throw ecosystems out of balance. If this study were to be repeated, a larger sample size would be incorporated to get a more definite result, as well as less variation in when the samples were collected.

<u>Acknowledgments</u>

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