

**Assessing the Environmental Health of Salish Creek: An Analysis of Water Quality for
Salmon**

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Abstract

This study investigates potential variations in pH, dissolved CO₂ (mg L⁻¹), oxygen levels (mg L⁻¹), and temperature (°C) across the years 2021, 2022, and 2023 in Salish Creek, offering valuable insights into the context of Salmon development. The analysis employed the Welch test for each metric, accompanied by post hoc Games-Howell tests, utilizing data collected during the fall of 2021, 2022, and 2023 by the Biol 342 class under the guidance of Professor Leander. Results reveal a significant difference in pH and oxygen levels, while no significant difference was observed for CO₂ or temperature. Post hoc tests indicate a noteworthy distinction in pH levels between 2021 and 2022, as well as 2021 and 2023. Furthermore, a significant difference in oxygen levels was identified between 2021 and 2022.

Assessing the Environmental Health of Salish Creek: An Analysis of Water Quality for Salmon

Salish Creek, located in the Pacific Spirit Forest at The University of British Columbia, is home to diverse fish species, including Cutthroat trout and Coho Salmon. In 2018, Metro Vancouver spearheaded a project to restore the stream, aiming to enhance its environmental suitability for its fish species (Ho, 2018). Yet, no publicly available data or analysis exists regarding the ecosystem's health before or after this restoration effort. This research aimed to assess Salish Creek's health by examining key water quality metrics, including pH, dissolved CO₂ concentration (mg L⁻¹), oxygen levels (mg L⁻¹), and temperature (°C), exclusively within the context of Coho Salmon. To achieve this, I conducted a Welch test (due to unequal variances) for each metric, systematically investigating variations across time (2021, 2022, 2023).

Literature Review

Although I conducted a generalized assessment of water quality, my focus remains predominantly on salmon within the literature review and research context. There's an abundance of literature available for Atlantic salmon, largely attributed to its extensive farming,

whereas there is comparatively less available for Pacific salmon. Consequently, I utilized existing literature to explore the general relationships between various water metrics and the development of salmon during their freshwater stages.

Dissolved CO₂ Concentration and Salmon Growth

Numerous studies have explored the relationship between CO₂ water levels (mg L⁻¹) and the developmental stages of salmon in freshwater environments. Concerning Atlantic salmon (*Salmo Salar*), Khan et al. (2018) revealed a negative linear relationship between CO₂ levels and production performance. Their findings indicated that acute CO₂ exposure in freshwater leads to diminished aerobic capacity, hinders growth, and adversely affects conversion efficiency. Graff et al. (2002) emphasized elevated CO₂ levels as a challenging factor for bone mineralization in salmon (*Salmo salar* L.) during the smoltification stage. Additionally, various studies have aimed to identify the optimal CO₂ levels for salmon growth. For instance, Gil Martents et al. (2006) identified a range of 15 to 20 mg L⁻¹ CO₂. The Norwegian Ministries of Fisheries and Coastal Affairs (2004) also endorsed an optimal concentration of 15 mg L⁻¹, a consensus derived from multiple experiments, including those conducted by Fivelstad et al. in 1999, 2003a, and 2003b (Fivelstad et al., 2015). In discussing the observed CO₂ levels at Salish Creek, I drew upon insights from the literature on these optimal CO₂ conditions.

pH Levels and Salmon Growth

In an experiment led by Fivelstad et al. (2004), Atlantic salmon smolts were exposed to different pH levels in freshwater for 35 days, ranging from normal (control group) to slightly acidic and more acidic conditions. The study found that the acidity in the water did not significantly impact gill structures, growth, or mortality during the freshwater stage. However, in the low-pH group, there was an increase in blood thickness and a decrease in salt levels in the blood three months after being transferred to seawater, suggesting potential long-term effects of acidic conditions on salmon physiology. This complements Kennedy & Picard's (2012) findings,

where exposure to low pH water during the freshwater life stage of Pacific salmonids led to adverse effects, including elevated plasma cortisol concentrations, lower body mass, and decreased seawater tolerance. It is noteworthy that both studies utilized a pH environment of 6.8 for the control group, emphasizing this level as conducive to salmon development, while anything below it begins to suggest critical conditions. Highlighting the significance of the pH environment, these studies underscore the necessity of comprehending the diverse impacts of acidity on various salmon species during critical life stages.

Oxygen (O₂) levels, Temperature, and Salmon Growth

The metabolic rate of ectotherms is primarily influenced by temperature, and the interplay between dissolved oxygen (mg L⁻¹) and temperature in aquatic environments is closely connected. Elevated temperatures raise the metabolic rate, leading to higher oxygen demand, while simultaneously decreasing the solubility of oxygen, which diminishes its availability (Pörtner & Knust, 2007). In exploring hypoxia tolerance thresholds for Atlantic salmon, Remen et al. (2013) discovered that the limiting oxygen saturation (LOS), defined as the oxygen level at which fish can no longer sustain routine metabolic processes, increased exponentially with temperature. The study's outcomes revealed mean (\pm SE) LOS values at 6, 12, 16, and 18 °C as 30 ± 1 , 39 ± 1 , 47 ± 1 , and $52 \pm 2\%$ of air saturation respectively. Stehfest et al. (2017) delved deeper into the interplay between oxygen and temperature in salmon. Their findings revealed that while salmon prefer temperatures of 16.5 to 17.5 °C, they prioritized avoiding low dissolved oxygen (<35% saturation), even when temperatures were within the preferred range but oxygen was insufficient. Notably, the salmon avoided not only low dissolved oxygen but also warmer surface waters (>20.1 °C). These findings bear significance, particularly illuminated by experiments such as those conducted by Burt et al. (2012), showcasing the repercussions of hypoxia. Their research suggested that intermittent hypoxia in Atlantic salmon results in diminished mass gain and compromised immune function, underscoring the critical impact when

dissolved oxygen levels fall below 6 mg L⁻¹. This threshold aligns with various pieces of literature, including works by Davis (1975) and Remen et al. (2012), emphasizing the consistency of this crucial oxygen concentration threshold. Given the vital role of these abiotic factors in salmon development, it is crucial to thoroughly examine the evolving measurements of dissolved oxygen and temperature in Salish Creek over the years.

Methods

Data Collection

The methods employed for data collection on each abiotic factor extend beyond the scope of this paper. I did not take part in the data collection for the years 2021 and 2022; my involvement was exclusive to the data gathered in 2023. While the methodologies employed will not be delved into, it is crucial to note that they were conducted under the meticulous guidance of Professor Leander, adhering to rigorous standards and characterized by both reliability and randomization.

In terms of the data characteristics, the data is cross-sectional information recorded predominantly over a week each fall, specifically in September. Notably, distinct sample sizes were collected annually, aligning with the class size for each academic year. To address any discrepancies in sample sizes, proper statistical methodologies were diligently applied.

Statistical Methodology

The Welch test is regarded as the preferred alternative to ANOVA analysis in the presence of variance heterogeneity (Jan & Shieh, 2014). In an unbalanced experimental design, particularly when sample sizes vary across testing groups, unequal variances can arise. This violation of one of the assumptions of ANOVA can diminish statistical power and lead to potentially inaccurate findings. Unless there is a notable similarity in standard deviations across unequal sample sizes, using the Welch test is advisable (Jan & Shieh, 2014; McDonald, 2014). In this study, I employed the Welch test to enhance the robustness of my analysis, considering

the variability in sample sizes across years for each abiotic factor. In instances where significant differences were detected across all groups, a post hoc Games-Howell test was conducted for further examination. This post hoc test is the preferred choice when the assumption of homogeneity of variance across samples is not met (McDonald, 2014).

Results

The ensuing five figures present box plots illustrating the distribution of water quality metrics—namely, pH level, oxygen levels, CO₂ levels, salinity, and temperature—across the years 2021-2023. Each boxplot delineates the 25th and 75th quartiles, median, mean, and highlights points of significance identified through the Welch test and subsequent Games-Howell post hoc test.

Comparison of pH Levels at Salish Creek for Years 2021, 2022, 2023

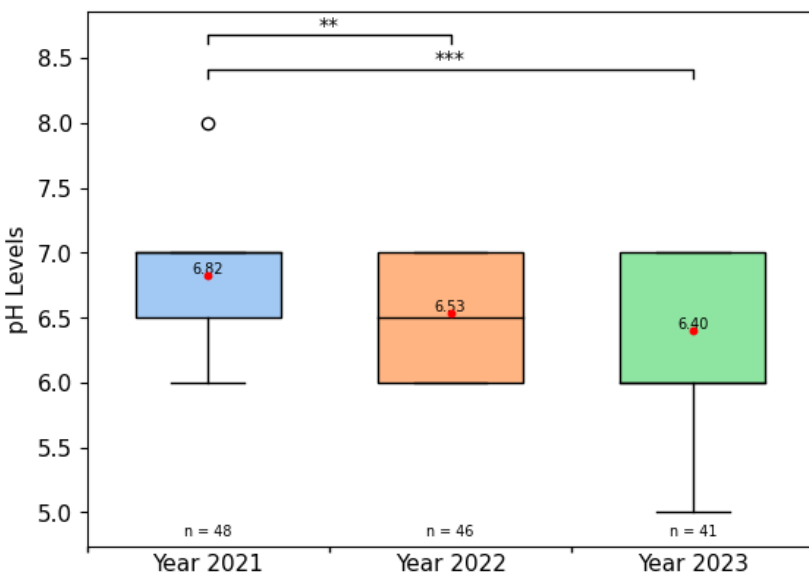


Figure 1. Comparison of pH Levels at Salish Creek for Years 2021, 2022, 2023. The boxplot represents the 25 and 75% interquartile percentiles, and illustrates the distribution of pH levels for the years 2021, 2022, and 2023. Red dots accompanied by annotated values represent the

mean values, while the central black line within each box represents the median. A significant difference in oxygen levels between the year 2021 and 2021 ($P < 0.01$) was found and also between the year 2021 and 2023 ($P < 0.001$)

Comparison of Oxygen Levels at Salish Creek for Years 2021, 2022, 2023

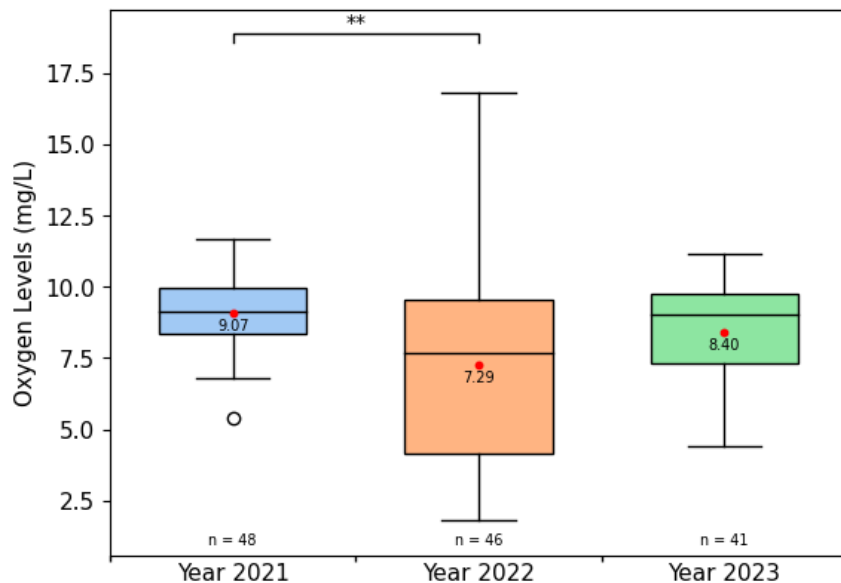


Figure 2. Comparison of Oxygen levels (mg L^{-1}) Levels at Salish Creek for Years 2021, 2022, 2023. The boxplot represents the 25 and 75% interquartile percentiles, and illustrates the distribution of oxygen levels for the years 2021, 2022, and 2023. Red dots accompanied by annotated values represent the mean values, while the central black line within each box represents the mean. A significant difference in oxygen levels between year 2021 and 2021 was observed ($P < 0.01$).

Comparison of CO₂ Levels at Salish Creek for Years 2021, 2022, 2023

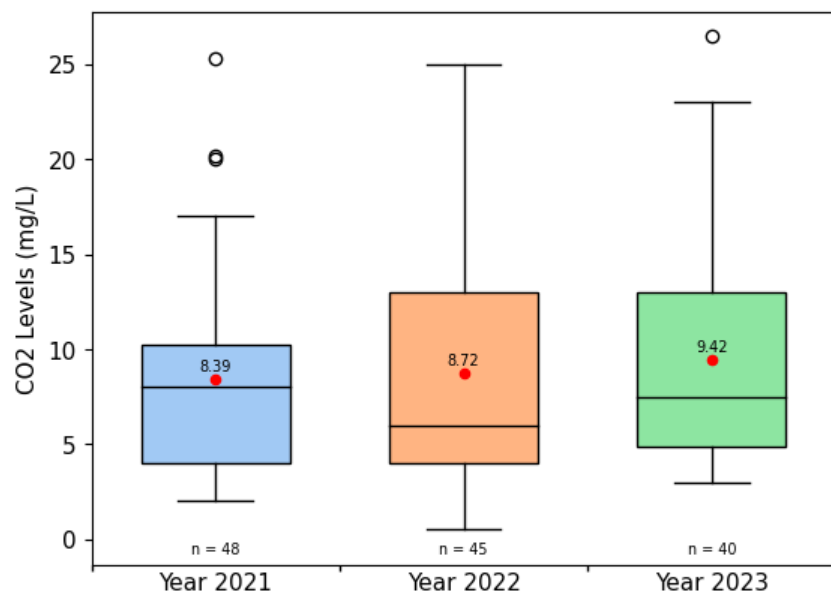


Figure 3. Comparison of CO₂ (mg L⁻¹) Levels at Salish Creek for Years 2021, 2022, 2023. The boxplot represents the 25 and 75% interquartile percentiles, and illustrates the distribution of CO₂ levels for the years 2021, 2022, and 2023. Red dots accompanied by annotated values represent the mean values, while the central black line within each box represents the mean. No significant differences in CO₂ levels are observed among the three years (P>0.05).

Comparison of Temperature (°C) at Salish Creek for Years 2021, 2022, 2023

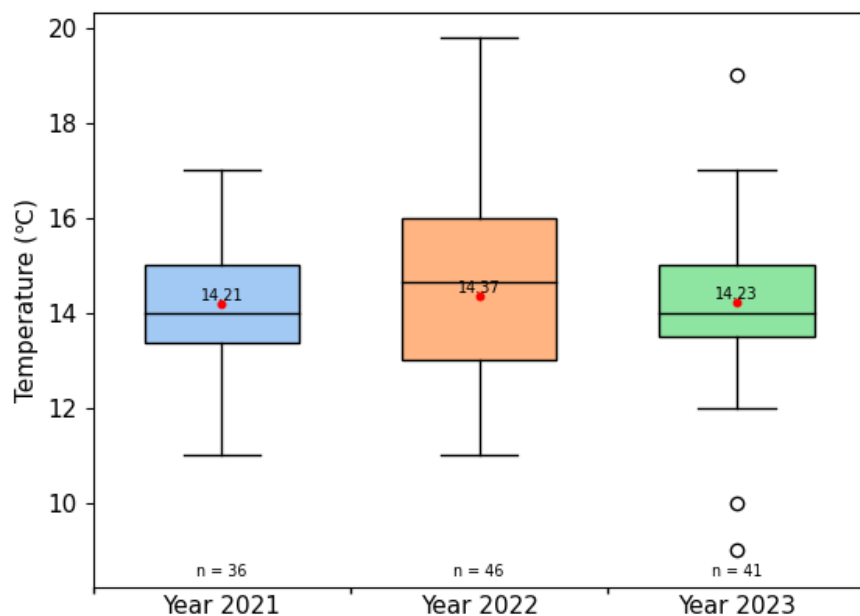


Figure 4. Comparison of Temperature (°C) Levels at Salish Creek for Years 2021, 2022, 2023.

The boxplot represents the 25 and 75% interquartile percentiles and illustrates the distribution of CO₂ levels for the years 2021, 2022, and 2023. Red dots accompanied by annotated values represent the mean values, while the central black line within each box represents the mean. No significant differences in temperature levels are observed among the three years ($P > 0.05$).

Discussion

The pH analysis shows notable differences between the years 2021, 2022, and 2023. Specifically, post hoc testing indicates significant disparities between 2021 and 2022, as well as between 2022 and 2023. The p-values further highlight the statistical significance of these differences, with values less than 0.01 for 2022 and less than 0.001 for 2023. Although the primary focus did not involve directional testing, a visual examination of box plots suggests a consistent decline in mean pH after 2021, culminating in a notable trough in the lower whisker of the 2023 data, recording a pH value of 5.0. This raises concern, as the literature consistently

emphasizes optimal pH ranges for control groups in the vicinity of 6.6-6.8, suggesting suboptimal conditions for salmon development (Fivelstad et al., 2014; Kennedy & Picards, 2012). The analysis signals a potential downward trajectory in pH levels at Salish Creek, necessitating further analyses, such as regression examinations, to validate and determine the significance of this trend. Vigilant monitoring and proactive measures may be imperative if the downward trend persists.

Oxygen levels in the water exhibit a more consistent pattern compared to pH results, with a notable distinction found only between the years 2021 and 2022, characterized by a p-value less than 0.01. Surprisingly, for both the comparisons between 2021 and 2023, and 2022 and 2023, no significant differences in the means were detected. This presents a more optimistic outlook than the pH findings, suggesting that while there was a significant deviation in the subsequent year after 2021, the oxygen levels returned to a comparable range in 2023. Notably, the distribution of points for the year 2022 is remarkably broader than in other years, with whiskers extending from 1.79 to 16.82 mg L⁻¹. In contrast, the spans for 2021 and 2023 are more confined, ranging from 6.8 to 11.7 mg L⁻¹ and 4.39 to 11.8 mg L⁻¹, respectively. Another noteworthy aspect to highlight is that, although no specific statistical test was undertaken to confirm this observation, the means for each year appear to consistently sit above the critical threshold of 6.0 mg L⁻¹ for oxygen levels, as documented in the literature. This is indicative of encouraging findings as values below this threshold have been associated with significant adverse effects on fish, including diminished mass gain and suppression of the immune system, as documented by sources such as Burt et al. (2012), Davis (1975), and Remen et al. (2012).

Regarding CO₂ levels, the findings are also reassuring. There are no statistically significant differences among the years, with mean concentrations for 2021, 2022, and 2023 measured at 8.39, 8.72, and 9.42 mg L⁻¹ for dissolved CO₂ in the water. This is promising, as none of these values approach or extend beyond the literature-reported threshold of 15 mg L⁻¹,

beyond which dissolved CO₂ in water starts to become detrimental to fish (Fivelstad et al., 2015). However, it is crucial to highlight that, firstly, no statistical test was conducted against the threshold value found in the literature. Secondly, individual data points exceeding 15 mg L⁻¹ were observed each year. The upper whiskers for 2021, 2022, and 2023 exceed this threshold at 17.0 mg L⁻¹, 25 mg L⁻¹, and 23 mg L⁻¹, respectively. Notably, even in 2021, two outliers surpassed the upper whisker of 17.0 mg L⁻¹, and in 2023, one outlier exceeded the upper whisker of 23 mg L⁻¹. Although the values nearing or surpassing the threshold may raise some concerns, it's crucial to understand that the data tends to lean more towards lower dissolved CO₂ levels. This is evident in the positive skewness, where the median consistently falls below the mean for each year, indicating that most data points are on the left side of the distribution.

Lastly, the temperature analysis, akin to CO₂ levels, did not unveil any statistically significant differences between the years. Notably, for the year 2022, the distribution exhibits a larger spread with upper and lower whiskers extending from 13.0°C to 19.8°C, while in other years, specifically 2021, the range is from 11.0°C to 17.0°C, and for 2023, it is from 12.0°C to 17.0°C. However, despite these variations in distribution, the means closely align, with values of 14.21°C, 14.37°C, and 14.23°C for 2021, 2022, and 2023, respectively. Comparatively, these mean temperatures, though lacking formal statistical analysis, do not deviate in great magnitude from the range of 16.5-17.5°C, as indicated in the literature, which is considered favorable for salmon (Remen et al., 2013). Furthermore, none of the means surpass the 21.5°C threshold, a temperature level that salmon typically avoid (Remen et al., 2013). These findings are promising, suggesting that the temperature ranges observed at Salish Creek consistently provide a suitable environment for salmon each year.

Limitations

The outcomes of this experiment are not exempt from limitations, and three key areas stand out for potential enhancement: statistical methods, data quality, and the inclusion of additional metrics.

Initially, I conducted my analysis assuming unequal variances due to the uneven sample sizes. While this possibility is recognized in the literature (Jan & Shieh, 2014), it's important to note that unequal sample sizes do not always imply unequal variances. If the homogeneity of variance is not violated, the ANOVA test is preferred over the Welch test, as using Welch when variances are equal can result in a loss of statistical power. A better approach would involve conducting a Levene test to check if the samples for each metric have unequal variances. Based on the results, one could then decide whether to use the Welch or ANOVA method accordingly. Another potential concern is the normality of the data. Although the Central Limit Theorem supports normality assumptions for sample sizes above 30, for more robust results, a Shapiro-Wilk test could have been conducted to confirm normality with a specified significance level. If normality assumptions were violated, an alternative to the Welch test, such as the Kruskal-Wallis test for non-parametric data, with a post hoc test like the Dunn test, could have been a more suitable alternative (McDonald, 2014). In summary, a more robust approach would involve examining each metric—pH, oxygen levels, CO₂ concentration, and temperature—by initially assessing normality using the Shapiro-Wilk test. Subsequently, for each metric, a decision between parametric methods (ANOVA or Welch) and non-parametric methods (Kruskal-Wallis test) could be made. If the data proved to be normal, the Levene test could then determine whether variances were equal or unequal, guiding the selection between ANOVA or Welch appropriately. Implementing this method would yield more comprehensive results for each water quality metric.

Moreover, the data utilized exhibits a cross-sectional nature, collected annually within a week during the Fall season. This limited timeframe may not entirely capture the variations throughout the entire year. The data collection approach aligns with Professor Leander's structure for the class. While a consistent year-round dataset could offer more comprehensive insights, the provided data, albeit constrained, is invaluable. Gratitude is extended to Professor

Leander and participating students for their dedicated data collection efforts in previous years. Yet, it is essential to acknowledge that a more consistent yearly dataset could facilitate in-depth analyses, such as trend exploration and regressions, providing richer insights.

Finally, an additional improvement could have been the inclusion of salinity analysis in the study. While data on salinity was available, time constraints prevented its incorporation into the analysis. This addition could have enhanced the comprehensiveness of the study by utilizing the available data.

Conclusion

In conclusion, this study provides valuable perspectives on the conditions at Salish Creek, particularly following the restoration project conducted by Metro Vancouver. Notably, substantial variations in pH and oxygen levels across the years 2021, 2022, and 2023 were observed, whereas no noteworthy differences were identified in CO₂ levels and temperature. The less optimistic findings pertain to pH trends, suggesting a potential downward trend over the three years.

Acknowledgments

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Appendix

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|---------------------|--|
| ph data 2021 | [7, 7, 7, 7, 6.5, 7, 7, 7, 6.5, 7, 6, 6.9, 7, 6, 6.7, 6.5, 7, 6, 6.5, 6.5, 7, 7, 7, 6, 7, 7, 7, 7, 7, 7, 7, 7, 8, 7, 7, 7, 6.25, 6.5, 6, 7, 6.5, 7, 7, 7, 7, 7] |
| ph data 2022 | [6, 7, 6.5, 6.2, 6.76, 6.5, 7, 7, 6, 7, 7, 6, 7, 6, 6.5, 6.2, 6, 6, 6, 6, 7, 7, 6, 7, 6.85, 6.8, 6.5, 6.5, 7, 7, 7, 6, 7, 6, 7, 7, 6, 6.5, 6.6, 7, 6.5, 6, 6, 6, 7, 6.5] |
| ph data 2023 | [7, 6, 6, 7, 6, 6, 7, 6, 7, 6, 5, 5.7, 6, 6.5, 6, 6, 7, 6, 6, 6, 7, 7, 7, 6, 6.5, 7, 7, 6, 7, 5.5, 6, 6, 7, 6.5, 6.5, 7, 7, 6, 6, 7, 7] |

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|---|---|
| oxygen (mg L⁻¹) data 2021 | [7.3, 6.8, 9.6, 8.5, 9.3, 9.2, 5.4, 8.7, 7.4, 9.5, 9, 9.9, 10.1, 8.3, 9.3, 10.3, 9.6, 9, 10.1, 8.4, 8.1, 9.8, 7, 8.1, 8.8, 7.4, 9.4, 9.9, 11, 8.9, 10.3, 9.6, 8.5, 10.5, 8.6, 10.7, 11.7, 7.3, 10.9, 10.2, 9.8, 10.7, 8.8, 10.4, 8.2, 8.5, 7.7, 9.1] |
| oxygen (mg L⁻¹) data 2022 | [15.7, 1.79, 5.75, 3.79, 5.3, 2.73, 3.73, 5.24, 5.73, 4.49, 8.51, 3.8, 2.45, 3.09, 3.28, 6.4, 2.35, 2.56, 4.01, 4.87, 9.27, 6.81, 4, 16.82, 10.45, 11.22, 9.54, 8.26, 9.55, 9.83, 9.59, 9.97, 8.73, 8.6, 10.54, 10.5, 10.41, 9.45, 8.5, 9.26, 6.36, 9.33, 9.34, 5.75, 10.44, 7.1] |
| oxygen (mg L⁻¹) data 2023 | [6.3, 6.62, 4.41, 8.61, 5.67, 8.68, 4.39, 9.02, 7.2, 7.92, 7.31, 5.42, 8.04, 5.62, 5.05, 8.93, 7.58, 9.09, 7.34, 9.2, 9.12, 10.04, 10.05, 9.3, 9.65, 9.66, 10.75, 9.02, 8.5, 9.75, 9.83, 7.42, 10.65, 9.82, 9.98, 9.71, 9.96, 7.75, 10.58, 11.18, 9.44] |

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|---|--|
| CO₂ (mg L⁻¹) data 2021 | [12.8, 13, 12, 9.25, 12, 20.2, 9, 9, 9, 9, 10, 25.3, 9, 10, 7.7, 13, 17, 12.1, 8, 7, 11, 9.5, 12, 20, 3.9, 2.5, 4, 2, 4, 9, 3, 3, 6, 4, 4, 4.5, 6, 6, 2.5, 6, 10, 8.1, 5, 4, 3.5, 3, 5, 7] |
|---|--|

| | |
|---|--|
| CO₂ (mg L⁻¹) data 2022 | [24.3, 15, 3.8, 17, 20, 0.95, 13, 9, 15, 12, 21, 25, 1.8, 20, 5, 14.2, 12, 18, 10.5, 5, 10, 13, 7, 5, 6, 6, 6, 3.33, 5.5, 6, 4, 4, 7, 4, 3, 0.5, 5, 3, 3, 2, 3, 5.5, 10, 4, 4] |
| CO₂ (mg L⁻¹) data 2023 | [8, 14, 11, 13, 10, 20, 5, 26.5, 13, 14, 7.5, 8, 11, 23, 10, 13, 20, 18, 20, 13, 6, 6, 4, 4.5, 3, 4, 5, 5, 6, 5, 3.8, 4, 4, 7, 3, 8, 5, 4, 7.5, 4] |

| | |
|------------------------------|--|
| Temperature data 2021 | [15, 14, 15, 15, 15, 15, 17, 16, 16, 15, 16, 15, 14, 15, 13, 13.5, 14, 12, 14, 14, 14, 13.5, 14, 12, 14.5, 15, 17, 15, 14, 15, 13, 13, 12, 12, 13, 11] |
| Temperature data 2022 | [16.5, 14.5, 13, 14, 12, 11, 14, 16.8, 19.8, 16.3, 15, 16.5, 16.7, 16.9, 12.5, 16.5, 13, 16, 15, 12, 15, 15, 16, 15, 11, 12, 13.5, 12, 13, 13, 16.5, 15.4, 14.9, 14.8, 12, 14.4, 13.1, 13.9, 14.2, 11.1, 16, 16, 11.3, 16, 13, 15] |
| Temperature data 2023 | [17, 14.5, 15, 15.8, 13.5, 16, 14, 14, 10, 14, 14, 15.7, 17, 14.5, 19, 16, 15, 14, 12, 15, 15, 13.5, 14, 14.5, 14.7, 13, 14, 14, 9, 14, 12, 15, 15, 13.5, 14, 15, 15, 13, 12, 13.4, 14] |

