Carbon dioxide levels at Salish, Canyon, & Musqueam Creek and its implications on salmon spawning and survival

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Abstract:

With increasing climate change, CO_2 levels in aquatic systems are becoming more important as high levels of CO_2 can prove to be lethal for salmon survival. In Pacific Spirit Regional Park, Musqueam Creek and Salish Creek have returning populations of salmon that spawn, while Canyon Creek does not. At each creek, CO_2 titrations were done to obtain CO_2 concentration measurements, in order to determine if there was a significant difference in CO_2 concentration between creeks. We predicted that there would be a significant difference between Salish Creek and Canyon Creek, and between Musqueam Creek and Canyon Creek, but not between Salish Creek and Musqueam Creek. Data were analyzed using a single factor ANOVA, and post-hoc analysis was done using Tukey's HSD test. A significant difference in CO_2 was detected between Salish Creek (M=13.7, SD=2.18) and Canyon Creek (M=9.31, SD=0.87) and between Salish Creek and Musqueam Creek (M=9.92, SD=0.67). Recent restoration efforts and photosynthetic activity in Salish Creek may be contributing to its higher CO_2 levels, relative to Musqueam and Canyon Creek. As all three creeks have mean CO_2 levels within a tolerable range for salmon (8-20 mg/L), we concluded that some factor other than CO_2 must be involved in the differential abilities of these creeks to support salmon.

Introduction:

With the increasing relevance of climate change, the investigation of carbon dioxide (CO_2) levels is of heightened importance. Climate change is being rapidly accelerated due to anthropogenic CO_2 emissions, causing current atmospheric CO_2 levels to rise at unforeseen rates (Cox et al., 2000). This atmospheric CO_2 is absorbed by aquatic ecosystems, such as oceans, as well as terrestrial ecosystems (Schrope, 2012). One means by which freshwater ecosystems (i.e. rivers and streams) acquire CO_2 is by erosion from the surrounding landscape (Schrope, 2012). Thus, as more CO_2 gets absorbed by terrestrial ecosystems, more CO_2 enters freshwater rivers and streams.

Salmon hatch and develop in freshwater, before migrating to seawater to live as adults, then return to freshwater to spawn ("Salmon life cycle", n.d.). However, salmon spawning and survival can be severely affected by CO_2 levels. When CO_2 dissolves in water, it forms carbonic acid, causing water acidification. In a study by Kitamura & Ikuta (2000), they concluded that spawning hime salmon were extremely sensitive to water acidification, as measured by the amount of nest-digging by spawning females. When only slightly acidified (pH 6.4), nest-digging was severely inhibited, and below pH 6.0, almost no nest-digging was observed (Kitamura & Ikuta, 2000).

Furthermore, Ou et al. (2015) conducted a study on the effect of projected increases in CO_2 on salmon survival. Studying pink salmon, Ou et al. (2015) observed that increasing CO_2 levels had various negative effects on early salmon development, such as decreased body length, decreased wet and dry mass, and decreased production efficiency during yolk absorption. They also found that when reared at high CO_2 levels, young salmon had reduced ability to detect olfactory cues to trigger predator escape behaviours. This may later reduce their ability to migrate to seawater and impair their ability to survive in the ocean (Ou et al., 2015).

In the current study, we are interested in investigating differences in CO_2 levels in different creeks in Pacific Spirit Regional Park, specifically Salish Creek, Canyon Creek, and Musqueam Creek. Though Vancouver once had over 100 salmon-bearing streams, Musqueam Creek is one of the very few to still see a consistent return of spawning salmon, and is the only one to have continuously supported a returning salmon population throughout Vancouver's history ("A contributing cycle", 2017). Though at its lowest point only six salmon returned to spawn, Musqueam Creek has since been restored and sees increasing levels of salmon return

("Musqueam Creek restoration", n.d., "Salish Creek", n.d.). Salish Creek is one of the most recent creeks to be restored; its restoration was completed in June 2018 (Ho, 2018). The Department of Fisheries and Oceans has since reported seeing salmon fry in Salish Creek (Ho, 2018). Canyon Creek, however, has had no reported evidence of salmon.

Our null hypothesis is that there is no difference in CO_2 levels between these creeks, while our alternative hypothesis is that there is a significant difference in CO_2 levels between at least two of these creeks. Based on differences in their ability to support salmon, and evidence of inhibited salmon spawning and survival in unfavorable CO_2 conditions, we predict that Musqueam and Salish Creek will have significantly different CO_2 levels than Canyon Creek, as Musqueam and Salish Creek have reports of salmon, while Canyon Creek does not currently support salmon. Musqueam and Salish Creek should not have a significant difference in their CO_2 levels.

Methods:

Materials

A permit was obtained from the Vancouver Park Board, allowing us to conduct research at Salish Creek, Canyon Creek, and Musqueam Creek in Pacific Spirit Regional Park. Measurements were taken at upstream locations at each creek (Figure 1). Upstream locations were chosen based on: 1) suitability for salmon spawning, as salmon are known to spawn in upstream locations where waters are generally calmer and better support the growth of salmon embryos ("Why do salmon", 2017), and 2) accessibility for the researchers. A measuring tape was used to place a 30 meter transect line along the length of the stream. CO_2 measurements were taken using a CO_2 titration kit, manufactured by LaMotte Company (Figure 2).

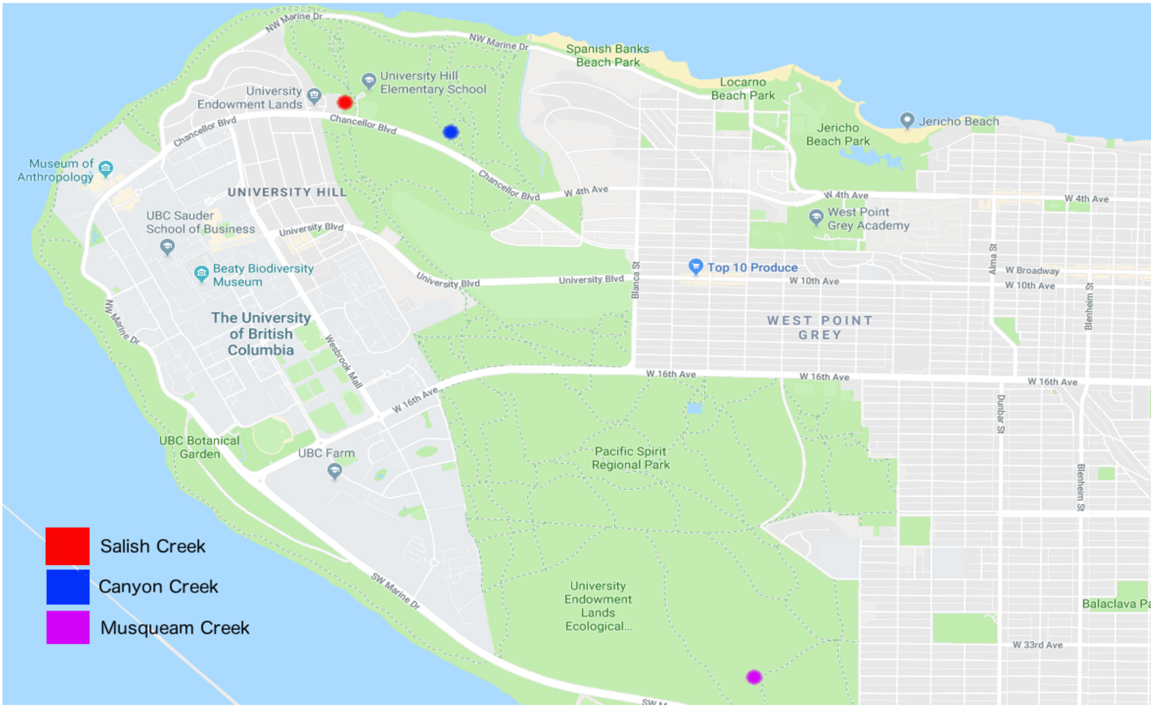

Figure 1. Pacific Spirit Regional Park map with creek locations

Figure 2. CO_2 titration kit by LaMotte Company

Procedure: Generation of random transect points

Using an online random number generator (random.org), 5 transect points for each creek were randomly selected. Since we required our transect points to have decimal points and random.org only generates integers, we first generated a random number (0 – 29) for the integral part, then generated a second random number (0 – 99) for the fractional part. This procedure was done 5 times for each creek.

Procedure: CO_2 measurements

CO_2 measurements were done in pairs and taken at Salish, Canyon, and Musqueam Creek. A 30 meter transect was placed along the length of the creek. At each transect point, a 20mL sample of stream water was obtained. To ensure exact measurement of 20mL, Pasteur pipettes were used. In the 20mL stream water sample, 2 drops of 1% Phenolphthalein indicator were added and mixed. The clear solution was then titrated with CO_2 Reagent B using a syringe to add drops. After each drop, the solution was mixed for 30 seconds. This was continued until a noticeable colour change was evident for 30 seconds. The solution was held against a blank white paper to aid in determining a colour change. The end-point was a pale pink colour. After the end-point was achieved, the volume on the syringe was recorded as our CO_2 measurement in ppm. After each measurement, our waste was disposed of in a waste bottle and our equipment was cleaned using distilled H_2O. The whole procedure was repeated 3 times at each point (i.e. 3 pseudo-replicates per transect point), in order to increase our confidence in our measurement. This made for a total of 15 measurements taken at each creek.

Data analysis

The 3 pseudo-replicates done for each transect point were averaged, making for a sample size (n) of 5 for each creek (Equation 1). The average measurement for each transect point was the value used in our data analysis. Data was analyzed for normality and equal variances using a normal probability plot and Levene's test, respectively. We then reciprocally-transformed our data in order for it to follow a normal distribution with equal variance (Equation 2). These data were then analyzed using a single-factor ANOVA with an alpha level of 0.05. For post-hoc analysis, Tukey's HSD test was used to determine which groups differed significantly in CO_2 concentration.

Results:

Our results from a single-factor ANOVA (alpha level 0.05) on reciprocally-transformed data found that there was a significant difference in CO_2 concentration between creeks ($F(2, 12) = 17.13$, $p = 0.00031$). Subsequent post-hoc analysis using Tukey's HSD test found that both Salish Creek (M=13.7, SD=2.18) and Canyon Creek (M=9.31, SD=0.87) differed significantly in CO_2 concentration, and Salish Creek and Musqueam Creek (M=9.92, SD=0.67) also differed significantly in CO_2 concentration (Figure 3). No significant difference in CO_2 concentration was detected between Canyon Creek and Musqueam Creek.

Equation 1. Mean CO_2 concentration at a given transect point:

$$x = \frac{pseudoreplicate1 + pseudoreplicate2 + pseudoreplicate3}{3}$$

Equation 2. Reciprocal transformation of data:

$$\frac{1}{x_1}, \; x_1 = \text{mean } CO_2 \text{ concentration at transect point 1}$$

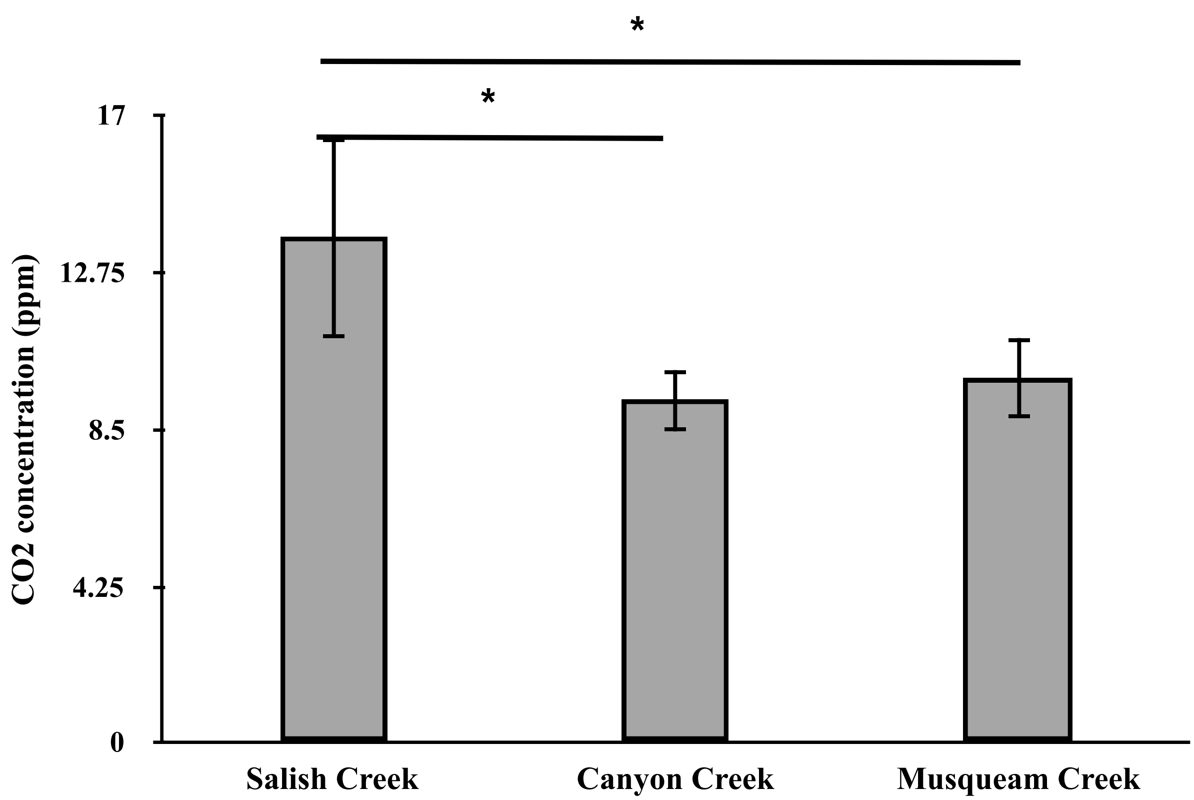

Figure 3. Mean CO_2 concentration in ppm at Salish Creek (n=5), Canyon Creek (n=5), and Musqueam Creek (n=5) in Pacific Spirit Regional Park. Error bars represent 95% confidence intervals, ANOVA $F(2,12) = 17.13$, $p=0.00031$. Asterisks indicate significant differences.

Water at each creek was observed to be running and flowing quickly. All creeks were situated in heavily forested areas within Pacific Spirit Regional Park. The temperature of water was qualitatively found to vary between creeks; Salish Creek and Musqueam Creek were observed to have cold, flowing water, while the water in Canyon Creek was noticeably warmer to the touch. Weather and air temperature were the same for all measurements (11°C). The depth and width of the creeks varied as well; both Salish Creek and Musqueam Creek were relatively wide (between 2-3 meters in width at different points along the creek) (Figures 4 and 5), while Canyon Creek was much narrower (~ 1 meter in width) (Figure 6). With regards to depth, Salish Creek was the deepest (~ 48 cm), followed by Musqueam Creek (~15 cm), with Canyon Creek as the most shallow (~10 cm).

Figure 4. Salish Creek

Figure 5. Musqueam Creek

Figure 6. Canyon Creek

Discussion:

There was a significant difference in CO_2 levels between the creeks, specifically between Salish and Canyon Creek as well as Salish and Musqueam Creek. Based on these results, we reject our null hypothesis and lend support to our alternative hypothesis. However, Canyon and Musqueam Creek did not differ significantly in CO_2 levels. This is different from what we had predicted. Both Musqueam Creek and Salish Creek are part of a restoration project and have returning salmon, whereas Canyon Creek shows no record of spawning salmon. Thus, we were expecting Musqueam and Salish Creek to have similar levels of CO_2, and both to be significantly different from Canyon Creek.

Salish Creek was restored by adding wooden panels, rocks, and other materials as well as removing invasive species in an attempt to improve spawning conditions for salmon (Ho, 2018). Dissolved CO_2 can enter streams and rivers from the oxidation of organic carbon from both allochthonous and autochthonous rock and sediment (Butman & Raymond, 2011). The addition of the rocks and removal of non-native species may account for the higher level of CO_2 observed in Salish Creek, relative to Musqueam and Canyon Creek. Higher photosynthetic activity from aquatic organisms also increases CO_2 levels in streams (Butman & Raymond, 2011; Brown et al., 2017).

Musqueam Creek and Canyon creek both have similar levels of CO_2, which may be a result of similar amounts of respiration from riparian vegetation or similar levels of photosynthetic activity. Both respiration and photosynthetic activity can alter levels of CO_2 in streams and rivers (Butman & Raymond, 2011; Brown et al., 2017). Further studies need to be conducted to compare respiration and photosynthetic activity among the three creeks.

It should also be noted that though both Musqueam and Canyon Creek have similar levels of CO_2, Musqueam Creek has always had salmon returning to spawn, while Canyon Creek shows no record of salmon. This suggests that there must be another external factor aside from CO_2 that is allowing for the support of salmon in Musqueam Creek, but not Canyon Creek. This suggestion is further supported by findings that the safe range of CO_2 lies within 15-20 mg/L (1 mg/L = 1 ppm) (Martens et al., 2006), with other studies finding that salmon can tolerate levels as low as 10.6 mg/L (Fivelstad et al., 1998) and 8 mg/L (Good et al., 2018). If found in inadequate or excess levels of CO_2, salmon suffer from decreased bodyweight (Martens et al., 2006) and show evidence of nephrocalcinosis (Fivelstad et al., 2003). As all three creeks had mean CO_2 levels within this tolerable range – albeit on the lower end – but exhibit differences in their ability to support salmon, it indicates that CO_2 is not the sole or deciding factor dictating salmon survival. This makes sense as in a natural system, several abiotic factors interact to influence the surrounding ecosystem, thus CO_2 may be interacting with other factors to influence salmon survival. Further research on various external factors should be conducted to determine the cause of this difference, whether by interaction with CO_2 and with other abiotic factors, or individually.

It is important to note design limitations and sources of error in this experiment. To conduct the experiment in a restricted time frame, the titrations were done in pairs of two, with each pair titrating at a different site within the same creek. Because each person perceives colour differently, the titration end-point is subjective to each person. In an attempt to reduce errors, all four members agreed on an end-point so measurements could be recorded consistently for the same end-point.

As part of our procedure, after each drop of CO_2 Reagent B, we counted to 30 seconds to ensure reaction completion. The time measurement was done without the use of a stop-watch or timer, thus, each pair could have counted seconds differently. This would impact whether another drop of CO_2 Reagent B was added or not, as time given for reaction completion was not exactly equal among pairs. Thus, the measurement obtained could differ slightly among sites. For further experiments, a stopwatch should be used to reduce measurement errors.

Another possible source of error was site selection at each creek. Due to time restrictions and natural obstacles (i.e. dense vegetation, steep banks, etc.), the measurement site at each creek was also the point of easiest access for the researchers. This is a possible source of bias because the location of the transect at each creek was not randomly selected (although the measurement points along the transect were). This may not have posed as serious a problem at Salish and Canyon Creeks because, as shown in Figure 3, the creeks are in a similar location and the easiest access point for each was a similar distance upstream. However, due to difficulty finding an accessible upstream site for Musqueam Creek, the measurements had to be taken at a site which was relatively downstream compared to the sites at Salish and Canyon Creeks. This is a possible source of error as many factors connected to CO_2 concentration vary with distance upstream, such as vegetation and erosion. For future research, ideally measurements should be taken at a comparable point upstream at each creek, to limit the confounding effect of these other variables.

Conclusion:

With the acceleration of climate change, investigations of CO_2 levels and their effects on the ecosystem are extremely relevant. With respect to CO_2 levels in creeks in Pacific Spirit

Regional Park, we found that CO_2 levels differed significantly between Salish Creek and Musqueam Creek, as well as between Salish Creek and Canyon Creek. Musqueam Creek and Canyon Creek did not differ significantly in their CO_2 levels. Despite differences in their observed ability to support salmon, all three creeks have mean CO_2 concentrations within a tolerable range for salmon survival. This suggests that an alternative factor must be involved in influencing these creeks' ability to support salmon.

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Appendix:

CO_2 concentration (ppm) / Transect point (m)	Replicate 1	Replicate 2	Replicate 3	Mean of replicates
19.9	16.0	19.0	16.0	17.0
14.4	12.0	13.5	12.0	12.5
18.26	11.5	11.5	12.5	11.83
18.45	11.5	12.0	13.5	12.33
19.21	15.5	14.0	15.0	14.83

Table 1. Carbon dioxide concentrations (ppm) for transect points (m) (n=5) measured at Salish Creek in Pacific Spirit Regional Park.

CO_2 concentration (ppm) / Transect point (m)	Replicate 1	Replicate 2	Replicate 3	Mean of replicates
8.85	9.3	9.5	12.0	10.27
10.22	10.0	10.0	9.0	9.67
13.07	8.9	9.5	9.3	9.13
4.81	9.8	9.5	9.3	9.53
25.18	7.0	8.0	8.8	7.93

Table 2. Carbon dioxide concentrations (ppm) for transect points (m) (n=5) measured at Canyon Creek in Pacific Spirit Regional Park.

CO_2 concentration (ppm) / Transect point (m)	Replicate 1	Replicate 2	Replicate 3	Mean of replicates
25.18	10.5	10.0	10.9	10.47

11.67	8.8	9.0	11.0	9.6
29.05	9.9	9.5	9.0	9.47
21.93	11.5	9.7	11.2	10.8
2.57	9.0	9.8	9.0	9.27

Table 3. Carbon dioxide concentrations (ppm) for transect points (m) (n=5) measured at Musqueam Creek in Pacific Spirit Regional Park.