EXPLORING THE
INTERACTIONS AND IMPLICATIONS
BETWEEN OCEAN ACIDIFICATION AND EUTROPHICATION
IN BUDD INLET

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ABSTRACT

Exploring the Interactions and Implications Between Ocean Acidification and Eutrophication in Budd Inlet

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Ocean Acidification is one of the greatest symptoms that climate change has inflicted on marine environments. Oceans naturally absorb carbon dioxide, however anthropogenic CO$_2$ has manifested greater adverse influences on marine life, which is stressing our ability to use these resources. Ocean pH has dropped 30% to 8.1 since the industrial age, however the pH reduction along coastlines and within estuaries has deteriorated even more, having a greater need to be monitored. Acidification is worse, especially around the Puget Sound because of high nutrient loads flowing into the Puget Sound from coastal communities, and other human industrial scale activities like agriculture. Nutrients, primarily in the form of nitrogen, increase algae and microbe primary productivity, eventually outputting new CO$_2$ through biological processes, resulting in amplification of the effect greenhouse gases are already exerting on marine ecosystems. This thesis project explored this relationship by looking at water samples collected from five locations in Budd inlet, and were tested for pH, nitrate, alkalinity. These variables were collected with the goal of determining if there was a noticeable difference between sample locations, and if there was a correlation between these variables all in context to the city of Olympia and Capitol Lake having some influence on findings. Results found no clear statistically significant differences between each variables and sample sites, however pH and nitrate concentrations had the greatest correlation. This suggests nutrients are indeed contributing significantly towards furthering acidification, more so than can be determined by CO$_2$ emissions levels alone. More research is warranted on establishing causal relationships between nutrient loads and acidification levels in all Puget Sound inlets.
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Introduction

Ocean acidification (OA) is one of many large climate change consequences that have arisen from anthropogenic carbon emissions. OA occurs when oceans absorb the carbon dioxide (CO$_2$) from the atmosphere, reacting with the water molecules to create hydrogen ions, which are directly responsible for acidification. Acidification impedes sea life functionality and survivability because organisms have adapted to a certain pH range, and the addition of new hydrogen ions to the system changes that pH. In addition, the hydrogen ions react with carbonate ions that organisms would normally absorb to form shells. Those organisms cannot grow shells effectively.

Researchers have conducted many OA experiments in the open ocean, but investigating the acidification affecting coastal and estuary systems is more difficult due to the many extra variables that could affect the carbonate chemistry, including freshwater input, tidal variance, and even eutrophication. This thesis will contribute to that literature on coastal and estuary systems by examining the interaction between eutrophication and OA, with the intent of improving upon the water quality knowledge of Olympia, WA’s Budd Inlet.

Eutrophication results from the overabundance of nutrients in the water, 66% of which is dissolved inorganic nitrogen, which leads to hypoxic conditions that kill off organisms in a localized ecosystem (McCarthy et al., 2017). These nutrients largely originate from agricultural fertilizer runoff flushing down our riverine systems. This in turn leads to an increase in the biodegradation of those phytoplankton by microbes. Microbes consume these algae in conjunction with a process of bacterial respiration, which uses up dissolved oxygen (DO) and organic carbon to produce new CO$_2$. This has
the net effect of increased CO₂ content within the water, which then worsens the local ocean acidification. Although the cycle described is a natural process, eutrophic conditions exacerbate the cycle and can have a considerable influence on pH levels. OA and eutrophication can have synergistic effects that worsen ecosystem health more than previously thought (Freely et al., 2010). This thesis project seeks to understand the influence of nutrients and the water’s ability to withstand these synergistic forces. The outcome of the project will have implications for the shellfish industry, whose products are directly affected by acidification. Coastal communities are also important as nearly 40% of the world’s populations live within 100km of coastlines, of which increasing nutrient loads are generating from (Wallace et al., 2014).

A few marine stations/buoys within the Puget Sound basin monitor the pH, alkalinity, and/or nutrients, which are at the heart of this thesis analysis. The alkalinity demonstrates the buffering capacity – the water’s natural capability to neutralize its acidic components. Alkalinity data will be compared to the pH, and nitrate + nitrite data, used here as a primary indicator for eutrophic conditions (Garcia-Martin et al., 2017).

The research question developed into two parts. First, “Is there a significant difference in Alkalinity, pH, and nitrate+nitrite concentrations individually between each sample site in Budd Inlet?” Second, “Is there a correlation between the alkalinity, pH, and nitrate+nitrite concentrations in Budd Inlet?” Four hypotheses manifested from these questions. First, there will be a significant difference for pH and nitrate+nitrite between each site, however there should not be a significant difference for alkalinity. Second, that there will be an inverse correlation between the pH and nutrient concentration. Third, there will be a positive correlation between the alkalinity and nitrate. Fourth, there will be
a positive correlation between the pH and alkalinity. Alternatively, the null hypotheses for each of these would be that there is no significant difference between sites, and there are no correlations/variables are independent of one another.

The framework I am using to approach my research question centers around pollution, specifically when it comes to Capitol Lake influences. The lake contributes a significant proportion of nitrogen and low dissolved oxygen into Budd Inlet. This project has an underlying assumption that dense urban populations, like Olympia, produce significant amounts of pollution entering into the local water system, having a significant influence on Budd Inlet acidification (McClelland et al., 1997; Roberts et al., 2015).

**Significance**

The purpose of this thesis project ultimately is to shed light on the influence that coastal communities contribute to acidification separate from carbon emissions. Human induced eutrophication is exacerbating acidification in coastal and estuary ecosystems, more so than is recognized since most acidification research is not being characterized in these terrestrial-marine boundaries in part due to the large number of variables impacting coastlines (Reum et al., 2014). Estuary habitat, like the southern Puget Sound, have more concern for eutrophic conditions due to the lack of flushing out of water this far inland from the sea. The water here in Budd Inlet has a longer residence time, meaning the water is more stagnant, in turn facilitating preferable algal habitat (Roberts et al., 2015). Increases in nutrient loads have made the inlet’s water quality an area of concern in terms of eutrophication and hypoxia for the state. Due to concern around eutrophication, this project aimed to better understand the its influence these nutrient inputs have on the
acidification of the region. Acidification is already of great concern along coastlines because low pH freshwater mixes with seawater, so any human action that worsens this delicate pH balance should send a red flag to communities whom will feel its effect first hand. This is why gathering data on nitrogen, a primary driver of eutrophication, was necessary for this project. This is one of the three most important variables needed to comprehend the impact humans have on OA. This project also sought to get an idea of Budd Inlet’s current capability to buffer against such impacts to acidification. To do that, alkalinity measurements needed to be made to understand the water’s natural buffering capacity. This capacity presents essentially a threshold for which the water is able to combat human influences to its pH balance. Knowing the alkalinity helps us learn how much the ecosystem can sustainably endure before long term damage will be done, or inversely till humans need to step in and help maintain the buffering capacity. The final primary variable was pH because of how integral it is to OA itself. This variable is especially crucial for marine organisms all of which are adapted for specific pH range. pH measurements were necessary to observe the relationship to nitrogen loading. This relationship makes up the backbone of this thesis. The goal of this project is neither to report exact proportions of influence on this region’s OA, nor to quantify causal relationships between each of the variables. The end goal is to simply point out that these variables are highly relevant for this research topic, they are related to one another, and they garner greater attention.
Literature Review

Ocean Acidification

Ocean acidification (OA) is concerning phenomena that occurs on a global scale due to the ocean’s natural propensity of being a significant carbon sink for atmospheric carbon dioxide (CO$_2$), absorbing 28% of anthropogenic carbon emissions in the last 200 years (Wang et al., 2014). A carbon sink is almost literal in meaning. The world’s oceans naturally absorb CO$_2$ when in equilibrium, getting shunted down into the water and reacting with the water molecules. Even though this is natural, the balance of absorption is being disrupted by the sheer volume of carbon being emitted by industrial processes. Oceans are being forced to absorb more carbon than they can manage without severe side effects.

This is due to the Earth’s natural carbon cycle, in which, oceans are a net carbon sink. Oceans normally exchange gases, including carbon, back and forth with the atmosphere, but overall more CO$_2$ is taken up in any given period of time. This is protecting people from the full brunt of atmospheric carbon threats, but it will not stay like this forever. As the oceans continue to warm, they will not be able to hold in carbon at the same rate, eventually becoming super saturated leading to the outgassing of carbon (Wang et al., 2014). This essentially turns the oceans into a net carbon source that will release carbon back into the atmosphere. This is not predicted to happen anytime soon, but the rate at which carbon is absorbed will decrease significantly by the year 2100 (Wang et al., 2014).

Currently the world’s oceans have dropped from about 8.2 to 8.1 pH, however this is a large percentage change in pH, about 30% (Bianucci et al., 2018; NOAA PMEL). By
the year 2100 the pH is expected to drop to 7.8, which is a 151% alteration from 8.2
(NOAA PMEL; Reum et al., 2014). The problem with coastlines and estuaries is they
usually experience more acidified conditions of less than a 7.7pH (Wallace et al., 2014).
On top of this, oceans are not expected to be able to absorb as much carbon by the year
2100 compared to now, reducing the mitigating effect they have on atmospheric carbon
(Wang et al., 2014). This is highly problematic since most of the marine life on earth live
near shallow coastal regions and are sensitive to changes made in the pH they are adapted
to (Fondriest, 2014). On top of that, even though many OA experiments have been done
on the open ocean, few have been performed for the effects on coastal and estuary
systems, emphasizing a need for this research.

The Chemistry

Acidification is a straightforward process with a few steps. First, once the CO\(_2\) is
absorbed into the ocean, it will dissolve into an aqueous solution where it can then
chemically react with the water molecules. Aqueous means that the gaseous CO\(_2\) is now
dissolved into the liquid, as opposed to being a stand-alone bubble within the water. This
is when the aqueous CO\(_2\) is able to react with H\(_2\)O as seen in the acidification equation
below. This will produce a new molecule known as carbonic acid (H\(_2\)CO\(_3\)), which is a
normally a short-lived molecule as only one percent of carbon is in this form. It then
dissociates into bicarbonate (HCO\(_3\)) and a one hydrogen ion, the ion directly responsible
for acidification. The bicarbonate speciation holds the largest proportion of initial carbon
at around 90% (Branch et al., 2013). The bicarbonate will further dissociate into a
carbonate ion (CO\(_3^{2-}\)) and another hydrogen ion, with carbonate comprising roughly nine
percent of the original carbon that was introduced to the system. This is illustrated well in figure 1 where the dotted line shows average ocean pH and associated carbonate proportions. This chain of reactions can go in either direction at each of the stages, but this is naturally pushed in the aforementioned direction and can be seen in this OA formula:

\[
H_2O + CO_{2(aq)} \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \rightarrow H^+ + CO_3^{2-}
\]

The problem is the last step of dissociating a carbonate and hydrogen ion is they will form back into the bicarbonate species of carbon when there is an abundance of hydrogen to react with carbonate. Normally the carbonate proportion is 9% of the initial CO$_2$ concentration, but OA is reducing that percentage (Branch et al., 2013). The overall shift in water chemistry is towards higher amounts of hydrogen ions and lower amounts of carbonate ions, the latter of which calcifiers need to be able to uptake.

*Figure 1: Relative speciation between carbon dioxide, bicarbonate, and carbonate depending on the pH (adapted from wordpress.com).*
Environmental Effects

Ocean acidification by definition has elevated levels of dissolved carbon dioxide within the water. pCO₂ (CO₂ partial pressure) has risen as a result of carbon emissions along and reduced carbonate species minerals (Reum et al., 2014). Partial pressure is a measure of the total volume that would be occupied by only the dissolved CO₂ in a given area as opposed to a general measurement of the total volume of all dissolved gases within that same given area. pCO₂ is an indirect measure used to determine pH, and it is also useful in OA research for its direct relation to anthropogenic emissions as well as its direct relation to aragonite concentrations (Reum et al., 2014).

Acidification itself exacerbates the overall pollution of the water. This feedback loop happens because lower pH water is absent of anions like carbonate and hydroxide (OH⁻) that would normally bind with inorganic heavy metals, and thereby buffering against OA (Zeng et al., 2014). There are not many toxic metals already in the water, especially since most are locked up in organic molecules, however, even small increases may result in higher water toxicity that animals may have a hard time coping with (Zeng et al., 2014).

Organismal Effects

OA causes many problems for sea life because the hydrogen ions react with carbonate to form bicarbonate when calcifying animals normally need carbonate to react with calcium ions to build calcium-carbonate shells, which renders the carbonate useless for those animals. These shell building and calcifying organisms are the species most affected by OA for this reason. Mollusks and crustaceans alike are of greatest concern because of their almost direct reliance on a pH balance and how much people rely on
them heavily as a food and economic source. Not only does lowering the pH interfere with their biological ability to form hard shells by making them more brittle and soft, but it can also impede metabolism function, growth, overall survivability especially at early life stages (Carter et al., 2013; Long et al., 2013; Swiney et al., 2017).

Calcification is a process by which creatures like crustaceans, mollusks, and coral take in calcium-carbonate to form their shell. OA has the effect of making it more difficult for crustaceans to physiochemically uptake calcium and carbonate ions in order to form a calcium-carbonate shell, although not to as great a degree as seen in mollusks (Branch et al., 2013). Early life stages are most vulnerable to external pressures including low pH values due to their sensitivity to water quality changes that they are specifically adapted for. Metabolism is also negatively affected by lower pH values, with the largest responses seen by embryos in most species. This is possibly due to the energy cost from longer development times before hatching. Calcifying organisms have a specific pH range for successful fertilization and falling outside of that range will reduce that number (Byrne, 2011). Researchers have also found evidence for “hypercapnia-induced metabolic depression” in embryos, which impairs their capability for internal acid-base regulation and suppresses their metabolic efficiency (Byrne, 2011; Carter et al., 2013). Hypercapnia is a condition in which there is too large a buildup in CO₂ within the blood stream. This lowering of the metabolic rate is initially good for the crab in the short term, but a continuous lowered metabolic rate will reduce any organism’s overall fitness and general metabolic maintenance (Carter et al., 2013).

Less available carbonate causes calcifying organisms will create a thinner and softer shells, making them susceptible to predation (NOAA, 2016). Calcifers were found to
have negatively correlated pH sensitivity to the usage of aragonite calcite types at the family, order, and class level (Busch & McElhany, 2017). Tolerance to acidification is partly dependent on degree of control over the process of calcification (Kroeker et al., 2013).

Researchers also found that acidified water decreased the molting success rate in crustaceans (Long et al., 2013). There larger implications for the stresses that crustaceans face during molting sessions since crabs molt multiple times in their lifecycle, more when they are young and less as crustaceans mature to adulthood. Calcifiers are disproportionately weakened in acidic water than they otherwise would be (NOAA Fisheries, 2016).

**Eutrophication**

One large environmental issue present in the south Puget Sound is an abundance of nutrient inputs causing a eutrophic environment. Eutrophic waters tend to cause algal blooms since the limiting factor of nitrogen is now in a surplus, thus increasing their primary productivity (Wallace et al., 2014). Eutrophication is happening for three main reasons in the south Puget Sound. First, the seasonal conditions are conducive to algal blooms such as in the late summer/early fall. Second, there is poor cycling and flushing out of the water in a certain region due to local geography. Third is the high level of pollution entering the water body (Wallace et al., 2014). The latter reason is the largest contributor to most eutrophic situations that occur. Large amounts of nutrients enter the water system from rivers and from overland runoff. Rivers tend to contain lots of excess nutrients from agricultural fertilizer, while urbanized coastal regions tend to have a lot of
storm water runoff that carries nutrients and pollutants over the impervious surfaces in a city. Eutrophication is a natural process however humans are making conditions more amenable to algal blooms.

The Chemistry

There are three parts in understanding eutrophication; in chronological order they are the nutrient load, algae primary productivity, then algal and microbial respiration. These three factors comprise the full eutrophication process. Eutrophication literally is the overabundance of nutrients, mainly nitrogen and phosphorous, that leads to an overgrowth of plant life. Whether the nutrients come from agricultural inputs into rivers, or storm water runoff through the city, they are greatly reducing the local water quality at the point of entry. Once the nutrients enter a water body, they begin to stimulate the primary production of plant life namely phytoplankton. Nitrogen is a limiting nutrient, so increasing the available concentration will allow phytoplankton to balloon in population (Garcia-Martin et al., 2017). Phytoplankton utilize photosynthesis to create their own energy by absorbing sunlight and CO₂ during the day. This process produces oxygen and glucose. At night they go through a process of respiration where they go through the reverse of the process. During respiration, the plankton uptake oxygen and produce CO₂ (Wallace et al., 2014). Altogether the process of photosynthesis and respiration is known as the biological pump (Wang et al., 2014). Photosynthesis would normally be helpful in controlling the amount of dissolved carbon, however the rates of respiration are greater, leading to a net increase in the dissolved carbon (Garcia-Martin et al., 2017).
**Environmental Effects**

Hypoxia is one of primary issues that occurs during an algae bloom in a eutrophic environment. Hypoxia is an extremely low dissolved oxygen (DO) condition with a standard threshold of about 2mg/L that prevents many organisms living in the water from thriving. (Freely et al., 2010). This occurs because overactive microbial populations consume oxygen in order to break down the algal blooms that resulted from excessive nutrient inputs faster than the oxygen can be replenished (Wallace et al., 2014).

Another result from the introduction of high nutrient loads and pollution is that organisms have a weakened capacity to photosynthesize in the first place (Zeng et al., 2015). With less total photosynthesis occurring, more CO₂ is free to react with water and further acidify.

**Organismal Effects**

The primary organisms that benefit from eutrophic conditions are algae/phytoplankton. They consume the excess nutrients and carbon dioxide to grow and reproduce. These algal blooms are problematic because they create acutely anoxic conditions (lacking oxygen) that few marine organisms can tolerate. Most organisms cannot survive in low oxygen waters because it prevents aerobic respiration of marine organisms, effectively suffocating them. Hypoxia (low dissolved oxygen) will typically kill off large numbers of fish, throwing the local ecosystem out of equilibrium.

Nitrogen and phosphorus are considered limiting nutrients in the environment because growth can only occur as long as they are available. Nitrogen is usually more important to plants than phosphorus because the ratio they require in their body makeup.
A ratio of 7.2:1 nitrogen to phosphorus tends to be the sweet spot that plants prefer (Roberts et al., 2015). In many freshwater environments, phosphorus tends to be the limiting nutrient, however, that is not the case in marine environments. Typically, in marine waters like the Puget Sound, nitrogen is less prevalent, therefore making it the limiting nutrient (Roberts et al., 2015). Excess nitrogen can be introduced through streams and runoff along coastal and estuarine environments. In recent decades, this has increased the amount of algal blooms around those areas (McClelland et al., 1997).

**Eutrophication – Acidification Interaction**

The linkage between eutrophication and OA occurs at the tail end of the eutrophication process where microbial activity contributes to CO₂ inputs. In eutrophic conditions, there is a prolific amount of nutrients including, but not limited to nitrate, phosphorous, and ammonia in the water as a result of runoff from agricultural practices or general pollution from urban populations. As indicated earlier, excess nutrients facilitate the ballooning of algae and phytoplankton growth and reproduction rates. Microbes then consume the algae and phytoplankton as they die, in a process called biodegradation. This process requires microbes to consume oxygen, so as more algae get eaten, the dissolved oxygen in the water column becomes depleted. This results in hypoxia, and is commonly defined as having equal to or less than 3mg/L of dissolved oxygen near the floor of the water body (since that is where detritus falls to and microbes consume the oxygen when breaking down organic material) (Wallace et al., 2014). DO values often fall close to hypoxic levels in the Puget Sound, which averages between 9-10.7mg/L (Freely et al., 2010).
Microbes increase their primary productivity and combine their consumed oxygen with the organic matter they break down to produce new CO₂, leading to a net increase in CO₂ in the system (Freely et al., 2010; Wallace et al., 2014). The CO₂ will react with the water molecules in the same way as CO₂ coming from the atmosphere, acidifying much more than compared to non-eutrophic water quality. The amount of microbe productivity is primarily determined by how much dissolved organic matter is present to consume to create energy (Garcia-Martin et al., 2016). Once they remineralize this organic material with DO, they respiate out CO₂, leading to the net increase in CO₂, despite algae taking CO₂ out of the water to photosynthesize (Freely et al., 2010). This entire process is illustrated in Figure 2 where eutrophication is introduced into the ecosystem in blue, on the left and goes through biochemical pathways, in black, to become converted into dissolved CO₂ that goes through the acidification process, shown in red. Since the level of algae and microbe productivity, and the observed acidification is much greater along coastal and estuary ecosystems than one would calculate solely based on carbon emissions. Scientists theorize that high nutrient loads are causing the increased intensity of acidification observed in these regions (Garcia-martin et al., 2016; Wallace et al., 2014).
Carbonate Chemistry

Carbonate system dynamics are integral to this project. Carbonate chemistry involves the relative proportions and interactions between different carbonate molecules and variables that influence these proportions and interactions. Carbonate (CO$_3$) manifests in different forms that influence water quality dynamics. In one example, calcium-carbonate is crucial for calcifying organisms to grow their shells. The availability of free carbonate becomes an issue when oceans uptake too much CO$_2$ with the end result being a lower concentration of available carbonate that can combine with calcium for these marine organisms (Branch et al., 2013).

There are three main forms of carbonate that occur during OA, as explained in the “OA chemistry” section. In short, carbonic acid, bicarbonate, and carbonate are formed through a series of reactions between CO$_2$ and water. Other important variables...
associated with carbonate chemistry include dissolved inorganic carbon (DIC) or pCO$_2$, pH, DO, and aragonite. The proportions of the three forms of carbonate depend on these other variables, but the key one is the pH. As seen in figure 1, a higher pH is needed to facilitate higher concentrations of carbonate. Oceans have an average pH of 8.1 meaning that available carbonate will likely always be a limiting factor. As pH levels decrease to the mid-range, the available carbonate drops even further, where high bicarbonate (HCO$_3$) proportions are favored. Decrease pH even further facilitates high CO$_2$ proportions with carbonate practically being snuffed out (also shown in Figure 1).

**Nitrogen Cycle/nitrification**

The nitrogen cycle explains how nutrients impact water quality. Nitrogen can come in organic, inorganic, dissolved, and particulate forms, but the form to pay most attention to in the context of OA would be dissolved inorganic nitrogen (DIN). DIN commonly denotes the nitrate concentration but authors and researchers may also include nitrite and ammonia in their calculation of nitrogen, depending on the experimental focus. Following that lead, both nitrate and nitrite were chosen in this project to represent nitrogen.

DIN is introduced into the ecosystem through agricultural practices, wastewater effluent, urban communities, runoff, etc. DIN goes thorough process of nitrification: different forms of nitrogen are oxidized, converting it from ammonia to nitrite, and oxidized again from nitrite into nitrate (Pelletier et al., 2017).
Aragonite Saturation

Aragonite, as a preferential form of carbonate, is one of the most important mineral resources for calcifying organisms and is highly dependent on the water’s pH balance. Many calcifiers preferentially select for aragonite due to how common and soluble it is, having a medium Mg-calcite content (Ries et al., 2016). Aragonite is the chosen polymorph of calcite or calcium carbonate (CaCO$_3$) used in many OA studies because it is so integral to carbonate chemistry. This mineral’s solubility is determined by its saturation state and is directly sensitive to pH changes; a lower pH has a large reduction effect on solubility (Long et al., 2016).

Researchers use $\Omega_{\text{arg}}$ to denote the aragonite saturation state, with oversaturation being [$\Omega$>$1$], equilibrium saturation [$\Omega$=$1$], and an undersaturated state being [$\Omega$<$1$] (Branch et al., 2012). Calcifying organisms prefer a saturation greater than one in order to easily uptake calcite without expending a lot of energy (Branch et al., 2013). Undersaturated aragonite will drive the reaction in the opposite direction, effectively dissolving calcium-carbonate, which requires organisms to use much more energy to uptake these ions (Branch et al., 2012). A low pH is associated with a low aragonite saturation state, with undersaturated states appearing near pH of 7.5 (Long et al., 2016).

Aragonite must have a saturation state equal to or greater than one in order for it to precipitate and be available to calcifying animals, whilst undersaturated water ($<$1) will dissolve the aragonite (Reum et al., 2014). This is illustrated in Figure 3 where all calcifying species’ shells began dissolving once the aragonite saturation state begin to approach “one.” Maintaining a saturation state greater than one is difficult with low alkalinity and high nutrient conditions present in Budd Inlet, as explained in the results.
section later. these forces hamper stable aragonite levels, thus creating unfavorable aquatic habitat for all calcifying organisms.

Figure 3: Aragonite dissolution rate by species of calcifier (Ries et al., 2016).

Budd Inlet Known/Unknown

Currently much of the South Puget Sound is an area of concern for Thurston County and the State of Washington due to the low oxygen and high nitrogen levels. In particular, since 2014 most of Budd Inlet has been designated for having major water quality impairments (McCarthy et al., 2017). The largest negative influence to Budd Inlet is Capitol Lake. Capitol Lake has had many water quality issues for decades and was
closed to all public usage in 2009. These negative water quality impacts consist primarily of high nutrient and low DO concentrations (Roberts et al., 2015).

Alkalinity data for Budd Inlet is limited. However, Taylor Shellfish of Shelton, WA takes measurements of alkalinity daily, albeit towards the northern Puget Sound. Nonetheless, water samples of Budd Inlet are anticipated to be similar to the overall content of Puget Sound water, which is primarily seawater, with some influx of freshwater from Capitol Lake and other smaller creeks and streams. Taylor Shellfish, during the time of sampling, reported average alkalinity concentrations to be around 180-200mg/L, which will be the assumed baseline measurement for Budd Inlet as well (IPACOA, 2019).

Nitrogen values in Budd Inlet are expected to fall within 0.1 and 1mg/L when mixing fresh and saltwater, with an average of 0.5mg/L during the time of sampling in late March (McCarthy et al., 2017; Roberts et al., 2015). Generally, nitrate values vary with the season, day, and depth.

Estuaries are unique ecosystems in that they are transitional zones between fresh and salt water. These coastal zones are necessary provide brackish water for certain organisms that are anadromous, meaning they are able to travel between fresh and saltwater systems. Estuaries provide a special transitional habitat for that migration. Many species are also adapted for such brackish waters, and estuaries have well suited habitat for many different species compared to a typical coastline. pH values in the more saline estuary portions tend to range between 8-8.6 today, while freshwater tends to have lower pH values between 7-7.5 (EPA, 2006). Due to the conductivity and salinity values measured in Budd Inlet being closer to average saltwater values, water samples were
expected to be most closely related to salt water than freshwater. pH values therefore are anticipated to be a mixture between salt and freshwater, likely being closer to the salt water range somewhere between 7.5 and 8.0 based off EPA ranges mentioned above.

Ocean Acidification and eutrophication are intrinsically linked at the junction where algal blooms lead to microbes creating anoxic conditions and introduce new CO$_2$ into an ecosystem. Understanding the carbonate chemistry and nitrogen cycle helps to inform how the components of OA and eutrophication propagate. Nutrient inputs are having compounded effects on pH reductions along with greenhouse gas emissions. This is stressing marine organisms by reducing the availability of carbonate, and creating a low saturation state that will cause the dissolution of shelled organisms. These impacts are happening in Budd Inlet through the influence of Capitol Lake nutrient inputs, prompting this research.
Methods

Field Sampling

Five points along a sampling transect were designated for sample collection with each of the five sites set to one kilometer distances between one another starting near the point of the peninsula towards the West Bay in the southern end of Budd Inlet. After the first sampling location was determined, subsequent locations were measured one kilometer north until five points were logged. The transect was chosen to be as close to the center of the inlet as possible while having a minimum 20 foot depth.

The GPS coordinates for each sampling site were saved into Garmin Echomap DV GPS so that each site could be revisited accurately for multiple sampling sessions shown in figure 4. At each site 60mL of unperturbed water was gathered using a Wildco 1100-1900 series vertical Van Dorn water sampler, captured at a 15 foot depth to perform an alkalinity and nutrient analysis. The 15 feet depth was chosen in order to be consistent with retail shellfish grower and seller Taylor Shellfish’s 15 feet measurement depths at Dabob Bay in Jefferson County. Taylor Shellfish collects and publishes live alkalinity and pH data, two major variables needed for my experimental analysis. A 15 foot depth is also beneficial because it lies below the surface air-water interactions that could skew data gathered (Moore et al., 2014).
In addition to the water samples, water column profile measurements were also collected at each site using a 2030 YSI Pro from 0-20 feet at five foot increments, for a total of five depths. This was intended to provide a more holistic snapshot of the water at each sampling site at that given time, although the 15 foot measurements are the most important to compare to gathered water samples. The variables acquired consisted of dissolved oxygen (DO), salinity, temperature, and conductivity. These measurements are also collected by Taylor Shellfish the Seattle Aquarium, King County’s Point Williams buoy, and many others. Data collection was repeated at each site three times: on 3/25/19,
3/27/19, and 3/30/19, starting at 10:30am, 12:33pm, and 3:41pm respectively. Start times were set to take place during receding tidal periods, which started 9:15am, 11:45am, and 2:15pm on the aforementioned days, shown in figure 5. Receding tides was chosen so the measurements would not be influenced by an influx of fresh sea water from north of Budd Inlet.

![Figure 5: Budd Inlet, Olympia shoal tide chart taken from Tides.net. Sampling time started at 10:30am on 3/25/19 (A), 12:33pm on 3/27/19 (B), and 3:41pm on 3/30/19 to be during the receding tidal period.](image)

Sampling yielded a total of 24 water samples, 15 unique and nine duplicate samples. Seventy-five data points were collected for salinity, conductivity, temperature, & DO each, however due to equipment issues, DO measurements from Day 1 were invalidated, leaving only 50 data points for DO.
All water samples were filtered through 0.45um cellulose filter syringe on site in order to remove larger particulates, such as chlorophyll, then stored in an ice cooler until they could later be transferred to a refrigerator to preserve them.

**Lab Analysis**

Two separate lab experiments were performed to determine the nutrient content and total alkalinity (TA) of the water samples. In order to figure out the nutrient content, the nitrate+nitrite concentration was specifically designated and is the most widely used nutrient measurement utilized in eutrophication research. These nitrogen species are associated with primary productivity and are a good proxy for eutrophic conditions (Garcia-Martin et al., 2016; Xu et al., 2014). In addition, nitrate (NO₃) is the only continuous nutrient measurement being taken in the Puget Sound by King County’s Point Williams buoy. Due to lab complications, the nitrite concentrations were not able to be measured, which will be elaborated in the results section. Other chemicals such as phosphorous or ammonia also add to the total contribution of nutrient loading in a given ecosystem, but the most commonly reported value is nitrate concentration. This, along with general time and resource limitations led to a focus on nitrate in this research. pH data were also taken independently as well as during alkalinity tests.

The nitrate analysis was performed using Schnetger & Lehners’ 2014 vanadium chloride reduction procedure. This procedure requires a stock solution of sodium nitrate (NaNO₃) and sodium nitrite (NaNO₂) being created. A 40mM stock solution of NaNO₃ and NaNO₂ was made. Both stock solutions were then diluted into nine molarities in order
to create a calibration curve. These molarities were 2uM, 4uM, 10uM, 20uM, 30uM, 40uM, 50uM, 60uM, and 70uM. This range was designed to encapsulate the full breadth of nitrate and nitrite concentrations that may be present in Budd Inlet based on the average nitrate values measured in real time by the Point Williams buoy in South Seattle along with other published materials. The nine points also increased the calibration curve’s accuracy. Four different primary reagents were then created for the reduction and isolation of nitrates, labeled as reagents A through D. Additional reagents, labeled E and F, were then created for the nitrate and nitrite extraction according to the ratios laid out in Schnetger & Lehners’ paper. Reagent E combined the first, second, and third reagents. The sixth and final reagent combined the first and second reagents.

The next step involved pipetting aliquots of these reagents and samples into the 96 well plate to be analyzed on a UV/vis Spectramax Plus spectrophotometer. A new calibration curve was created for each batch of samples being tested. Two batches of every sample were created, with each batch being run twice on the spectrophotometer to increase the sample size. An R^2 significance value of 99.5% or higher was required to validate accurate measurements.

The total alkalinity (TA) experiment was done to determine the water’s buffering capacity – the water’s natural capability to neutralize its acidic components. The main neutralizing components in this test was the bicarbonate concentration which is dependent on its pH. The TA was assessed using the USGS’ 2012 standard water quality and field sampling titration procedures along with Dr. Erin Martin’s titration procedures, which were adapted from the USGS methods (Martin, E., 2019).
The alkalinity test required a Sulfuric acid (H\textsubscript{2}SO\textsubscript{4}) titrant solution to be created; a final concentration of 0.025M or 0.05N was created. The pH probe and meter was calibrated prior to each sample titration. Water samples were set out at room temperature until their temperature stabilized in order to perform the titration consistently, although this was a limitation, not being able to perform titrations at their original temperature. Each sample was titrated to a pH below 4.0 utilizing a Gilmont micrometer buret. Samples were titrated in larger increments to start, then in smaller increments until the desired pH was reached. The pH and temperature was recorded after each allotted acid titrant.

**Data Analysis**

An Analysis of Variance (ANOVA) statistical test was performed on nitrate, alkalinity, and pH measurements using the JMP 2014 program provided by Evergreen State College. ANOVA is applicable here because there were five distinct groups that needed to be tested against each other (i.e. each sampling location). ANOVA was also useful since it is very robust against deviations around the mean. This was a concern here due to smaller sample sizes present in the experiments and measurements, as well as the data not all being normally distributed, having high variability. ANOVA allows for a determination of statistically significant differences between each site from another. This test was able to point out which of the five site locations, if any, were significantly different. ANOVA has a primary assumption that the means have equal variance. The
null hypothesis for an ANOVA test for any of the variables was that the mean between all five sites were the same ($H_0: u_1 = u_2 = u_3 = u_4 = u_5$).

The alternative hypothesis for each test was that at least one of the sample locations was different from the others, however an additional Tukey-Kramer HSD test is required to determine which mean is different from the others. pH and nitrate data did not have a normal distribution and were transformed by taking the square and log respectively, which were the most normally distributed histograms chosen for running the ANOVA.

Three major variables were plotted against each in a normal scatter plot to see if there was a correlation between the variables. This included graphing the alkalinity values versus the pH, alkalinity versus nitrates, and pH versus nitrates. Once a graphed, a trendline and $R^2$ value were generated to see if there is a positive or negative trend and the level of correlation between variables. The correlation explained what percent of a change in Y can be explained by a change in X.
Results

The data explained below encompasses lab analysis results for pH, alkalinity, and nitrate concentrations, as well as field measurements for DO, salinity, conductivity, and temperature. pH, alkalinity, and nitrate constitute the most important reported results and have their own dedicated sections in the literature review explaining the historical and current expected figures for these variables.

There are two research questions that require pH, alkalinity, and nitrate in order to answer. First, “Is there a significant difference in pH, alkalinity, and nitrate between each of the five sampling sites?” This question based on the knowledge that high nutrient loads are coming primarily from Capitol Lake as explained in the literature review. Concentrations are more influenced closer to southernmost sampling site compared to the northernmost site. The second research question being answered here is “Is there a correlation between pH, alkalinity, and nitrate?” This research does not aim to determine causal relationships but seeks to determine if there is a trend of association between these variables.

Nitrite concentrations will not be used to answer the research question, as explained later in the results section. The end of the results section includes a paragraph speaking to the limitations of these results.

pH

The observed pH values came out to be close to expected values for a mixture of fresh and saltwater. These measurements confirm that estuaries, like Budd Inlet, have lower pH levels than the open ocean. Ocean acidification has caused the pH of the open
ocean to drop from 8.2 to 8.1. This is in contrast to the average pH value of 7.86 found in this project. If a reduction in pH from 8.2 to 8.1 amounts to about a 30% lower pH, then the difference between 8.2 to 7.86 amounts to almost a 125% lower pH than the open ocean (NOAA PMEL, 2018). This is a much lower pH, however these results are not too surprising because mixed freshwater and saltwater could range from 7.5-8 (EPA, 2006). This average pH value of 7.86 suggests the water more closely resembles saltwater, which is consistent with other variables measured in the field.

At first glance, Figure 6 below shows a slight positive trend in mean pH between the five sites. Site 1 (closest to Olympia, WA and Capitol Lake) had the lowest mean pH by far, which suggests that something is influencing the pH, and pH seems to taper off the farther out into the inlet. Upon further examination, there was only a statistically difference between site 1 and site 4 with a p-value of 0.0214, significant at the 0.05 level. Unfortunately, the ANOVA test showed an unequal variance despite data being transformed. This is likely due in part to the low sample size present for each site (sites 1, 3, & 5 n=12; sites 2, & 4 n=6), as well as the large spatial and temporal variability present in the Puget Sound (Roberts et al., 2012).

Hypothesis 1 states that “there will be a significant difference in pH between each site.” A difference between only the first and fourth sites means that my data did not fully support the hypothesis. Therefore the null hypothesis, that all sites are similar/same, is not rejected. This is partly because the pH measurements not meeting ANOVA assumption standards, and because there was not a clear distinction between one site and the next—the water could flow between sites. Furthermore, even though the means show
a roughly linear gradient going from the first to the last site, the variation within each site was too great to say the sites were actually different from one another.

The research results cannot determine the relative proportion of impact to pH from greenhouse gasses or nutrient loading. It will, however, provide insight into the observed pH level drops.

![Figure 6: Mean pH values at each site, showing a shallow overall increase in pH by site.](image)

**Alkalinity**

Titration data was calculated with a USGS alkalinity calculator, along with alkalinity hand calculations made from the same set of titration data to increase the total sample size. Data was then entered into ANOVA to be analyzed for site differences. At face value, the mean alkalinity values in Figure 7 look low at the first site and increase as one goes further out into the inlet. This makes some sense since freshwater influx would contain lower alkalinity levels and contribute significantly to the decrease in overall alkalinity (Fry et al., 2015). The second hypothesis for alkalinity states that “there will be
a positive relationship in alkalinity by distance going north up Budd Inlet,” with site 1 having the lowest value and site 5 having the highest value. The null is that there is no difference between any of the sites. It is important to say that the expectation is for the null hypothesis to be true because there should not be significant influences to alkalinity coming from southern end of Budd Inlet.

ANOVA results showed no statistical difference between the alkalinity of the five sites. These results met the assumption of equal variance. This means the null hypothesis cannot be rejected. Despite not having any statistical difference, similar values make the most sense because the biggest influences on alkalinity are the mixing of fresh and saltwater, and alkalinity tends to have more stable variation throughout the year (Fassbender et al., 2018). Small changes between each kilometer in distance away from Olympia should not result in values that are distinguishable from each other. Additionally, there should be no difference between sites for alkalinity since alkalinity showed little to no correlation to either the highly variable pH or nitrate levels. The alkalinity should remain relatively homogenously mixed within the water column because alkalinity is less variable and more stable over time (Bianucci et al., 2018; Fassbender et al., 2018). Alkalinity is not significantly influenced by changing pH or nitrate levels, therefore expected values should not change regardless of pH and nitrate influences.
Having the same alkalinity, but with differing nitrate values could lead to different pH values between sites. This would mean the nitrates would correlate to the pH, as is shown to be the case in Figure 8. Assuming the buffering capacity (as reflected in alkalinity) of Budd Inlet is constant, the increased concentration in nutrients entering into the inlet will reduce the pH. It is important to note that the buffering capacity is not changing here; there is a reduced capability of the water to maintain a stable pH.

Figure 7: Mean alkalinity results by site, showing a slight positive trend from site 1 to 5.

Figure 8: Graph overlaying corresponding pH and nitrate values at depth -15ft. R² shows a moderate correlation.
Alkalinity values averaged 101.87mg/L or (1018umol/kg) across all sites. These measurements are consistent with average estuarine alkalinity values, which average 116mg/L (EPA, 2006). The main factor contributing to this difference in buffering capacity is the influence of freshwater flowing in from the Deschutes river, as well as other freshwater sources (Thurston County Water Resources Report, 2018). Freshwater alkalinity values fall between 30-90mg/L, and this combines with the fact that the water in the lower Puget Sound does not get flushed out easily, having a longer residence time (EPA, 2006). The aggregate effect results in a below average buffering capacity.

Nitrate

Nitrate concentrations, after being measured in the lab, were analyzed using an ANOVA statistical test to determine if there was a significant difference between sites. The third nitrates hypothesis states that “nitrogen concentrations would show a negative gradient” with higher values in the southern most sampling location (Site 1) and lowest the location furthest away (Site 5). This hypothesis is based on the idea that sources of nitrates would originate from Capitol Lake and runoff from the ports, and dissipate/dilute as one goes further out into the inlet due to mixing of incoming seawater containing lower background concentrations of nitrates (Roberts et al., 2012; Roberts et al., 2015). The null hypothesis reflects this: There is no difference between any of the sampling sites or that all sites would have equal concentrations. ANOVA results show there is a statistically significant difference in concentrations with an overall p-value of 0.0032; however, this itself does not allow me to confirm or deny the hypothesis. A secondary
Tukey-Kramer HSD test showed specifically that site 1 had a significantly higher concentration than the third and fifth sites.

These results normally would allow me to reject the null hypothesis that all five sites are the same and say that site one is different from site three and five. Unfortunately, the ANOVA showed unequal variances between the five sites; equal variance is a required assumption of this test. The null hypothesis cannot be officially rejected, despite having the largest samples sizes of any variable in this project (site 1, 3, & 5 \( n=36 \); site 2, & 4 \( n=18 \)).

**Nitrite**

The nitrite (NO\(_2\)) concentrations analyzed in this experiment were expected to be a mainstay for the nutrient results. Originally these results were to include nitrate+nitrite combined levels, however, because nitrite results were so low, most dropping below zero, they could not be incorporated into the nutrient analysis.

Nitrite concentrations were surprising in that all concentrations were found to be negative; results were between -1 to 0 micromoles per liter, suggesting concentrations could be an order of magnitude smaller. These results were replicated four times for every primary and duplicate sample tested all with an \( R^2 \) significance higher than 0.995 (99.5%). This led to the conclusion that the values do not indicate there was an error with the instrument or a failure of procedural execution. Instead, it is likely that the nitrite concentrations are so small that the instrument could not distinguish between the samples and the blanks used as the neutral standard. This seems reasonable given that concentrations are being measured in micromoles per liter (10\(^{-6}\) moles in every liter or
33 fluid ounces). This is compounded by the fact that nitrite concentrations naturally are often less than one micromolar, as well as that the detection limit for this procedure is 0.07uM, meaning anything smaller will not get picked up by the spectrophotometer (Schnetger & Lehners, 2014). Nitrites are therefore insignificant compared to nitrate concentrations.

I speculate these values could be so low in Budd Inlet because nitrite concentrations in seawater typically are miniscule in the first place, usually less than one micromole per liter (Hallock, 2009). Seawater concentrations tend to be so low when there is adequate levels of oxygen present. Bacteria are then able to go through the natural process of aerobic nitrification, in which nitrite is combined with oxygen to form nitrate (Pelletier et al., 2017). Budd Inlet had sufficient levels of dissolved oxygen from which bacteria could consume, ultimately reducing the overall concentrations of nitrite.

Nitrates, pH, & Alkalinity

Nitrates, pH, and alkalinity were compared to each other in order to examine the research questions and discuss implications of findings. Three graphs compare nitrate vs alkalinity, pH vs alkalinity, and pH vs nitrates, each with a correlation coefficient to determine the level of possible relatedness [0.1=weak, 0.3=medium, ≥0.5=strong (scale from 0-1)]. (See Figures 8, 9, 10, and 11).

The first graph, Figure 9, shows the relationship between measured pH and alkalinity values (n=48). Within the sample locations and time period, this graph shows a very weak positive correlation between these variables with a correlation coefficient (R²) of 0.0156 (1.56%). As the pH increases so too did the alkalinity. This graph shows lots of
scatter that would indicate there is no meaningful relationship and therefore they do not correlate with one another. Thus, there is no support for the hypothesis that they are positively correlated. However, this is not an unexpected result. It makes sense that pH does not influence alkalinity because of how they are defined and measured. Total alkalinity is a measure of all the basic components within a substance, meaning the amalgamation of negatively charged ions within said substance. pH and alkalinity are related in the sense that hydrogen is included in alkalinity equations but is relatively small compared to a substances basic (negatively charged) components. However, the concentration hydrogen ions says nothing about whether the alkalinity will be high or low. The lack of a correlation between pH and alkalinity could be due to in part to the relatively small sample size (n = 48) – a more holistic view of Budd Inlet is needed to gain a more accurate understanding of their relationship. This would mean increasing sample size to account for seasonal and daily time variation, and to create a three-dimensional image of the entire Inlet. This should include a method of random sampling along a transect rather than the sampling methods regularly spaced method employed here.
Next, Figure 10 shows the correlation between alkalinity and nitrate levels. Figure 10 reveals a weak negative correlation between these two variables with an $R^2$ of 0.0753 (7.53%). As the nitrate concentrations increased, there was a decrease in the alkalinity levels. Even though this relationship is weak, the figure shows a negative correlation between the two. The correlation coefficient was lower than expected, due to the scatter in the data. Nonetheless, I would not say their correlation is strong enough to definitively answer the research question of correlation. Based on this project’s experiments, there was no significant correlation between the level of nitrogen and the buffering capacity. This could also be due to the fact that there was little discernible difference between each of the five sampling locations.
The final correlation, displayed in Figure 8 above, shows the correlation between pH and nitrate levels with a correlation coefficient of 0.2745 (27.54%). These two variables had the greatest relationship of all three pairs, with a medium correlation between them. Plotting the pH versus the nitrates produced a negative inverse relationship; as the nitrate concentrations increased the pH decreased. This confirmed my third hypothesis that there would be an inverse correlation between pH and nitrates. This is practically significant since it indicates nitrates from runoff, etc. could be contributing to lower pH readings. Additionally, this correlation means nitrates should be a central focus in acidification research, especially since estuary and coastal ecosystems contain higher nitrogen levels than the open ocean (Roberts et al., 2015; Roberts et al., 2012). Figure 11 displays a clearer inverse relationship between pH and nitrate means. Nitrates (in blue) start out higher towards the first site and decreases further out, whilst pH (in red) starts out low and increases further out.
Dissolved Oxygen

Dissolved oxygen (DO) levels found during field measurements were higher than originally anticipated. Measurements averaged 22.3mg/L at the 15 foot study depth. The vertical profile measurements (0-20ft depth) show a range from 18-28mg/L. As stated in the literature review, Budd Inlet’s water quality is considered to be impaired with lower DO levels than is suggested for a healthy inlet according to a 2014 water quality assessment of the region by McCarthy et al. The minimum DO water quality standard considered for most of the Puget Sound is 7mg/L, and in some inlets is 5-6mg/L, however Budd Inlet tends to fall below these levels (McCarthy et al., 2017). Their findings suggest the Inlet often falls close to hypoxic levels, which are defined as 3mg/L or below (Wallace et al., 2014).
Data from this study show a much higher level of dissolved oxygen than reported in the literature; however, the short time span and space studied may not have been indicative of seasonal and daily variation. The higher values recorded may not necessarily be a sign of recovery of the water or a balanced ecosystem. DO values tend to be higher towards the surface as opposed to the floor bottom throughout certain Puget Sound inlets, although this project did not capture deep enough measurements to confirm this (Fondriest, 2014). This is counterintuitive because typically the deeper water has more pressure and a lower temperature, allowing for more DO to be present. This is also compounded with the fact that DO levels are lowest in the late summer/early fall, which is several months removed from the time of this project’s recorded DO measurements (LOTT, 2000). The historically low DO values reported for Budd Inlet at those times put a major stress on organisms that rely heavily on stable, high DO concentrations.

Salinity

Salinity is defined as the total amount of dissolved salts in the water, specifically using potassium chloride (KCl) concentrations – also known as the chlorinity – as a standard since KCl is a major salt ion within all water sources, especially saltwater. Salinity values at the 15 foot study depth averaged 28.62 parts per thousand (ppt). Looking at the vertical profile, results ranged from 19-29ppt. It is important to note that the lowest salinity values were consistently found at the surface in nearly every group of measurements. This suggests surface sea-air interactions, along with freshwater separation could be influencing salinity, then they stabilize out the deeper measurements are taken.
Interestingly, the first sampling site consistently had the lowest values, although a statistical test for significance was not conducted on salinity. These lower numbers can be explained by incoming freshwater sources at the southern tip of the inlet, which is similar to most freshwater-saltwater boundaries. The range of numbers suggest there is a mixture between seawater and freshwater, being skewed more towards seawater. Average seawater consists of roughly 32-37ppt, while freshwater typically is less than 0.5ppt (Fondriest, 2014). As salinity increases, the pH will decrease (Fine et al., 2016).

**Conductivity**

Conductivity measures the resistivity of the water, or inversely, the capability for electrical flow through dissolved ions in the water. Due to this, salinity and conductivity are directly related: both are measured using dissolved salts. Conductivity measurements averaged a little over 44,600μS/cm (microsiemens per centimeter) at the 15 foot study depth. The conductivity’s vertical profile usually ranged from 40-45,000μS/cm, however a few surface measurements as low as 30,000μS/cm appeared in the data. It is important to note that the lowest conductivity measurements were observed at the surface and the numbers began to stabilize in deeper water. It is interesting to see that the first site had the lowest average conductivity values, much like the salinity values. Again, this may be explained by the incoming freshwater at the southernmost portion of the inlet. Likewise with salinity, as conductivity increases, the pH may decrease.
**Temperature**

Temperature was measured in degrees Celsius in this research. Temperature readings averaged just below 9°C at the 15 foot study depth. Temperature ranged from 8-11°C in vertical profile measurements. Temperature, in every case, was highest at the surface and gradually decreased linearly the deeper one measures. This is intuitive and consistent with water profiles around the region and around the world.

It is important to note that all lab work on water samples were conducted at room temperature as I did not have the means to perform experiments at the temperatures in the field. Room temperature was chosen as it was the only stable temperature that could be taken advantage of. Temperature was not expected to significantly influence results since temperature was not notably related to any variables being tested in the lab.

**Results Caveat**

It should be noted that these results cannot be extrapolated at any larger scale than what was specifically studied. This includes temporal and spatial scales. Nor do the results imply causal relationships. Sampling took place over a week period towards the end of March in 2019, meaning these results apply only to this time period and may not necessarily be indicative of any other time of year and even by times of day. Spatially, these results only include a 5km distance within Budd Inlet at a specific depth and are not necessarily indicative of the contents of the entire water body - it is unknown if these variables exist homogenously or not throughout the entire inlet. It should also be noted that determinations on the source of specific inputs, or how much of a substance is coming from separate locations cannot be determined from the results found here.
Finally, conclusions cannot be drawn about the volume of nitrates coming into Budd Inlet from Capitol Lake versus how much is background level coming from the Pacific Ocean. Despite these caveats, this study does provide important background data on the water quality and pH levels of Budd Inlet.
Discussion

pH

pH results varied greatly between days, which was to be expected since large diurnal changes in pH are natural, especially in estuaries where the presence of nitrogen and other factors influence pH (Fassbender et al., 2016). These variations shown in Figure 12 for a number of locations around the world. pH is also heavily influenced by plant photosynthesis, which removes CO$_2$ (directly proportional to pH) from the water and increases the pH during the day, but decreases pH at night when photosynthesis is not happening. Temperature also adds a dynamic to this: increasing the water by 1°C will decrease pH by 0.001 (Fine et al., 2016). Fine et al. also found that an 1ppt increase in salinity will lead to a 0.003 decrease pH. Figure 12 best illustrates the difference in pH variability between separate water body types.

These factors make characterizing estuary pH difficult and lead to variation being greater than open ocean measurements due to the plethora of additional dynamic components. The prominent contributors to Budd Inlet’s pH profile include Capitol Lake, Olympia’s stormwater runoff, as well as the low circulation and flushing of Budd Inlet (Roberts et al., 2015). Factors influencing the pH contributed to the wide and unequal variation seen in Figure 13. This variation makes it hard to determine actual difference between site means. Still, these pH results are still intriguing because they encourage a follow up study to determine if pH levels are actually lower towards the southern edge of Budd Inlet.
Figure 12: Comparing pH variability between month long open ocean (top) vs estuarine (bottom) measurements (Branch et al., 2013).

Figure 13: Graph showing squared pH value quantiles, showing a significant difference between sites 1 and 5, but with large, unequal variance.
The fact that Figure 11 shows a seemingly clear inverse relationship between pH and nitrate, suggests that nutrients are playing a larger role in acidification than is generally given credit and needs to be explored more in OA research. Acidification along coastal and estuary regions is of greater concern than the open ocean for this reason. Only 24-49% of the observed drop in pH within the Puget Sound can be explained by anthropogenic CO\(_2\) input, meaning there is a significant, alternate cause for the total observed acidification (Freely et al., 2010). OA will have acute influences over the health of Budd Inlet, and the entire Puget Sound estuary, as well as regional economies that rely on these nearshore resources (Zeng et al., 2014).

Alkalinity

The alkalinity result was slightly surprising because of how low the observed values were, resembling freshwater values more than saltwater ones. Other variables like the conductivity, salinity, and pH more closely resembled those for saltwater. Alkalinity levels for seawater average about 230mg/L or 2300umol/kg (Fine et al., 2016). Taylor shellfish alkalinity samples, for instance, averaged close to 200mg/L during the time of sampling, though they are much closer to the Pacific Ocean (IPACOA, 2019). Before completing the lab analysis for alkalinity, this led to the assumption that the results would skew towards saltwater values; however this was not the case.

Figure 14 illustrates that alkalinity was not statistically different between sites and fell around 100mg/L. Recalling that alkalinity reflects the ability of a water body to neutralize \(H^+\) ion, this result means Budd Inlet has a lower buffering capacity regardless of measurement site, presenting problems for regional acidification all throughout the Inlet.
A low buffering capacity means there is a reduced ability of the water system to cope with the increasing effects of acidification from carbon emissions as well as increasing nutrient loads entering the inlet. With values being twice as high towards the Puget Sound’s northern end, alkalinity is of greater concern here in the south Puget Sound. The alkalinity will likely remain constant as time goes on, however influences that reduce pH values will continue to afflict the area and will further impede suitability for marine life and some human uses.

Figure 14: Alkalinity quantiles for each site, showing no statistical difference between sites, with an equal variance.

**Nitrate**

Nitrate results are of practical significance, despite not being able to judge on statistical significance, because there was a noticeable negative trend that is backed up by the literature. Higher concentrations of nitrates were expected closer to Capitol Lake, and the measured values reflect this, however there was not a clean linear gradient between
Dissolved inorganic nitrogen (DIN) in the form of nitrate concentrations ranged from 3-10µM or 0.19-0.62mg/L, and averaged 0.395mg/L between all sample locations. This is consistent with Department of Ecology 1997 measurements, which averaged about 0.5mg/L DIN within late March when this thesis project’s samples were collected (Roberts et al., 2015). The Ecology measurements are over 20 years old, however this may indicate relatively unchanged conditions overall within this part of the estuary.

Figure 15 shows a general decrease in nitrate levels, however there was a large variation in measurements at each site. Variability is normal for Budd Inlet, so a larger sample size would be needed in order to make any definitive conclusions. Nonetheless, that is what is intriguing about these results. Nitrates are suggested to be coming from Olympia/Capitol Lake. Taking into account the full variation in these data points, some values exceeded the recommended nitrate concentration threshold of 0.8mg/L (Xu et al., 2014). These results suggest that follow up studies must be conducted that expand upon spatial and temporal limitations that are showing up in via the high variation.

Figure 15: Nitrate quantiles for each site, showing significant difference between sites 1 and 3, and between sites 1 and 5, but with unequal variances.
Nitrate, pH, & Alkalinity

I argue that nitrates and pH co-occur for indirect reasons. First, this co-occurrence seems to confirm the respiratory CO₂ intrusion from microbes and bacteria in Budd Inlet. While this connection is not immediately apparent, nitrates are nonetheless one of the greatest contributors to the observed drop in pH within the Puget Sound. This process does not involve the buffering capacity directly, but the magnitude to which the environment can cope with the CO₂ intrusion is nonetheless dependent on the buffering capacity. Climate change will acidify the water more, and human activities increase nitrate concentrations over coming years. Neither of these, however, will likely alter alkalinity levels, meaning Budd Inlet will only acidify at accelerated rates.

While alkalinity did not show any notable correlation towards pH or nitrates, alkalinity is still important to study because it provides a different perspective on OA. It gives insight into how well the natural environment will fare against the forces of OA. It would be worth further investigation to determine alkalinity levels all around the Puget Sound. This information would inform law makers about which areas have the best chances to cope with OA, and which areas need human intervention the most. Still, the average pH values found in this research will likely decline further as greenhouse gases and nutrient loads increase, creating a positive feedback loop. Since Budd Inlet has such a low alkalinity, acidification will intensify sooner in this specific region. This will impact local species first who depend on stable water quality conditions, and who are already struggling from poor conditions already present in Budd Inlet. This is of immediate concern for Olympia and should be handled by monitoring sources and concentrations of nitrogen, hopefully to curb its introduction into our water bodies. Olympia is a major
source for nitrogen inputs, primarily from Capitol Lake. If the lake was turned back into an estuary, DO and nitrogen levels would significantly improve seasonally over its current outlook (Roberts et al., 2015). This would likely stabilize pH levels to a certain degree.
Future Research

There are several aspects of this project I would change were I to repeat it. The biggest difficulty was accounting for seasonal and daily alterations in the all the variables collected. A future study should have this as a central aspect of the study because it is impossible to truly describe and interpret results without an entirely holistic view of regional conditions. This is conceptually easy to accomplish, the primary constraints being time and resources. Having samples from an entire season and over different segments of the day would greatly improve the ability to answer the research questions. The sample size would increase and results could be indicative of a much broader time period. I would also like to see an increase in the number of sampling locations, randomly choosing spots to sample that were not necessarily equidistant from one another.

Second, I would also like to study the aragonite saturation state of the water samples as well. This would provide more information in relation to calcifying organisms. Aragonite saturation data could allow for characterizations of which organisms at greatest risk in the region, as well as describing its relationship/correlation to the other variables collected in this study.

Third, I would collect data on microbe respiration rates. This information would tell me exactly how much nutrients in the water impact the respiration process, and how much oxygen is consumed and how much CO2 is released. This would better establish the relationship that eutrophication is directly contributing to acidification.

Fourth, I would improve the initial focus and justification for sampling locations. Originally, I set out to see if the LOTT outfall site was contributing nitrogen into Budd
Inlet, as most wastewater treatment plants around the Puget Sound do. I had since learned that LOTT is the only wastewater treatment plant that is required to filter out their nitrogen (LOTT, 2000). I would also have required isotope signatures of the water coming from the facility to distinguish it from ambient nitrogen in seawater as well as from Capitol Lake. This is also where I would shift the focus of this research, by testing water with the intent of investigating the influence of Capitol lake on Budd Inlet. There is more research on the impact of Capitol Lake on its surrounding environment as compared to information on general conditions in Budd Inlet itself.
Conclusion

Ocean Acidification is an ever growing global problem that is very hard to mitigate. It remains relevant for coastal communities to be cognizant of what acidification is doing to their water and what are the influences other than carbon emissions contributing to this acidification. Eutrophication is one other major contributor to acidification and should be given greater attention in OA research. For the purposes of this project, buffering capacity, pH, and nitrates were identified as variables needed to understand current water quality conditions and relationships for Budd Inlet, but all three are valuable data that will inform future decision making to deal with OA along coastlines.

The context that sparked this project included sources of high nutrient inputs from human induced activities. This includes Capitol Lake and downtown stormwater runoff. There were two primary goals that involved alkalinity, nitrate, and pH values. The first, was to determine if there was a visible difference in these variables along a transect traveling north in Budd Inlet. The driving hypothesis was that the southern end of Budd Inlet would have the highest nitrate concentrations and lowest pH levels. The second goal was to determine if there was a correlated relationship between the three variables. Water samples were collected from five sites over three days so that alkalinity, nitrate, and pH could be measured, along with in situ temperature, salinity, conductivity, and DO measurements.

Water samples tested for alkalinity showed an average of 101.87mg/L, with no statistical difference between sites. This is 12% below average alkalinity values for estuaries, suggesting that Budd Inlet has a reduced ability to compensate for acidifying forces, especially as more nutrients enter the inlet over time. Water samples tested for
nitrate concentrations revealed extremely low levels of nitrites, enough so that they could not be measured and were removed from the focus of this project. Nitrate levels were found to be around 0.395mg/L, which is within the average range for estuaries. Nitrates were highest at the first site, but statistical tests essentially show no statistical significance between sites. Regardless, these concentrations are below the recommended threshold of 0.8mg/L according to Xu et al. The biggest problem is that nitrate concentrations have been steadily increasing in Budd Inlet in the past few decades (Roberts et al., 2012). pH measurements were found to be around 7.86, which is within the expected range. pH was found to be lowest at the first site, which coincided with the highest nitrate concentration, however pH was also found to not be statistically different between sites. Nonetheless, pH and nitrate levels showed the greatest correlation. This correlation has significance for acidification research conducted within estuaries and the connection should be integrated into OA research as the magnitude of nutrient impact will only increase from human induced activities. This presents a problem for water quality concerns as it will likely get worse.

A quote by Max Planck in 1949 expresses well the attitude needed for OA research as a whole. “An important scientific innovation rarely makes its way by gradually winning over and converting its opponents...What does happen is that its opponents gradually die out, and that the growing generation is familiarized with the ideas from the beginning” (Brewer, 2013). This encapsulates the approach needed to be taken with scientific research going forward. Science is at a consensus on humans being the root cause for increasing greenhouse gases. People must shift the focus away from trying to convince climate deniers that we need to fix this problem we have created, to allocating more
attention towards educating younger generations of all the different human causes of OA.
This will promote greater awareness of the problem, and get people thinking about how to holistically tackle OA in the years to come.
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Results Figure Appendix:

ANOVA Data Alkalinity

A) Figure 16: ANOVA alkalinity results (A) showing no statistically significant differences between sites at the 0.05 p-value level. Secondary Tukey-Kramer statistical test (B) showing that all sites are statistically similar to one another. ANOVA test for equal variance (C) among alkalinity values. Results show no differences in variance, accept the null of similar variance.

ANOVA Data Nitrates
Figure 17: ANOVA results (A) for log transformed nitrates showing a statistically significant difference with a p-value of 0.0032, at the 0.05 level. Secondary Tukey-Kramer statistical test (B) showing that site 1 is statistically different from site 3 and 5 for nitrates at the 0.05 level. ANOVA test of equal variance (C). Results show unequal variance between nitrate sites at the 0.05 level.

ANOVA Data pH
Figure 18: ANOVA test (A) of squared transformed pH showing statistically significant p-value of 0.0214, at the 0.05 level. Secondary Tukey-Kramer test (B) for squared pH showing statistically significant difference between site 1 and 4 at the 0.05 level. ANOVA test of equal variance (C) showing that variance between sites is not equal.
Figure 19: Titration figure for sample “A1-1” with an equivalence point at pH 4.47 and an alkalinity of 102.6mg/L. Titration figure for sample “A1-2” with an equivalence point at pH 4.55 and an alkalinity of 100.1mg/L. Titration figure for sample “A2-1” with an equivalence point at pH 4.58 and an alkalinity of 100.7mg/L. Titration figure for sample “A3-1” with an equivalence point at pH 4.47 and an alkalinity of 102.6mg/L.
Figure 20: Titration figure for sample “A3-2” with an equivalence point at pH 4.68 and an alkalinity of 99.5mg/L. Titration figure for sample “A4-1” with an equivalence point at pH 4.59 and an alkalinity of 101.3mg/L. Titration figure for sample “A5-1” with an equivalence point at pH 4.64 and an alkalinity of 101.3mg/L. Titration figure for sample “A5-2” with an equivalence point at pH 4.51 and an alkalinity of 103.2mg/L.
Figure 21: Titration figure for sample “B1-1” with an equivalence point at pH 4.53 and an alkalinity of 103.2mg/L. Titration figure for sample “B1-2” with an equivalence point at pH 4.46 and an alkalinity of 102.6mg/L. Titration figure for sample “B2-1” with an equivalence point at pH 4.53 and an alkalinity of 103.2mg/L. Titration figure for sample “B3-1” with an equivalence point at pH 4.38 and an alkalinity of 105.7mg/L.
Figure 22: Titration figure for sample “B3-2” with an equivalence point at pH 4.42 and an alkalinity of 103.8mg/L. Titration figure for sample “B4-1” with an equivalence point at pH 4.62 and an alkalinity of 103.2mg/L. Titration figure for sample “B5-1” with an equivalence point at pH 4.63 and an alkalinity of 103.2mg/L. Titration figure for sample “B5-2” with an equivalence point at pH 4.58 and an alkalinity of 101.3mg/L.
Figure 23: Titration figure for sample “C1-1” with an equivalence point at pH 4.64 and an alkalinity of 98.2mg/L. Titration figure for sample “C1-2” with an equivalence point at pH 4.56 and an alkalinity of 99.5mg/L. Titration figure for sample “C2-1” with an equivalence point at pH 4.65 and an alkalinity of 99.5mg/L. Titration figure for sample “C3-1” with an equivalence point at pH 4.67 and an alkalinity of 100.1mg/L.
Figure 24: Titration figure for sample “C3-2” with an equivalence point at pH 4.60 and an alkalinity of 100.7mg/L. Titration figure for sample “C4-1” with an equivalence point at pH 4.47 and an alkalinity of 103.2mg/L. Titration figure for sample “C5-1” with an equivalence point at pH 4.54 and an alkalinity of 99.5mg/L. Titration figure for sample “C5-2” with an equivalence point at pH 4.68 and an alkalinity of 97.6mg/L.